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# HISTO-ANATOMICAL AND ECOLOGICAL ASPECTS ON MEDICINAL SPECIES (*LAMIACEAE*) FROM MEDITERRANEAN CLIMATE

## ASPECTE HISTO-ANATOMICE ȘI ECOLOGICE LA PLANTE MEDICINALE (*LAMIACEAE*) DIN CLIMATUL MEDITERANEAN

GRIGORE M.N.<sup>1</sup>, TOMA C.<sup>1</sup>, ZAMFIRACHE Maria Magdalena<sup>1</sup>, BOȘCAIU  
Monica<sup>2</sup>, IVĂNESCU Lăcrămioara<sup>1</sup>  
e-mail: mariusgrigorepsyche@yahoo.com

**Abstract.** *In the present study we discuss several structural features regarding three medicinal species from Lamiaceae: Lavandula dentata L., Rosmarinus officinalis L. and Thymus vulgaris L. Within all histo-anatomical features, attention should be paid on protective and secretory hairs, having a large structural diversity.*

**Key words:** medicinal, hairs, Mediterranean, gypsophytes

**Rezumat.** *În lucrarea de față, prezentăm particularitățile structurale pentru trei specii de plante medicinale din familia Lamiaceae: Lavandula dentata L., Rosmarinus officinalis L. și Thymus vulgaris L. Dintre toate trăsăturile histo-anatomice, rețin atenția în mod deosebit perii tectori și secretori, care prezintă o înaltă plasticitate arhitecturală.*

**Cuvinte cheie:** plante medicinale, peri, mediteranean, gipsofite

### INTRODUCTION

*Lamiaceae (Labiatae)* comprises about 7000 species included in 230-234 genera (Takhtajan, 2009). A large number of these species have aromatic and medicinal value (De Laage, 2001; Dewick, 2002; Duke et al., 2002; Barnes et al., 2007; Ebadi, 2007), as is the case of taxa discussed in the current study.

The Mediterranean climate is characterized by strong seasonality which involves the association of a drought period when temperatures are at their hottest and a cool (and cold in many areas) moist period (Thompson, 2005).

There is a rich literature related to histo-anatomical features of *Lamiaceae* species; general studies have been conducted especially by foreign botanists (Lemaire, 1882; Mansfield, 1916; Jackson and Snowdon, 1990; Svoboda K. P. and Svoboda T. G., 2000). In Romania, such investigations were focused mainly on anatomy of *Lavandula angustifolia* (Toma and Niță, 1982; Toma and Rugină, 1998; Robu et al., 2011), and *Thymus* (Toma and Berciu, 2007; Berciu and Toma, 2008; Boz et al., 2009). Secretory tissues and secreted volatile compounds have been recently summarized by Burzo and Toma (2012).

However, a large part of investigations conducted until now have a

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<sup>1</sup> "Al. I. Cuza" University Iași, Romania

<sup>2</sup> Instituto Agroforestal Mediterráneo, Universidad Politécnica de Valencia, Spain

predominantly descriptive character. In this work, we try to propose, starting from our results, an interesting discussing context, related to possible ecological implications of secreted volatile oils. Attention should be paid on the fact that we refer on Mediterranean climate with all ecological factors emerging from it (Grigore, Toma, Boşcaiu, 2011a). In addition, two of investigated species (*Thymus vulgaris* and *Rosmarinus officinalis*) have been collected from gypsic soils that represent striking and incompletely elucidated ecosystems; the nature of plant adaptations vegetating in such soils has been recently critically discussed and questioned (Grigore, Toma, Boşcaiu, 2011b).

## MATERIAL AND METHOD

In this study, we included histo-anatomically observations on three *Lamiaceae* species collected from Spain, in 2010: *Lavandula dentata* L. (collected from Gandia), *Rosmarinus officinalis* L., and *Thymus vulgaris* (collected from gypsic soils in Tuejar). Anatomical investigations were conducted following the standard method fixed by our group working in plant anatomy from Faculty of Biology Iaşi (for a detailed description of this method, see: Grigore, Toma and Boşcaiu, 2010b).

## RESULTS AND DISCUSSION

In *Lavandula dentata*, in the aerial stem (fig. 1), we noticed a relatively thin cortex with an unusual collenchyma and parenchyma in the remaining area. Underneath, a cork (3-4 layers of large cells) and phelloderm (2-3 layers of small cells) follow. Central cylinder is delimited by a discontinuous ring of thin strands of sclerenchymatic fibers, separated by the cells belonging to internal layers of cortical parenchyma. The stele has a secondary structure, derived from the cambium activity: a thick phloemic ring, a very thick xylemic ring, and an internal, thin ring of primary xylem. The lamina has a bifacial-heterofacial structure; it is worth noting the large presence of protecting multicellular, branched hairs (fig. 2); we also noticed secretory, multicellular hairs, with multicellular gland, covered by a prominent cuticle (fig. 2 – note in this case that cuticle is complete, non disintegrated).

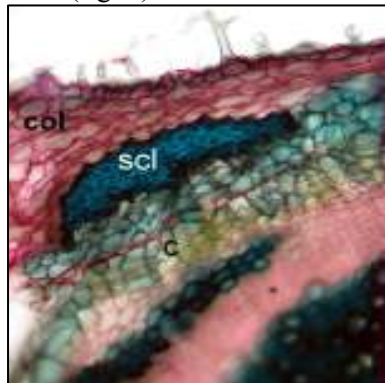


**Fig.1** - Cross section through the aerial stem of *Lavandula dentata* (X 200); c – cork; c.p. – cortical parenchyma; x.v. – xylemic vessels

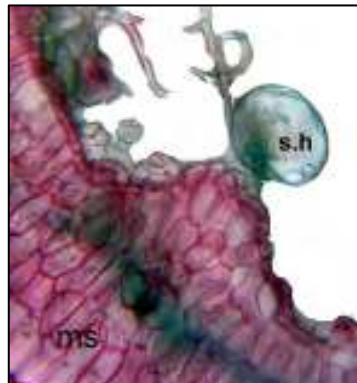


**Fig. 2** - Cross section through the lamina of *Lavandula dentata* (X 200); p.h. – protective hair; s.h. – secretory hair

In *Rosmarinus officinalis* epidermis of aerial stem presents protective and secretory hairs (fig. 3). The first 3-4 layers underneath epidermis form an angular collenchyma, consisting of cells with moderately thick walls. This area is followed by parenchyma, where several strands of sclerenchymatic fibers may be found (fig. 3). In close proximity to endodermis or pericycle, 1-2 layers of large cork cells are located. The upper epidermis of lamina has secretory and protective hairs (fig. 4).



**Fig. 3** - Cross section through the aerial stem of *Rosmarinus officinalis* (X 200); col – collenchyma; ck – cork; scl - sclerenchyma



**Fig. 4** - Cross section through the lamina of *Rosmarinus officinalis* (X 200); s.h. – secretory hair; ms - mesophyll

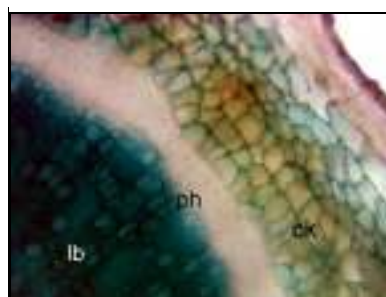
Secretory hairs are abundant, different in length, with uni-, bi- or tricellular stalk and spherical unicellular gland. The basal cell of tricellular stalk has thick walls; following the length of stalk, several branches may be evidenced. We also found scattered short secretory hairs with multicellular gland covered by a prominent cuticle. On lower epidermis, multicellular protective hairs are present; they are relatively long, branched with 2-3 unicellular branches in the point where the cells forming the axis of protective hairs are jointed (fig. 5).

In *Thymus vulgaris*, in the aerial stem, the phellogen (derived from pericycle) produced 3-4 layers of cork and 1-2 layers of phelloderm, having intensely and radially flattened cells (fig. 6).

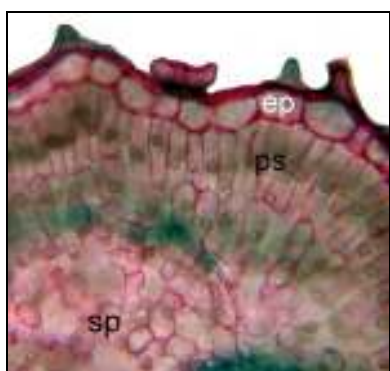
The stele is thick with secondary structure; cambium produced a very thin phloemic ring and two xylemic thick concentric rings, with scattered vessels in the libriform mass (fig. 6). Epidermis of lamina presents protective hairs; some of them are very short, unicellular, with basis clearly flattened (fig. 7), while others are long, unicellular, bicellular, or even tricellular (fig. 8). Secretory hairs are of two types: some of them are longer, with unicellular gland, and others are very short, with multicellular gland, covered by the same cuticle, very prominent (fig. 7).



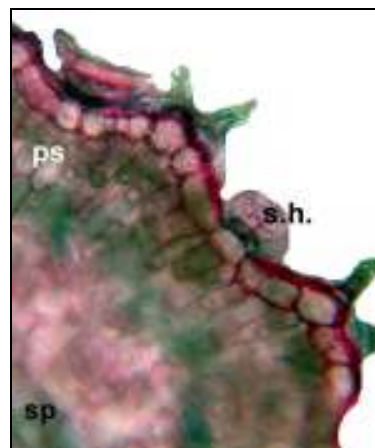
**Fig. 5** - Protective hair on the lamina epidermis of *Rosmarinus officinalis* (X400)



**Fig. 6** - Cross section through the aerial stem of *Thymus vulgaris* (X 200); ck – cork; lb – libriform; ph - phloem



**Fig. 7** - Cross section through the lamina of *Thymus vulgaris* (X 200); ep – epidermis; ps – palisade tissue; sp – spongy tissue



**Fig. 8** - Cross section through the lamina of *Thymus vulgaris* (X 200); ps – palisade tissue; sp – spongy tissue; s.h. – secretory hair

All histological features in investigated species are generally circumscribed in the typical structural plan of *Lamiaceae*. Apart from other *Thymus* species from Romania (Berciu, 2007), the aerial stem of *T. vulgaris* shows less significant collenchyma in cortex.

The important challenge is that at least in the case of *T. vulgaris* and *R. officinalis*, their adaptations could be correlated to gypsic soils, where from these taxa have been collected. Although recognized as gypsophytes (Mota et al., 2010), ecological significance of their histo-anatomical features is still obscure and difficult to specified; this is because *Lamiaceae* is a very heterogeneous taxonomic family and species here included have large ecological spectra.

However, taking into consideration that gypsic soils are mainly specific for Mediterranean climate and secretion of essential oils is an important property

of these species, several ecological correlations may be found.

Aromatic species from *Lamiaceae*, as perennial, evergreen and xeromorphic shrubs are, most likely, the most relevant adaptive profile in respect to ecological factors in Mediterranean. It has been proposed that the presence and accumulation of essential oils may improve tolerance of water constraints and high solar radiation (Thompson, 2005). Moreover, glandular structures containing volatile oils on the leaf surface may enhance tolerance of elevated leaf surface temperature and reduce excessive water loss. The secondary compounds may be reconverted and re-utilized after their release. In some species, neo-methyl glycoside produced by leaves is transported to roots and rhizome where it is converted into other lipid-like metabolites; further, these metabolites may be reutilized in the developing of underground organs. Not in the last, researchers assumed that essential oils might play a role in enzyme maintenance during summer when metabolism and growth are depressed. Other hypothesis suggests that the biosynthesis of essential oils could maintain the appropriate enzyme systems in a state which could allow the rapid reactivation of the metabolic system once favourable conditions for rapid growth occur (Thompson, 2005).

## CONCLUSIONS

Secretory hairs and biosynthesis of essential oils – with all associated functions - suggest that their secretion could be involved in an ecological integration of these species in habitats from Mediterranean climate. Other morphological and anatomical features such as perennial, xeromorphic shrub -like appearance could be also related to the ecology of these species.

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# CONTRIBUTIONS TO THE KNOWLEDGE OF *VERONICA* *PERSICA* SPECIES ANATOMY

## CONTRIBUȚII LA CUNOAȘTEREA ANATOMIEI SPECIEI *VERONICA* *PERSICA*

RĂDUȚOIU D.<sup>1</sup>, RĂDUȚOIU Amira<sup>2</sup>

e-mail: radutoiudaniel@yahoo.com

**Abstract:** *Veronica persica* is one of the few *Veronica* species with solitary flowers and an abundant rooting at the level of the main stem nodes. The plant physiognomy is variable depending on the substrate on which develops, on exhibition and competition with other species. The lack of anatomy studies for this plant prompted us to study this aspect in order to contribute to a better knowledge of this plant.

**Keywords:** anatomy, *Veronica persica*, Romania.

**Rezumat:** *Veronica persica* este printre puținele specii de *Veronica* cu flori solitare la care se observă o înrădăcinare abundentă la nivelul nodurilor tulpinii principale. Fizionomia plantei este variabilă în funcție de substratul pe care se dezvoltă, de expoziție și concurența cu alte specii. Lipsa studiilor de anatomie la această plantă ne-a determinat să abordăm și acest aspect pentru a contribui la o cunoaștere cât mai bună a acestei plante.

**Cuvinte cheie:** anatomie, *Veronica persica*, Romania.

### INTRODUCTION

*Veronica* genus is considered to be among the richest genera of the Romanian spontaneous flora (and elsewhere). In the Flora Europaea (Tutin et al. 1972) 62 species with many infraspecific taxa are presented and in the Romanian Flora - Vol VII - 41 species and 3 hybrids are presented (Ghișa, 1970). Recent works on the Romanian flora present 41 species, 13 subspecies and 2 varieties (Ciocârlan, 2009).

If data on the *Veronica* species morphology and chorology of the Romanian flora are found in many papers on flora and vegetation of a certain territory (Roman, 1976, Dihoru, 1979, Sirbu, 2003), data on their anatomy are totally sporadic (Răduțoiu, 2007, 2009).

In the Romanian literature the studied species is assigned to the *Scrophulariaceae* family; although the recent studies (Albach, 2004) based on phylogenetic analysis using DNA sequences assign it to the *Plantaginaceae*.

The objectives of this paper are: the evidence of some anatomical characters that help to an easier recognition of the species, illustration of essential characters through original photographs.

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<sup>1</sup> University of Craiova, Romania

<sup>2</sup> "Gh. Bibescu" Secondary School no. 36, Craiova, Romania

## MATERIAL AND METHOD

The first stage of the study was to perform numerous field trips in different locations of the country (Craiova, Păușești, Pietrari, Copăceni (VL), Alba Iulia, Bucharest, Cluj Napoca, Iași, Baia de Aramă (MH) in order to collect the study material.

The collected material was brought to the laboratory where it was preserved by pressing it in blotting paper or in liquid environment in a mixture formed of equal parts of absolute ethyl alcohol, glycerine and distilled water. It was harvested at flowering.

To complete the description of species and to highlight its structure, we performed cross-sections with anatomical razor through the roots, stems and leaves of the studied plants. The segments were cut at about the same level on the analyzed plants.

To study the leaf epidermis and epidermal productions we used the excoriation technique (tangential sections).

The microscopic sections were analyzed using a binocular microscope type Krüss (objectives 10, 20, 40), then photographed with a Nikon system.

The measurements were made at MC-3 microscope. 100 measurements and counts were made (epidermal cells, stomata). The maximum singular value ( $V_M$ ), the minimum singular value ( $V_m$ ) and the average value ( $\bar{X}$ ) were considered that represent the arithmetic mean of the 100 singular values.

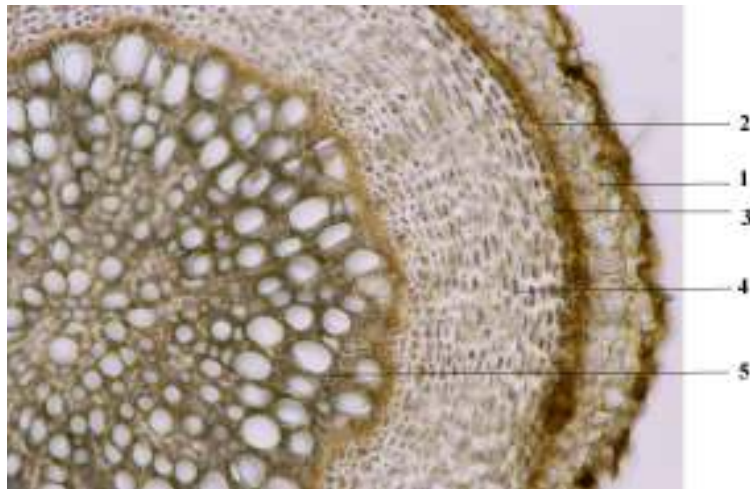
The identification of the plant material was made using the literature (Ghișa, 1960, Tutin et al., 1972, Ciocârlan, 2009).

The authors' abbreviations were made after Brummitt & Powell (1992).

## RESULTS AND DISCUSSIONS

After analyzing the preserved material the following anatomical characters were observed:

**The root** has an obvious secondary structure in the central cylinder and has a thickness of 1170  $\mu\text{m}$  (fig. 1).



**Fig. 1** - Cross- section through the root of *Veronica persica* (Ob 10 x Oc. 10 - orig): 1. bark, 2. endoderm, 3. pericycle, 4. secondary bast 5. secondary xylem (wood).



The rizoderm is not obvious, and the bark is not differentiated in exoderm, cortical parenchyma and endoderm but is composed of three layers of large cells with strong colenchimatized walls. It has a thickness of 90  $\mu\text{m}$ .

Between the bark and central cylinder there is a layer of small cells arranged orderly and with highly thickened walls. This layer is the endoderm because under it there is still a layer of larger cells which are arranged orderly representing the pericycle.

The secondary bast consists of numerous layers of cells arranged more or less ordered in radial rows. It has the thickness of 135  $\mu\text{m}$ .

The cells of the bast have strongly thickened walls; those near the cambium have thinner walls.

The wood has a diameter of 585  $\mu\text{m}$  and consists of wood vessels including the medullary rays in radial direction.

The wood vessels diameter is variable ( $V_m = 15.75 \mu\text{m}$ ,  $V_M = 40.50 \mu\text{m}$ ), and the mean is of  $\bar{X} = 27.22 \mu\text{m}$ .

The cambium is formed of several layers of rectangular cells, slightly elongated in the tangential direction.

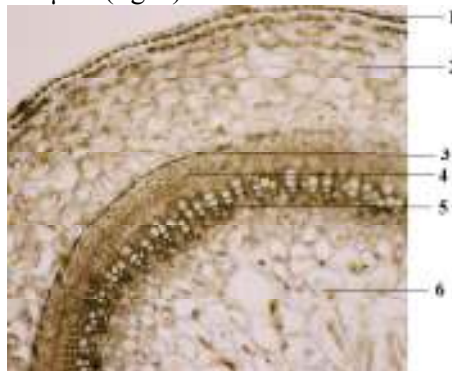
There cannot be measured the Liberian vessels sizes because of the thickening of walls and the strict un-delimitation of cells.

The centre of the root is occupied by wood vessels and by wood parenchyma belonging to the primary xylem.

The central cylinder is very developed in relation to the bark, having a diameter of 882  $\mu\text{m}$ .

The endoderm thickness is of 9  $\mu\text{m}$ , and the pericycle is of 13.5  $\mu\text{m}$ .

**The stem** has an obvious secondary structure in the central cylinder and an average thickness of 1998  $\mu\text{m}$ . (fig. 2).



**Fig. 2** - Cross- section through the stem of *Veronica persica* (Ob. 40 x Oc. 10 – orig.):  
1.epidermis, 2. bark, 3. amiliphera sheath, 4. secondary bast, 5. secondary xylem, 6.  
primary fundamental parenchyma.

The epidermis has only one layer and it has cells with strong cutinized walls and presents a thick outer cuticle of 4.5  $\mu\text{m}$ .

The bark has several layers; it has a thickness of 283.5  $\mu\text{m}$ , and is differentiated in an outer and inner bark and an amiliphera sheath (fig. 3).

The first cells layer of the outer bark has colenchimatized walls. The cells of this layer are arranged orderly, just below the epidermis. Under it there are cells that grow in size until the inner bark. The cells are ovoid, with spaces between them and with chloroplasts inside that give the green colour of the stem.

The inner bark is formed of large, ovoid cells, with spaces between them without inner chloroplasts and which decrease in size to the amiliphera sheath.

The amiliphera sheath has one layer, it has elongated cells in tangential direction which do not have spaces between them, and they have thin walls and contain reserve substances.

The central cylinder has an outer one- layered pericycle, composed of cells arranged orderly, smaller than the ones of the amiliphera sheath.

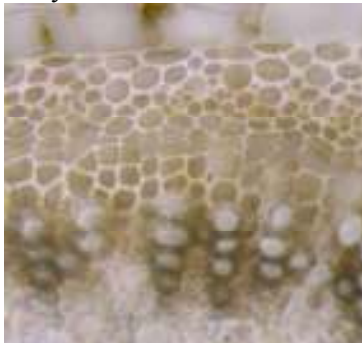


**Fig. 3** - Epidermis, bark and a part of the central cylinder of the stem of *Veronica persica* (Ob. 40 x Oc. 10 – orig.)

The bast and wood are arranged as two concentric circles uninterrupted. It is difficult to distinguish between the secondary elements of the phloem and xylem.

The cambium is composed of two layers of rectangular cells, located between the bast and the wood fibres. The wood fibres and vessels are arranged orderly in radial rows.

The wood vessels decrease in diameter in centripetal direction (Fig. 4), and between them and the cambium there are wood fibres. Under the wood vessels there are smaller cells arranged in several layers, unordered, that belonged to the primary fundamental parenchyma.



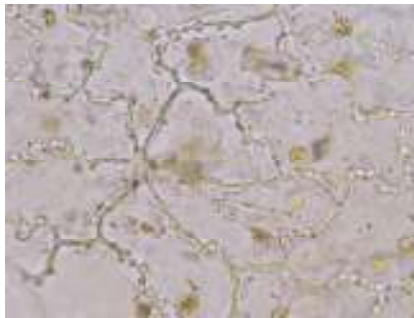
**Fig. 4** - Segment of central cylinder with bast and secondary xylem of the stem of *Veronica persica* (Ob 40 x Oc. 10 – orig.)

The centre of the stem is occupied by the medullar parenchyma, composed of heterodiametrics, large cells with spaces between them and without inner reserve substances.

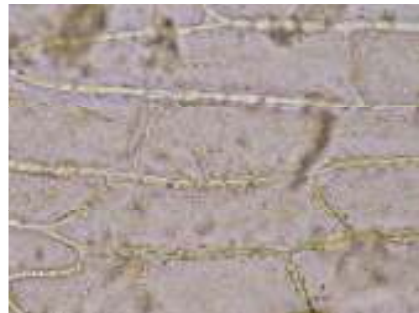
**The leaf** has a bifacial structure and a thickness of 270  $\mu\text{m}$ .

The superior epidermis (adaxial) has one layer, with a thick cuticle of 3.38  $\mu\text{m}$ . The epidermal cells length is of:  $V_m = 85.50 \mu\text{m}$ ,  $V_M = 157.50 \mu\text{m}$ ,  $\bar{X} = 118.35 \mu\text{m}$  and the width of  $V_m = 40.50 \mu\text{m}$ ,  $V_M = 99 \mu\text{m}$ ,  $\bar{X} = 63.90 \mu\text{m}$ . The stomata have lengths ranging from:  $V_m = 29.25 \mu\text{m}$ ,  $V_M = 31.50 \mu\text{m}$ ,  $\bar{X} = 30.60 \mu\text{m}$ . Their average density is 0.0942 stomata /  $\text{mm}^2$ .

The epidermis between veins has cells with strong sinuous walls and with visible simple punctuations (fig. 5). The epidermal cells have walls almost straight near the median vein (fig. 6). The simple punctuations on walls can be also seen in this area.



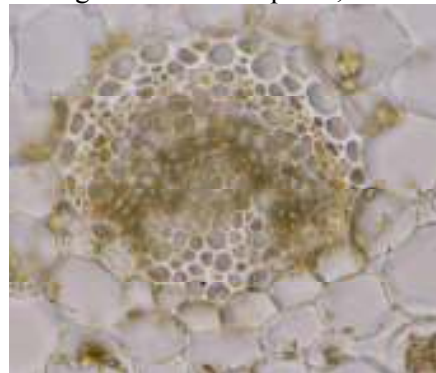
**Fig. 5** - The superior epidermis between veins at the leaf of *Veronica persica* (Ob. 40 x Oc. 10 – orig.)



**Fig. 6** - The superior epidermis near the median vein of *Veronica persica* (Ob. 40 x Oc. 10 – orig.)

The palisade parenchyma consists of 2-3 layers of radially elongated cells and has a thickness of 112.5  $\mu\text{m}$ .

The lacunar parenchyma is composed of ovoid or elongated cells in the tangential direction, with large intercellular spaces; it has a thickness of 78.75  $\mu\text{m}$ .



**Fig. 7** - Detail on the conductor beam of the leaf of *Veronica persica* (Ob. 40 x Oc. 10 – orig.)

The abaxial epidermis cells have different dimensions (length:  $V_m = 65.25 \mu\text{m}$ ,  $V_M = 112.50 \mu\text{m}$ ,  $\bar{X} = 89.32 \mu\text{m}$  and width:  $V_m = 27 \mu\text{m}$ ,  $V_M = 54 \mu\text{m}$ ,  $\bar{X} = 41.62 \mu\text{m}$ ) and has a thick outer cuticle of  $2.25 \mu\text{m}$ . The wood bast conductor beam from the median vein is of open collateral type, it has the wood and the bast arranged in a crescent form that extend until the endoderm. Above the wood and bast there are several layers of cells with sclerenchimatized walls (fig. 7).

## CONCLUSIONS

From the analysis of the studied material we draw some conclusions:

- it is an annual species of the *Veronica* genus with the highest values of the average thickness of the root and stem;
- unlike *Veronica filiformis* with which can be mistaken at first sight, it distinguishes by high values in all structures of the vegetative and sometimes reproductive organs (i.e. *Veronica persica* capsule may be of 7-10 mm while that of *Veronica filiformis* is of 4-5 mm);
- although this is an annual species there can be noticed an obvious secondary structure in the central cylinder of the root and stem.
- the knowledge of the vegetative organs structure of this species allows to take effective measures to control it, knowing that this plant is a weed common in hoed crops (especially during spring), which together with other species form some specific associations in these places.

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# PHOTOSYNTHETIC PIGMENTS (CHLOROPHYLL a, CHLOROPHYLL b) DINAMICS IN SILVER FIR TREES NEEDLES INFECTED BY MISTLETOE

## DINAMICA PIGMENȚILOR ASIMILATORI (CLOROFILA a ȘI CLOROFILA b) ÎN ACELE ARBORILOR DE BRAD DIN DIFERITE CLASE DE PARAZITARE CU VÂSC

**BARBU Cătălina<sup>1</sup>**

e-mail: barbu\_catalina2003@yahoo.com.com

**Abstract.** *In silver fir stands of Bucovina located on the eastern border of silver fir distribution area, the presence of a pathogen agent with an important role in fir decline - mistletoe (*Viscum album ssp. Abietis*) – has been noticed. Mistletoe infections are located in mature and aged stands wherein the proportion of silver fir exceeds 40%, located below the elevation of 700 meters. Our objective was to evaluate the intimate transformation that occurs in silver fir needles because of mistletoe infection. In order to assess the differences of photosynthetic pigments (chlorophyll a and b) in needles of trees from different infection classes we collected samples for analysis from three trees in each infection class. Photosynthetic pigments were extracted from needle material with 80% acetone. The amount of chlorophyll increases with needle age but decreases with infection degree. The chlorophyll a/ chlorophyll b ratio decreases from the healthy to the very infected trees..*

**Key words:** Chlorophyll a, chlorophyll b, silver fir, mistletoe, infection class

**Rezumat.** *In brădetele din Bucovina situate la limita estică a arealului de distribuție se semnaleză prezența unui agent patogen cu un rol important în declinul bradului – vâscul. Atacurile de vâsc sunt localizate în arboretele mature în care proporția bradului depășește 40%, situate la altitudini mai mici de 700 de metri. Cercetările efectuate urmăresc să surprindă transformările ce apar la nivelul aparatului foliar ca urmare a parazitării cu vâsc și vizează determinarea conținutului de pigmenți asimilatori (clorofilă a și clorofilă b) precum și a raportului clorofilă a/ clorofilă b în acele brazi din diferite clase de parazitare. Pigmentii asimilatori au fost extrasi din materialul foliar cu acetona 80%. Am concluzionat ca raportul clorofilă a/clorofilă b descrește de la arborii sănătoși la arborii puternic parazitați. În același timp cantitatea de clorofilă crește cu vârsta frunzelor, dar scade cu gradul de parazitare.*

**Cuvinte cheie:** Clorofila a, clorofila b, brad, vâsc, clase de parazitare

### INTRODUCTION

Like in many European countries, coniferous forests in Romania are also declining (Barbu, 1991; Bîndiu, 1996). The silver fir stands of Bucovina are located on the eastern border of silver fir distribution area (Barbu et Barbu, 2005).

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<sup>1</sup> University “Ștefan cel Mare” of Suceava, Romania

The mistletoe infections are located in mature and aged stands wherein the proportion of silver fir exceeds 40%, located below the elevation of 700 meters. At altitudes higher than 700 m, the infection is incipient.

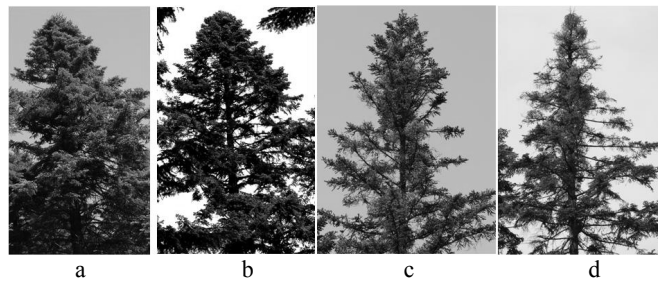
Studies and investigations regarding the transformation that occurred in needles of damaged firs are numerous (Ianculescu et Tisescu, 1992; Bačić et al., 2003; Peguero-Pina et al., 2007; Rigling et al., 2010). The main objective of this paper is to underline the intimate transformation that occurs in silver fir needles because of mistletoe infection. The research aimed the determination of photosynthetic pigments - chlorophyll a and chlorophyll b and chlorophyll a/ chlorophyll b ratio.

## MATERIAL AND METHODS

We selected one stand mainly of silver fir trees in the Northern part of Romania in the forest district Solca (Eastern Carpathians). This stand site was selected because 70 % of the trees are affected by mistletoe attack.

The sample was made of silver fir trees gathered into infection classes using 4 class rating system (Barbu, 2009) (fig. 1). To determine whether there were differences of photosynthetic pigments in needles of trees from different infection classes, we collected samples for analysis from three trees in each infection class.

Each tree crown was divided in 4 sections (section 1 – upper third of the crown, section 2 – middle third, section 3 – lower third and section 4 – compensation crown made of epicormic branches). In each section, 5 needle samples were collected as follows: a sample of 1 year needles, a sample of 2 years needles, a sample of 3 years needles, a sample of 4 years needles, a sample of 5 years needles. In the end, a total of 240 needle samples were collected and analyzed (4 infection classes \* 3 trees/class \* 4 section/tree \* 5 samples/section=240).



**Fig. 1** - Examples of trees from each infection class: a) class 0 of infection; b) 1<sup>st</sup> class of infection; c) 2<sup>nd</sup> class of infection, d) 3<sup>rd</sup> class of infection

Photosynthetic pigments were extracted from needle material with 80% acetone by grinding in the presence of quartz sand (Arnon, 1949; Holden, 1965; Ianculescu et Tisescu, 1992, Porra, 2002; Bačić et al., 2003). For quantitative determination of chlorophyll a and b as well as of total chlorophyll formulas proposed by Porra (2002) were used:

$$\text{Chlorophyll a (chl a) } (\mu\text{g/ml}) = 12.25 (A_{663,6}) - 2.55 (A_{646,6})$$

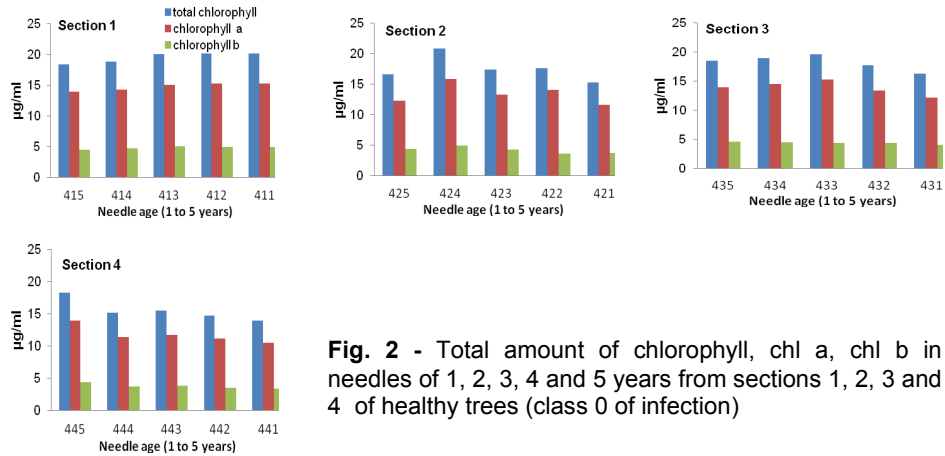
$$\text{Chlorophyll b (chl b) } (\mu\text{g/ml}) = 20.31 (A_{646,6}) - 4.91 (A_{663,6})$$

$$\text{Total chl } (\mu\text{g/ml}) = 17.76 (A_{646,6}) + 7.34 (A_{663,6})$$

$A_{646,6}$  and  $A_{663,6}$  are the absorbances of the solution at 646,6 nm and 663,6 nm.

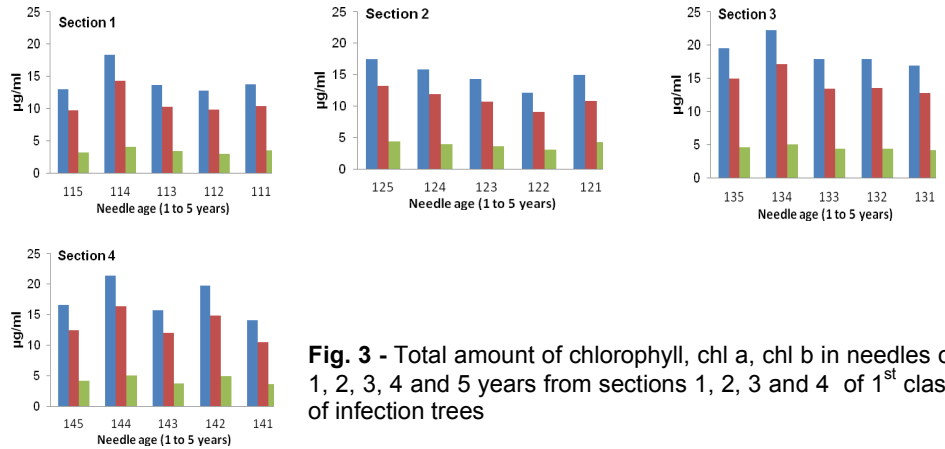
## RESULTS AND DISCUSSIONS

In healthy trees, (class 0 of infection) total chlorophyll in 1 to 5 years needles harvested from different parts of the crown varies between 13.888  $\mu\text{g/ml}$  for 1 year needles of the compensation crown and 20.765  $\mu\text{g/ml}$  in those of 4 years of middle third of the crown (fig. 2). The amount of chlorophyll a varies between 10.514  $\mu\text{g/ml}$  for 1 year needles of the compensation crown and 15.882  $\mu\text{g/ml}$  in those of 4 years for the middle third of the crown. Related to chlorophyll b, its amount varies between 3.377  $\mu\text{g/ml}$  for 1 year needles of the compensation crown and 5.001  $\mu\text{g/ml}$  for 3 years old needles of the upper third of the crown.



**Fig. 2** - Total amount of chlorophyll, chl a, chl b in needles of 1, 2, 3, 4 and 5 years from sections 1, 2, 3 and 4 of healthy trees (class 0 of infection)

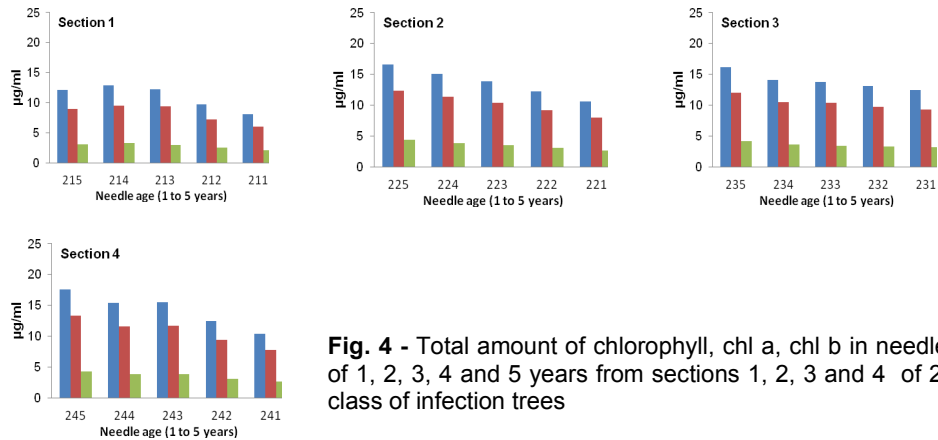
For trees of 1<sup>st</sup> infection class, (low infection) the total amount of chlorophyll in 1 to 5 years old needles harvested from different crown sections varies between 12.146  $\mu\text{g/ml}$  - for 2 years needles of middle third of the crown – and 22.229  $\mu\text{g/ml}$  – for 4 years needles from lower third of the crown (figure 3).



**Fig. 3** - Total amount of chlorophyll, chl a, chl b in needles of 1, 2, 3, 4 and 5 years from sections 1, 2, 3 and 4 of 1<sup>st</sup> class of infection trees

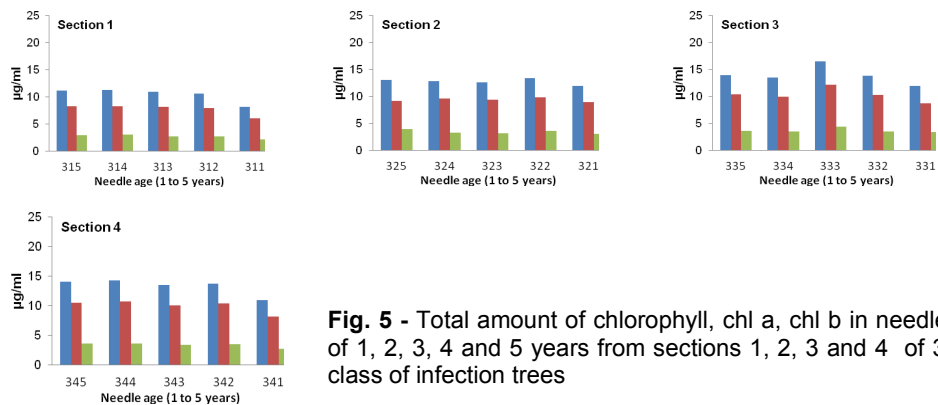
The amount of chlorophyll a varies between 9.039  $\mu\text{g/ml}$  for 2 years needles of the middle third of the crown and 17.169  $\mu\text{g/ml}$  in those of 4 years of the lower third of the crown. Related to chlorophyll b, its amount varies between 2.958  $\mu\text{g/ml}$  for 2 years needles of the upper third of the crown and 5.066  $\mu\text{g/ml}$  for 4 years old needles of the lower third of the crown.

**For trees of 2<sup>nd</sup> infection class** (moderate infection) total amount of chlorophyll varies between 8.106  $\mu\text{g/ml}$  - for 1 year needles of upper third of the crown – and 17.562  $\mu\text{g/ml}$  – for 5 years needles of the compensation crown (figure 4). The amount of chlorophyll a varies between 5.970  $\mu\text{g/ml}$  for 1 year needles of the upper third of the crown and 13.315  $\mu\text{g/ml}$  in those of 5 years of the compensation crown.



**Fig. 4** - Total amount of chlorophyll, chl a, chl b in needles of 1, 2, 3, 4 and 5 years from sections 1, 2, 3 and 4 of 2<sup>nd</sup> class of infection trees

**For trees of 3<sup>rd</sup> infection class** (heavy infection) total amount of chlorophyll varies between 8.134  $\mu\text{g/ml}$  - for 1 year needles of upper third of the crown – and 14.291  $\mu\text{g/ml}$  – for 4 years needles of the compensation crown (figure 5).



**Fig. 5** - Total amount of chlorophyll, chl a, chl b in needles of 1, 2, 3, 4 and 5 years from sections 1, 2, 3 and 4 of 3<sup>rd</sup> class of infection trees



The amount of chlorophyll a varies between 6.049 µg/ml for 1-year needles of the upper third of the crown and 12.149 µg/ml in those of 3 years of the compensation crown. Related to chlorophyll b, its amount varies between 2.087 µg/ml for 1-year needles of the upper third of the crown and 4.352 µg/ml for 3 years old needles of the lower third of the crown.

Table 1

**Photosynthetic pigment (Chl a and Chl b) concentration (µg/ml) in young and mature (1, 3 and 5 years) needles of silver firs from different infection classes**

| Needle age                   | class 0 of infection | 1 <sup>st</sup> class of infection | 2 <sup>nd</sup> class of infection | 3 <sup>rd</sup> class of infection |
|------------------------------|----------------------|------------------------------------|------------------------------------|------------------------------------|
| <b>Chlorophyll a (Chl a)</b> |                      |                                    |                                    |                                    |
| 1                            | 12,40±1,78           | 11,08±1,12                         | 11,08±1,12                         | 7,94±1,78                          |
| 3                            | 13,81±1,42           | 11,61±1,43                         | 11,61±1,43                         | 9,56±0,47                          |
| 5                            | 13,50±0,71           | 13,10±2,16                         | 13,10±2,16                         | 9,94±1,34                          |
| <b>Chlorophyll b (Chl b)</b> |                      |                                    |                                    |                                    |
| 1                            | 3,98±0,67            | 3,82±0,37                          | 2,64±0,42                          | 2,78±0,74                          |
| 3                            | 4,32±0,45            | 3,79±0,08                          | 3,30±0,36                          | 3,41±0,38                          |
| 5                            | 4,45±0,11            | 4,63±0,27                          | 3,94±0,58                          | 3,51±1,48                          |
| <b>Total chlorophyll</b>     |                      |                                    |                                    |                                    |
| 1                            | 16,39±2,46           | 14,95±1,22                         | 10,39±1,79                         | 10,54±1,79                         |
| 3                            | 18,14±1,86           | 15,41±1,60                         | 10,44±1,31                         | 12,53±2,35                         |
| 5                            | 17,95±0,76           | 18,51±3,10                         | 15,60±2,09                         | 13,07±1,31                         |
| <b>Chl a/Chl b</b>           |                      |                                    |                                    |                                    |
| 1                            | 3,11±0,06            | 2,87±0,21                          | 2,93±0,10                          | 2,86±0,1                           |
| 3                            | 3,19±0,20            | 3,05±0,08                          | 3,06±0,11                          | 2,92±0,08                          |
| 5                            | 3,03±0,13            | 3,28±0,27                          | 2,95±0,13                          | 2,73±1,26                          |

Table 2

**Photosynthetic pigment (Chl a and Chl b) loss (%) in silver fir needles of 1,3 and 5 years**

| Infection class        | Chlorophyll |     |       |     |       |     |
|------------------------|-------------|-----|-------|-----|-------|-----|
|                        | Chl a       |     | Chl b |     | total |     |
|                        | µg/ml       | %   | µg/ml | %   | µg/ml | %   |
| <b>1 year needles</b>  |             |     |       |     |       |     |
| Class 0                | 12,40       | 100 | 3,98  | 100 | 16,39 | 100 |
| Class 1                | 11,08       | 89  | 3,82  | 96  | 14,95 | 91  |
| Class 2                | 7,75        | 64  | 2,64  | 66  | 10,39 | 64  |
| Class 3                | 7,94        | 62  | 2,78  | 70  | 10,54 | 63  |
| <b>3 years needles</b> |             |     |       |     |       |     |
| Class 0                | 13,81       | 100 | 4,32  | 100 | 18,14 | 100 |
| Class 1                | 11,61       | 84  | 3,79  | 87  | 15,41 | 85  |
| Class 2                | 10,44       | 76  | 3,30  | 76  | 10,44 | 69  |
| Class 3                | 9,56        | 69  | 3,41  | 79  | 12,53 | 57  |
| <b>5 years needles</b> |             |     |       |     |       |     |
| Class 0                | 13,50       | 100 | 4,45  | 100 | 17,95 | 100 |
| Class 1                | 13,10       | 97  | 4,63  | -   | 18,51 | -   |
| Class 2                | 11,66       | 86  | 3,94  | 88  | 15,60 | 87  |
| Class 3                | 9,94        | 73  | 3,51  | 79  | 13,07 | 73  |

The amount of chlorophyll increased with leaf age (Peguero-Pina et al., 2007; Bačić et al. 2003), but decrease with the degree of infection. Chlorophyll a/chlorophyll b ratio decreases from healthy trees (average of 3.1) to heavy infected trees (average 2.8) (tab. 1).

Decline of photosynthesis results in substantial reduction of growth. Most pronounced quantitative reduction of chlorophyll (-35-40 %) is observed at 1 and 3 years needles of moderate and heavy infected trees (class 2 and 3 of infection). For trees of 1<sup>st</sup> class of infection (low infection) quantitative reduction of chlorophyll is on average 10-15 % for 1 and 3 years old needles (tab. 2).

## CONCLUSIONS

1. The chlorophyll a/chlorophyll b ratio decreases from the healthy to heavy infected trees.

2. Most pronounced quantitative reduction of chlorophyll is observed in needles of moderate and heavy infected trees (class 2 and 3 of infection).

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# MORPHO-ANATOMICAL STUDIES ON THE SHOOT'S INTERNODE IN SOME GRAPEVINE VARIETIES CULTIVATED IN IASI AND COTNARI VINEYARDS

## CERCETĂRI MORFO ANATOMICE ÎN AXUL LĂSTARULUI LA UNELE SOIURI DE VIȚĂ DE VIE CULTIVATE ÎN PODGORIILE IAȘI ȘI COTNARI

*IFRIM Camelia<sup>1</sup>, JITĂREANU Carmen Doina<sup>2</sup>,  
SLABU Cristina<sup>2</sup>, MARTA Alina Elena<sup>2</sup>, TOMA Liana Doina<sup>2</sup>*  
e-mail: camicris@yahoo.com

**Abstract.** *The morphology and anatomy of the shoot and root are criteria for the classification of taxonomists in the vine. Moreover, the anatomical variability of different genotypes is in direct relationship with the efficiency of water use. The adaptation to water stress is based on the hydraulic conductance of the plant, which is determined by the diameter and density of vessels in the xylem. In the study we analyze the morpho-anatomical structure of the shoot internode in the flowering phenophase at Frâncușă, Fetească albă, Grasă de Cotnari and Tămâioasă românească vine varieties cultivated in Iasi and Cotnari vineyards, under the conditions of 2011. The differences between the varieties are visible in the secondary structure which result from the activity of cambium and phelogen meristems, especially in conducting tissues and refers to the number of layers of collenchyma, number of conducting beams (vascular bundles), presence of concentric beams and calotte of lignified cells in the liberian parenchyma.*

**Key words:** *Vitis vinifera* varieties, water stress, shoot anatomy

**Rezumat.** *Morfologia și anatomia lăstarului sunt criterii de clasificare a taxonilor la vița de vie. Mai mult, variabilitatea anatomică a diferitelor genotipuri este în relație directă cu eficiența utilizării apei. Adaptarea față de stresul hidric se bazează pe conductanța hidraulică a plantei, care este determinată de diametrul și densitatea vaselor din xilem. În lucrare analizăm structura morfo-anatomică a axului lăstarului în fenofaza de înflorire la soiurile de viță de vie Frâncușă, Fetească albă, Grasă de Cotnari și Tămâioasă românească, cultivate în podgoriile Iași și Cotnari, în condițiile anului 2011. Diferențele dintre soiuri sunt vizibile în structura secundară care rezultă din activitatea meristemelor cambiu și felogen, în special în țesuturile conducătoare și se referă la numărul de straturi de colenchim, numărul de fascicule conducătoare, prezența fasciculelor concentrice și calota celulelor lignificate din parenchimul liberian.*

**Cuvinte cheie:** soiuri *Vitis vinifera*, stres de apă, anatomia lăstarului

### INTRODUCTION

The morpho-anatomical study of the grapevine represented the subject of several important papers (Metcalf and Chalk, 1950) or individual studies (Chatelet et

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<sup>1</sup> Botanical Garden "Anastase Fătu" of Iasi, Romania

<sup>2</sup> University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

al., 2008), of specialists in anatomy (Zanoschi et Toma, 1985) or oenologists (Jackson, 2008). The characteristics of the secondary phloem and their role in classifying the cultivars was initially demonstrated by (Esau, 1965) and confirmed by further research studies (Aloni and Peterson, 1991; Swanepoel et al., 1984).

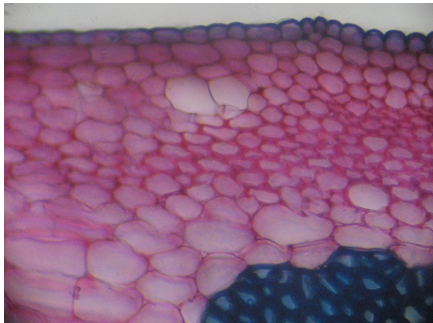
## MATERIAL AND METHOD

The botanical material taken into the study is represented by fertile and sterile shoots from seven different varieties of grapes belonging to the *Vitis vinifera L.* species: Băbească gri, cultivated in Iași; Fetească albă, cultivated in Cotnari; Fetească regală, cultivated in Iași; Frâncușă, cultivated in Iași; Grasă de Cotnari, cultivated in Iași and Cotnari; Riesling italian, cultivated in Iași; Tămâioasă românească, cultivated in Iași and Cotnari.

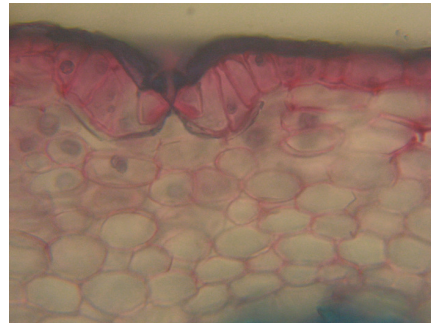
## RESULTS AND DISCUSSIONS

The cross section of the shoot's internode presents a circular ribbed contour. All the varieties present an incipient secondary structure, so that the epidermis is not yet replaced by the periderm and the level of suberin and pheloderm is quite low.

The epidermis, characteristic to the primary structure, consists of closely united polygonal cells with convex outer walls that are thicker than the rest and costated. At the level of the collenchyma, the epidermal cells are relatively tall, while in the parenchymal area they are slightly tangentially oblong (fig. 1). Here and there tector hairs generally pluricellular, but also simple can be seen as well as stomata. The latter ones, in the case of the varieties we refer to, have a variable position; thus, most often the stomata are situated at the level of the epidermic cells and form a substomatal chamber, not very obvious.



**Fig. 1** - Riesling italian *variety*, cultivated in Iași – Cross section of fertile shoot pointing out the epidermis

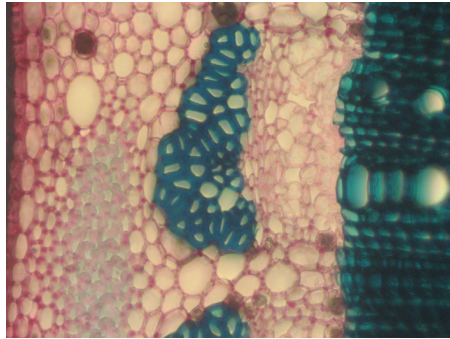


**Fig 2** - Fetească albă *variety*, cultivated in Cotnari - Cross section of fertile shoot pointing out the stomata

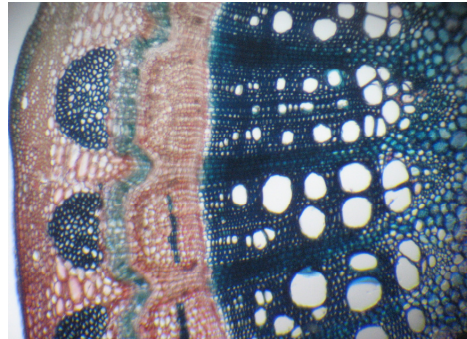
In other cases (Fetească albă fertile shoot cultivated in Cotnari) they are situated under the level of the epidermic cells and form an obvious suprapostomatal

chamber (fig. 2). This suggests the existence of some xerophyte adjustments determined by environmental conditions.

The bark is thin, formed by assimilator cellulosic parenchyma areas alternating with angular collenchyma areas (fig. 3) that are most often situated at the level of the vascular bundle. The central cylinder occupies most part of the section and consists of numerous conducting liberian and wooden fascicles of open collateral type with a circular structure. At this level we can notice the beginning of the development of the secondary structure based on lateral meristems. The moment the phellogen is formed depends of the variety's precocity. Suber and phelloderm tissues are poorly represented.



**Fig. 3** - Fetească albă *variety*, cultivated in Cotnari - Cross section of fertile shoot pointing out the absence of the phellogen

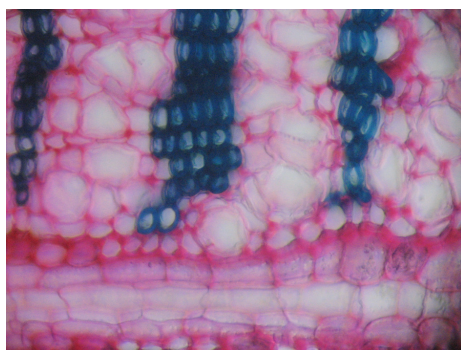


**Fig. 4** - Fetească albă *variety*, cultivated in Cotnari - Cross section of sterile shoot pointing out the continuous phellogen

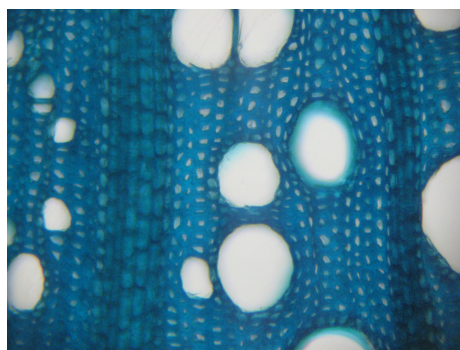
The sections performed at different varieties pointed out the presence of phellogen (fig. 4) in the fertile shoot of the following varieties Frâncușă, Grasă de Cotnari and Tămâioasă românească, cultivated in Iași and Fetească albă cultivated in Cotnari. Pericyclic fiber (periphloemic) cordons are highly present at the periphery of the phloem (fig. 3).

The cambium has an annular shape in cross section and a bifacial functioning system, producing towards the outside the secondary liber and towards the inside the secondary wood; the latter one presents annual rings with young wood vessels (fig. 4) with a diameter larger than of the late wood vessels. Once the secondary phloem and wood are formed, the primary liber is pushed towards the outside and crushed so that it can hardly be recognized at the tendrils with a typical secondary structure.

The secondary liber is characterized by an alternation of the bands of lignified liberian fibers with thick walls (hard phloem) and screened tubes (soft phloem), annex cells and liberian parenchyma cells. The structure is different depending of the variety presenting up to 3 bands of hard phloem (fig. 5) each of them with a variable number of layers (2-5).



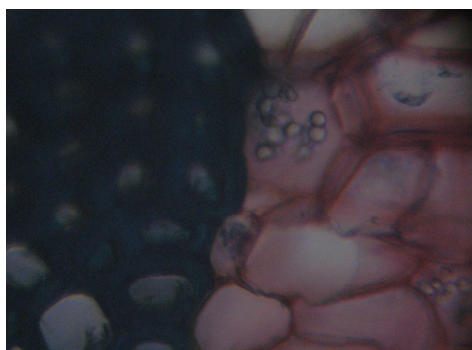
**Fig. 5** - Băbească gri *variety*, cultivated in Iași - Cross section of sterile shoot pointing out the liber



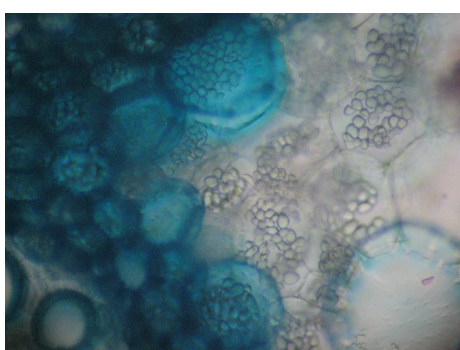
**Fig. 6** - Tămâioasă românească *variety*, cultivated in Iași - Cross section of fertile shoot pointing out the wood

The wood occupies a wider area than the phloem, presenting medullary rays narrower than the ones from the liber. The secondary wood consists of scleriform and dotted vessels, septate wood fibers and wood parenchyma cells all having thick lignified walls. Towards the medulla, the primary wood can also be noticed with cellulosic parenchyma cells between the radiant vessels rows.

The diameters of the wood vessels are highly different despite being on the same section and the width of the medullary rays varies between 2 – 5 or even 7 layers (fig. 6).



**Fig. 7** - Grasă de Cotnari *variety*, cultivated in Iași - Cross section of fertile shoot pointing out the starch granule next to the liber

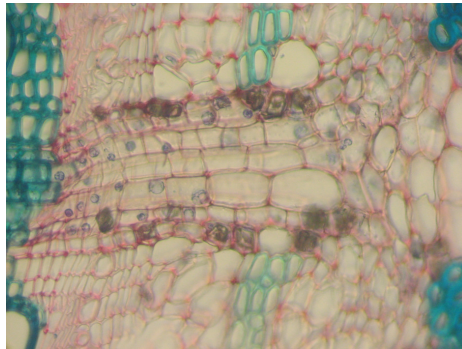


**Fig. 8** - Fetească regală *variety*, cultivated in Iași - Cross section of fertile shoot pointing out the starch granule from the cortical parenchyma

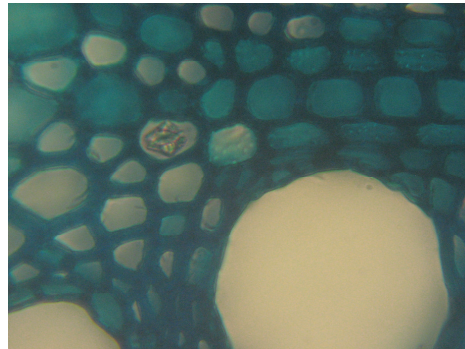
Usually, towards the end of the vegetation season, the liberian parenchyma cells and the liberian medullary rays present starch and tannin. In the case of the varieties included in the present study we noticed only rare granules of starch at the liberian medullary rays, scarcely next to the pericyclic fibers (fig. 7) and very numerous at the wood medullary rays (for example the fertile shoots of Fetească albă cultivated in Iași). In the case of the fertile shoot of Fetească regală that is

cultivated in Iasi, numerous granules of starch were noticed in the outer cellular layers of the cortical parenchyma (fig. 8).

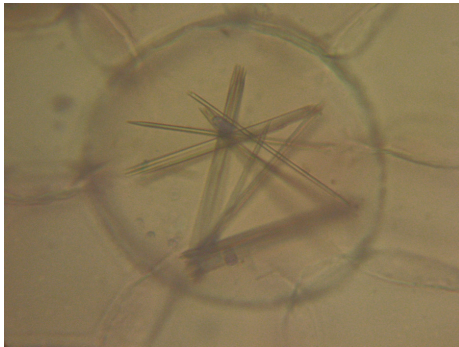
From the papers consulted and included in the biography resulted that the medullar rays cells contain simple crystals (often needle like crystals) or calcium oxalate ursini (fig. 11). In the case of the varieties included in the present study, the types of crystals and their distribution was more varied. We noticed its massive presence in the marginal layers of the medullar rays from the liberian level (fig. 9) and the rare presence in the wood medullar rays (in the fertile shoot of Fetească albă, cultivated in Cotnari – fig. 10). In the medullar parenchyma cells, the presence of raphides was noticed (at the sterile shoot of Fetească albă, cultivated in Cotnari – fig. 12, at the fertile shoot of Fetească regală cultivated in Iasi).



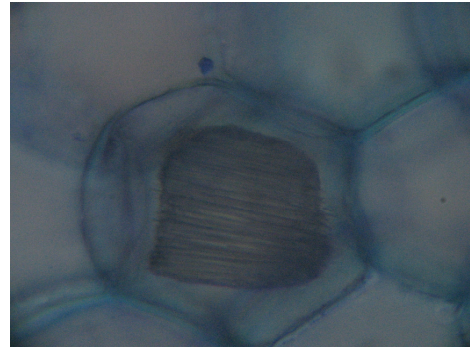
**Fig. 9** - Fetească albă *variety*, cultivated in Cotnari - Cross section of fertile shoot pointing out the chrystals



**Fig. 10** - Fetească albă *variety*, cultivated in Cotnari - Cross section of fertile shoot pointing out the chrystals



**Fig. 11** - Fetească albă *variety*, cultivated in Cotnari - Cross section of fertile shoot pointing out the niddle like chrystals



**Fig. 12** - Fâncușă *variety*, cultivated in Iași - Cross section of fertile shoot pointing out the raphides

## CONCLUSIONS

1. The varieties taken into consideration for the present study are in the developing phase of the secondary structure.
2. The phellogen's apparition and the well functioning depend of the variety's precocity.
3. In the case of Fetească albă variety, the presence of stomata under the level of the epidermis suggests it's adaptation to the hydric stress.
4. The development of the hard phloem at the secondary phloem is different depending on the variety's precocity, the information presented in the specialized literature.
5. The distribution and the types of calcium oxalate crystals are very varied, resulting differences from what is presented in the specialized literature.

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# ECOPHYSIOLOGICAL RESEARCHES ON SOME GRAPEVINE CVS. CULTIVATED IN IASSY AND COTNARI VINEYARDS IN 2011 YEAR

## CERCETĂRI ECOFIZIOLOGICE LA UNELE SOIURI DE VIȚĂ DE VIE CULTIVATE ÎN PODGORIILE IAȘI ȘI COTNARI ÎN ANUL 2011

*MARTA Alina Elena<sup>1</sup>, JITĂREANU Carmen Doina<sup>1</sup>,  
SLABU Cristina<sup>1</sup>, TOMA Liana Doina<sup>1</sup>*  
e-mail: martaalinaelena@yahoo.com

**Abstract.** *In the context of climatic changes that take place on the planet and that are felt more and more in our country, the vines in areas of Moldova have to deal with two major problems: drought and excessive temperatures. Although vine has a great capacity to adapt to extreme conditions of hydrophily or xerophily, the water deficit during the dry years disturbs the main plant physiological processes, having negative effect on vine stock vegetation and their production capacity. In the Iasi and Cotnari vineyards, the growing season of 2011 was characterized by drought conditions caused by strong water deficit in May and July to September. This study analyzed the effect of climatic conditions on quantitative and qualitative parameters of grape production and the dynamics of chlorophyll content in leaves of Frâncușă, Fetească albă, Grasă de Cotnari and Tămâioasă românească vine cvs.*

**Key words:** vineyard, eco-physiology, photosynthetic pigments

**Rezumat.** *În contextul schimbărilor climatice care au loc la nivel planetar și care se fac simțite tot mai pregnant și în țara noastră, cultura viței de vie în arealele din Moldova se confruntă cu două probleme majore: seceta și temperaturile excesive. Deși vița de vie are o mare capacitate de adaptare la condiții extreme de hidrofilie sau xerofilie, deficitul de apă din anii secetoși dăunează principalelor procese fiziologice ale plantei, având efecte negative asupra vegetației butucului și a capacității de producție. În podgoriile Iași și Cotnari sezonul de vegetație a anului 2011 s-a caracterizat prin condiții de secetă, provocate de un deficit hidric pronunțat în lunile mai și iulie - septembrie. În lucrare a fost analizat efectul acestor condiții climatice asupra unor parametri cantitativi și calitativi ai producției de struguri, precum și asupra dinamicii conținutului de clorofilă în frunzele soiurilor de viță de vie Frâncușă, Fetească albă, Grasă de Cotnari și Tămâioasă românească.*

**Cuvinte cheie:** viță de vie, eco-fiziologie, pigmenți fotosintetici

### INTRODUCTION

Several research studies have pointed out the importance of photosynthesis in the quantitative and qualitative production of grapevine (Cifre et al., 2005; Lebon et

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

al., 2008). Photosynthesis is the most sensitive process to the summer drought (Palliotti et al., 2009; Zulini et al., 2005). The stress caused by the lack of water and the water deficit that is closely related to it have severe effects especially when they appear suddenly, when they are extremely intense and accompanied by high temperatures (Cifre et al., 2005; Jităreanu et al., 2011).

The present study focuses on the ecophysiological reaction of certain grapevine varieties, which was determined on the basis of the chlorophyll content in the leaves and in relation to the drought conditions present in the north of Moldavia.

## MATERIAL AND METHODS

The researches were conducted in the vegetation season of 2011 on the following grapevine varieties Fetească albă, Grasă de Cotnari, Tămâioasă românească and Frâncușă that were cultivated in Iasi and Cotnari vineyards. The evolution of climatic conditions in January - October was estimated taking into consideration the average value of the temperatures recorded, the monthly minimum and maximum values (°C) as well as the monthly sum of precipitations (mm). These data were related to the normal values registered in Iasi and Cotnari vineyards. The grape production values (kg/stock) and the sugar content in the must (g/l) were used as production indicators.

The ecophysiological reaction was estimated using a photosynthesis parameter, more precisely, the chlorophyll content index in the leaves (CCI), the field method was used and a CCM 200 plus device. These values were recorded in dynamics, during the blooming phenophases, the grapes' growth and maturation.

## RESULTS AND DISCUSSIONS

### *The evolution of climatic conditions in Iasi and Cotnari vineyards*

In 2011 the climatic conditions in Iasi vineyard recorded average monthly temperatures higher than the normal, with deviations ranging between 0.1°C in April and 2.3°C in September; the maximum temperatures recorded varied between 31.6 °C in May and 35.5 °C in July. The precipitation quantity showed a monthly sum characterized by a very high hydric deficit in May and July-September. In April and June, the values recorded were very close to normal, the hydric deficit being of only 4.6 mm.

In Cotnari vineyard, the average monthly temperatures recorded were also higher than the normal, with values raging between 0.8°C in April – May and 3.2°C in September. The maximum temperatures ranged between 22.6°C in April and 33.6°C in July. A severe rainfall deficit was recorded in May and August. On the other hand, in June it was registered a precipitation excess of 35.8 mm higher than the normal. Just like in Iasi vineyard, in Cotnari, the months May – August – September 2011 were characterized by severe drought, while in June the rainfall excess from Cotnari vineyard diminished the effects of the drought in July when the hydric deficit was also lower (tab. 1).

### *The effect of climatic conditions on some production indicators*

The climatic conditions of the vegetation season in 2011 have lead to the production of a higher quantity of grapes in Cotnari vineyard than in Iasi vineyard

for all the grape varieties that were analyzed; it was expressed in grape production (kg/stock), the average number of grapes/stock and the average weight of one grape (tab. 2). The quality of the production, expressed by the sugar content in the grapes (g/l) and the glucoacidimetric index was higher especially at the following varieties - Grasă de Cotnari and Tămâioasă românească. At the same time, the mass of 1000 berries (g) was superior in the case of Grasă de Cotnari (tab. 2).

Table 1

**Humidity and temperature climatic conditions in Iasi and Cotnari vineyards in 2011**

|                                    | I     | II    | III   | IV   | V     | VI    | VII   | VIII  | IX    | X    |
|------------------------------------|-------|-------|-------|------|-------|-------|-------|-------|-------|------|
| Iasi vineyard                      |       |       |       |      |       |       |       |       |       |      |
| Temperature °C                     |       |       |       |      |       |       |       |       |       |      |
| Monthly average                    | -2,4  | -2,3  | 3,5   | 10,3 | 16,7  | 20,7  | 22,9  | 21,6  | 18,1  | 8,8  |
| Deviation from normal              | -0,7  | -1,1  | 0,1   | 0,1  | 0,4   | 1,0   | 1,7   | 1,1   | 2,3   | -1,3 |
| Maximum                            | 10,3  | 15,3  | 21,6  | 24,7 | 31,6  | 34,6  | 35,5  | 33,1  | 31,9  | 28,5 |
| Monthly sum of precipitations (mm) |       |       |       |      |       |       |       |       |       |      |
| Monthly sum                        | 13,2  | 13,7  | 8,4   | 82,2 | 32,3  | 84,1  | 37,8  | 32,1  | 18,7  | 40,2 |
| Deviation from normal              | -17,3 | -14,7 | -24,4 | 33,1 | -26,8 | -4,6  | -45,0 | -24,8 | -33,3 | 7,4  |
| Cotnari vineyard                   |       |       |       |      |       |       |       |       |       |      |
| Monthly average                    | -1,3  | -2,6  | 3,5   | 10,4 | 16,3  | 20,0  | 22,0  | 21,2  | 18,7  | 9,3  |
| Deviation from normal              | 1,3   | -1,3  | 0,5   | 0,8  | 0,8   | 1,2   | 1,6   | 1,2   | 3,2   | +0,8 |
| Maximum                            | 11,2  | 15,9  | 19,9  | 22,6 | 28,8  | 32,2  | 33,6  | 31,0  | 29,9  | 27,1 |
| Monthly sum of precipitations (mm) |       |       |       |      |       |       |       |       |       |      |
| Monthly sum                        | 8,7   | 28,0  | 14,0  | 58,2 | 12,8  | 114,4 | 70,8  | 13,4  | 18,0  | 31,0 |
| Deviation from normal              | -12,6 | 6,6   | 13,4  | 8,5  | -46,2 | 35,8  | -13,8 | -46,3 | -26,0 | 1,8  |

It can be seen that the thermal factor from the two vineyards taken into consideration respected the optimum ecological requirements for grapevine. The amount of precipitations registered in June diminished the effects of the drought in July for Cotnari vineyard, it being responsible for the production values that are net higher than those registered in Iasi vineyard.

Lebon et al. (2008) point out the role of sugars in the blossoming phase of the grapevine; the decrease of sugars' disponibility disturbs the formation of flowers and their abortion. At the same time, sugars can be seen as signal molecules involved in the stress reaction. The source of sugars is represented by photosynthesis; drought reduces the photosynthetic capacity during summer (Palliotti et al., 2009). Reducing the drought conditions in the flowering phenophase and at the beginning of the growth stage in Cotnari vineyard it was stimulated the apparition of inflorescences and fruits,

reducing the risk of flower abortion; this led to the appearance of a higher number of grapes/stock and implicitly to the growth of grapes production.

At the same time, the temperature and rainfall values from September and October have had a positive effect on the maturation and the quality of the grapes from Cotnari, increasing the sugar content, the glucoacidimetric index and the mass of 100 berries. The highest values were recorded at Grasă de Cotnari variety.

The extreme climatic conditions favored the over-maturation of grapes, bringing about a slow process of sugar and flavor accumulation.

Table 2

**Grape production for the grapevine varieties studied in 2011**

| Variety              | Vineyard | Grape production (kg / plant) | Calculated production per hectare (t / ha) | Grapes on the plant (average) | The average-weight of a grape (g) |
|----------------------|----------|-------------------------------|--|-------------------------------|-----------------------------------|
| Fetească albă        | Iași     | 2,77                          | 10,5                                       | 26,4                          | 105                               |
|                      | Cotnari  | 3,02                          | 8,4  | 30,5                          | 99                                |
| Frâncușă             | Iași     | 2,69                          | 10,2                                       | 25,8                          | 104                               |
|                      | Cotnari  | 4,86                          | 13,5                                       | 42,2                          | 115                               |
| Grasă de Cotnari     | Iași     | 1,19                          | 4,5  | 8,21                          | 145                               |
|                      | Cotnari  | 2,98                          | 8,3  | 13,9                          | 213                               |
| Tămâioasă românească | Iași     | 2,13                          | 8,1  | 19,0                          | 112                               |
|                      | Cotnari  | 3,56                          | 9,9  | 21,8                          | 163                               |
| Mean values          | Iași     | 2,19                          | 8,32                                       | 19,8                          | 11,6                              |
|                      | Cotnari  | 2,58                          | 7,02                                       | 27,1                          | 14,7                              |

Table 3

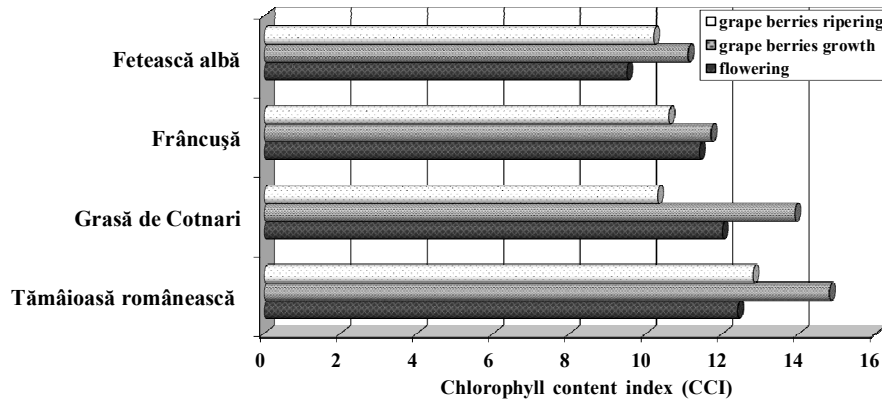
**Production's quality for the grapevine varieties studied in 2011**

| Variety              | Vineyard | Sugars (g/L) | Weight of 100 grains (g) | Gluco-acidimetric index |
|----------------------|----------|--------------|--------------------------|-------------------------|
| Fetească albă        | Iași     | 195          | 177                      | 48,75                   |
|                      | Cotnari  | 205          | 173                      | 48,23                   |
| Frâncușă             | Iași     | 211          | 171                      | 45,37                   |
|                      | Cotnari  | 220          | 169                      | 51,40                   |
| Grasă de Cotnari     | Iași     | 238          | 327                      | 58,04                   |
|                      | Cotnari  | 298          | 339                      | 71,12                   |
| Tămâioasă românească | Iași     | 181          | 199                      | 40,67                   |
|                      | Cotnari  | 231          | 204                      | 56,47                   |
| Mean values          | Iași     | 206,2        | 218,5                    | 48,21                   |
|                      | Cotnari  | 238,5        | 221,2                    | 56,80                   |

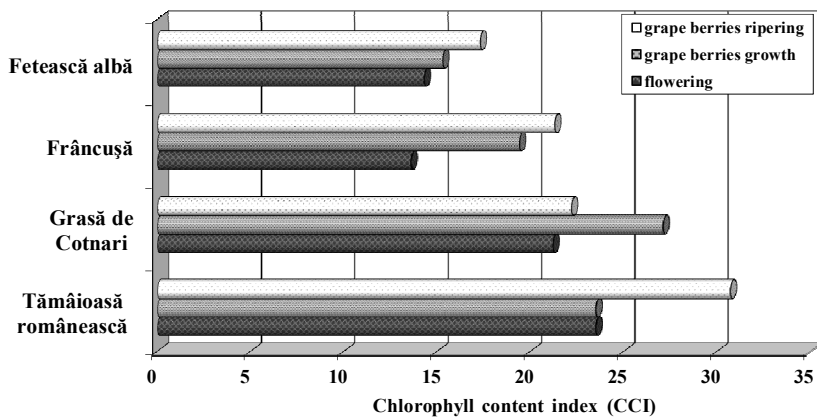
***The dynamic of chlorophyll content in the leaves of grapevine varieties***

The dynamic of chlorophyll content in the leaves of grapevine varieties cultivated at Iasi vineyard during the vegetation period of 2011 can be represented by a uniapical curve for all the varieties studied, with maximum values during the growth phenophase of berries. The chlorophyll content reaches its maximum level at Tămâioasă românească variety and its minimum at Fetească albă (fig.1).

The resulted data confirm the varieties mentioned in the specialized literature referring to the color of the leaves, that is light green for Fetească albă variety (Rotaru, 2009). At the same time, they can be used to point out the high efficiency of photosynthesis during blooming and maturation phenophase of fruits that is characteristic for grapevine. The peak registered in the period July – August can be related to the low photosynthetic activity due to drought. Pigments can also play a part in absorbing and dissipating the excess solar radiant energy.



**Fig. 1** - The dynamic of chlorophyll content in the leaves of the grape varieties cultivated at Iasi vineyard



**Fig. 2** - The dynamic of chlorophyll content in the leaves of the grape varieties cultivated at Cotnari vineyard

In Cotnari vineyard, the dynamic of CCI values maintains its shape of unipical curve at only one variety of grape – Grasă de Cotnari; this variety is sensitive to drought during berry growth, while for the other varieties, an ascendant curve is registered. The same minimum values were recorded by Fetească albă variety and the highest values were recorded by Tămâioasă românească (fig. 2).

The comparative analysis of the average value of the CCI in the leaves for the varieties analyzed in Iasi and Cotnari during vegetation reveals a much higher

content of chlorophyll in the leaves of the varieties cultivated in Cotnari. In both vineyards, the chlorophyll content is minimum at Fetească albă and maximum at Tămâioasă românească varieties.

Relating these data to the grape production, it results the ecophysiological reaction of different varieties of grapes to the climatic conditions of 2011, pointing out the role of the photosynthetic pigments in controlling the hydric stress that diminishes the normal quantity produced in both vineyards, but also their different effect on the production of the two vineyards (t/ha) and the sugar content of grapes (g/l). It is noticed the action of some photosynthetic parameters – foliar pigments in the formation and the supramaturation of grapes, in the accumulation of sugars and flavored compounds, that are more intense at the autochthonous varieties from Cotnari vineyard.

## CONCLUSIONS

1. The climatic conditions in 2011 from Iasi and Cotnari vineyards are generally characterized by pronounced in May and July-September; in June, the effect of the drought is considerably reduced by the excess rainfall recorded in Cotnari vineyard.

2. The qualitative and quantitative grape production was greater in Cotnari than in Iasi vineyard.

3. The dynamics of the chlorophyll content in the leaves shows the participation of photosynthesis in the formation and the maturation of grapes in the given ecological conditions.

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# cDNA-AFLP ANALYSIS OF GENE EXPRESSION IN RED RASPBERRY (*Rubus idaeus* L.) DURING WATER STRESS

## ANALIZA EXPRESIEI GENICE LA ZMEUR (*Rubus idaeus* L.) ÎN CONDIȚII DE STRES HIDRIC PRIN TEHNICA cDNA-AFLP

CIOBOTARI Gh.<sup>1</sup>, EFROSE Rodica Cătălina<sup>1</sup>, BRÎNZĂ Maria<sup>1</sup>, SFICHI-DUKE Liliana<sup>1\*</sup>

e-mail: lilianasfichi@hotmail.com

**Abstract.** Drought stress has negative effects on plant growth and productivity. A cDNA-AFLP (amplified fragment length polymorphism) analysis was performed to identify differential gene expression in response to drought stress in red raspberry (*Rubus idaeus* L.) plants. The expression profile was compared between well-watered control plants (100±10% field capacity) and stressed plants (40±5% field capacity) cultured in greenhouse conditions. Screening with 64 primer combinations identified multiple transcript-derived fragments (TDFs) that are differentially expressed in drought stress conditions. The differences between control and stressed plants were qualitative when TDFs were either present or absent or quantitative when TDFs showed different levels of expression. The results show that cDNA-AFLP is a valuable technique for studying expression patterns of genes involved in sensitivity/tolerance mechanisms to drought stress in red raspberry.

**Key words:** cDNA-AFLP, water stress, *Rubus idaeus*.

**Rezumat.** Stresul hidric are efecte negative asupra creșterii și productivității plantelor. Analiza cDNA-AFLP a fost efectuată la zmeur (*Rubus idaeus* L.) pentru a identifica modificări în expresia genică induse de stresul hidric. Profilul genic a fost comparat între plante bine hidratate (100±10% din capacitatea de câmp) care au fost folosite în calitate de control și plante stresate hidric (40±5% din capacitatea de câmp), ambele categorii fiind cultivate în condiții de seră. Screeningul efectuat cu 64 combinații de primeri a identificat multiple fragmente de transcripte (TDFs) care sunt exprimate diferențial în condiții de stres hidric. Diferențele între plantele control și cele stresate au fost, fie de ordin calitativ, când fragmentele TDF erau prezente sau absente, fie de ordin cantitativ când aceste fragmente arătau nivele de expresie diferite. Rezultatele obținute demonstrează că cDNA-AFLP este o tehnică valoroasă pentru studierea paternelor de expresie a genelor implicate în mecanismele de sensibilitate/toleranță la stresul hidric la zmeur.

**Cuvinte cheie:** cDNA-AFLP, stres hidric, *Rubus idaeus*.

## INTRODUCTION

According to the World Atlas of Desertification, UNEP (United Nations Environment Programme), dry lands cover 40% of the world's land surface (more than five billion ha), and they are the habitat and source of livelihood for about 1

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

billion people. With the growth of population and the development of modern agro-industry and the other industries, extension of the dry land surfaces becomes a growing concern all over the world. This concern is reflected in the Convention on Biological Diversity's dry lands work program, and in the establishment of the UN Convention to Combat Desertification (Xiao-Cheng Jiang et al., 2004). To mitigate the effects of drought, it is very important first to promote the effective and efficient use of existing plant resources, second to investigate the physiological processes and mechanisms that help plants to acclimatize to the water deficiency conditions. It is assumed that molecules and compounds such as proline, glycine betaine and soluble sugars etc. synthesized and accumulated during desiccation play an important role in the protection of the plants from stress (Pan et al., 2000). However, at this time the molecular basis for these protective mechanisms is almost unknown. In this experiment, the screening of the differentially expressed genes in water deficit conditions was accomplished by means of the high reproductive technique cDNA-AFLP. By this method we identified in stressed plants multiple polymorphic transcript-derived fragments (TDFs) that highlight specific plant reactions on molecular level.

## MATERIAL AND METHOD

### Plant material

Raspberry (*Rubus idaeus* L.) plants were grown in the greenhouse of the "V. Adavachi" Research Station (University of Agricultural Sciences and Veterinary Medicine of Iasi). When plants have reached maximum development they have undergone stress conditions by reducing soil moisture to 40±5%. The control plants were normally irrigated. Raspberry leaves were collected from mature greenhouse-grown plants and were immediately frozen in liquid nitrogen and stored at -80 °C.

### RNA extraction

Total RNA was isolated from raspberry very fine grinded leaves, in liquid nitrogen, with the SpectrumPlantTotal RNA Kit (Sigma) according to the manufacturer's protocol. RNA quality was checked using Bioanalyzer 2100 and RNA 6000 Nano Kit (Agilent Technologies) that allow visualization of 18S and 28S subunits and calculation of RNA Integrity Number (RIN).

### First-strand cDNA synthesis

The first-strand cDNA synthesis was accomplished with SuperScript II Reverse Transcriptase (RT) Kit (Invitrogen) that is an engineered version of MMLV RT with reduced RNase H activity and increased thermal stability.

### Second-strand cDNA synthesis

The second-strand cDNA synthesis was performed with SuperScript Double-Stranded cDNA Synthesis Kit (Invitrogen) and an oligo(dT) primer (Promega) according to manufacturer protocols.

### Restriction Endonuclease Digestion

The restriction fragments were obtained by digestion with two restriction endonucleases: **EcoR I** and **Mse I**. EcoR I has a 6-bp recognition site, and Mse I has a 4-bp recognition site. When used together, these enzymes generate small DNA fragments that will amplify well and are in the optimal size range (<1 kb) for separation on denaturing polyacrylamide gels. To digest the cDNA template, we used the AFLP Analysis System I (Invitrogen) following the instructions provided by the manufacturer with some modifications.



### **Ligation of Adapters**

After heat inactivation of the restriction endonucleases, the cDNA fragments are ligated to EcoR I and Mse I adapters to generate DNA template for amplification. These common adapter sequences flanking variable cDNA sequences serve as primer binding sites on these restriction fragments. Using this strategy, it is possible to amplify many DNA fragments without having prior sequence knowledge.

### **Amplification Reactions**

The cDNA-AFLP reactions were performed twice, with RNAs from independent samples, using the standard AFLP protocol. Duplicate samples gave similar banding patterns, indicating that the amplification was specific.

PCR was performed in two consecutive steps. In the first step, called preamplification, cDNAs are amplified with AFLP primers each having one selective nucleotide. The resulted PCR products were diluted and used as template for the selective amplification with EcoR I (E) and Mse I (M) selective primers, each containing three selective nucleotides. We tested a number of 64 E-M primer combinations

### **Separation of Amplified Fragments**

Products from the selective amplification were separated on a 4% agarose gel. The banding pattern was analyzed using a fluorescence/chemiluminescence gel documentation system (ECL3/UVP) for the identification of differentially expressed fragments (TDFs).

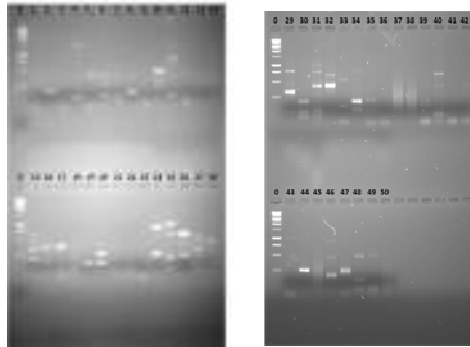
## **RESULTS AND DISCUSSION**

Plant growth and productivity are affected by various abiotic stresses such as drought, heat, cold, etc. The mechanism of drought tolerance is one of the most important research topics in horticultural sciences as drought stress decreases worldwide fruits and berries production.

Plant responses to dehydration involve many different proteins that have specific roles such as proteins for the biosynthesis of osmolytes, transport proteins, proteins involved in degradation and repair processes etc (Campalans et al. 1999). However, the molecular responses involved in drought tolerance remain largely unknown, especially in woody plants. Although many genes induced by dehydration have been identified in various plant species, there is little evidence for the involvement of a specific gene in desiccation protection. Tolerance to abiotic stresses, and in particular to drought stress, is a complex mechanism involving many different genes.

In our work we used cDNA-AFLP technique to compare gene expression profiles of red raspberry (*Rubus idaeus* L.) cultivar 'Opal' in response to drought stress and well-watered conditions. The cDNA-AFLP technique gives highly reliable expression profiles (Millioni et al., 2002). Screening with *EcoR* I and *Mse* I selective primers revealed many of transcript-derived fragments (TDFs) per each primer combination (picture 1) in both stressed and control plants.

Approximately 400 transcript-derived fragments (TDFs) were obtained and analyzed. About 92 TDFs were differentially expressed in stress conditions. Among these fragments, a number of 11 TDFs increased in response to water stress. We also found 2 TDFs that decreased in water stressed plants. Further characterization of these transcribed sequences will indicate potential candidate genes that might be involved in drought stress.



**Fig 1** - A cDNA-AFLP gel with 25 *EcoRI* and *MseI* selective primer pairs: 0 – ladder; 1, 2 - E-ACC / M-CAG; 3, 4 - E-ACC / M-CAC; 5, 6 - E-ACC / M-CAT; 7, 8 - E-ACC / M-CTT; 9, 10 - E-ACC / M-CTG; 11, 12 - E-ACT / M-CAA; 13, 14 - E-ACT / M-CAC; 15, 16 - E-ACT / M-CTA; 17, 18 - E-ACT / M-CAT; 19, 20 - E-ACT / M-CTC; 21, 22 - E-ACT / M-CTG; 23, 24 - E-ACT / M-CAG; 25, 26 - E-AAG / M-CAG; 27, 28 - E-AAG / M-CAA; 29, 30 - E-AAG / M-CAC; 31, 32 - E-AAG / M-CAT; 33, 34 - E-AAG / M-CTC; 35, 36 - E-AAG / M-CTT; 37, 38 - E-AGG / M-CAT; 39, 40 - E-AGG / M-CAA; 41, 42 - E-AGG / M-CTA; 43, 44 - E-AGG / M-CTC; 45, 46 - E-AGG / M-CTT; 47, 48 - E-ACA / M-CTA; 49, 50 - E-ACA / M-CAT. Even numbers – stressed plants, odd numbers – control.

## CONCLUSIONS

Screening with 64 primer combinations identified multiple transcript-derived fragments (TDFs) that are differentially expressed in drought stress conditions. The differences between control and stressed plants were qualitative when TDFs were either present or absent or quantitative when TDFs showed different levels of expression. The results show that cDNA-AFLP is a valuable technique for studying expression patterns of genes involved in sensitivity/tolerance mechanisms to drought stress in red raspberry.

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# THE USE OF qRT-PCR APPROACH FOR COMPARATIVE PHENYLPROPANOID GENE EXPRESSION STUDIES IN TWO *Rubus* spp

## UTILIZAREA METODOLOGIEI qRT-PCR ÎN STUDII DE ANALIZĂ COMPARATIVĂ A EXPRESIEI GENELOR IMPLICATE ÎN METABOLISMUL FENILPROPANILOR LA DOUĂ SPECII DIN GENUL *Rubus*

EFROSE Rodica<sup>1</sup>, CIOBOTARI GH.<sup>1</sup>, MORARIU Aliona<sup>1</sup>, PASCU D.<sup>1</sup>,  
BRÂNZĂ Maria<sup>1</sup>, SFICHI-DUKE Liliana<sup>1</sup>  
e-mail: lilianasfichi@hotmail.com

**Abstract.** *Rubus* spp. is economically-important crops worldwide and represents an invaluable source of healthy-related compounds, being used traditionally for therapeutic purposes. In berries, development of fruit quality traits is critically dependent on flavonoids-derived metabolites. Moreover, these compounds are often involved in resistance against biotic and abiotic stress. Better knowledge and understanding of the flavonoid biosynthetic pathway and its regulation is crucial for identifying strategies for enhancement product accumulation and quality, in specific environments. In the present study, a qRT-PCR approach was performed in order to assess the accumulation in the transcript of several genes (*pal1*, *pal2*, *chs*, *4cl1*, *4cl2*, *4cl3*), involved in flavonoid biosynthesis pathway. Comparative transcriptional profiling was carried out in raspberry (*Rubi* cv.) and blackberry (*Lochness* cv.) plants grown under controlled, greenhouse conditions. The determination of the relative expression levels of *pal1* and *chs* genes showed that in raspberry, these transcripts were 3- and 150-fold, respectively more abundant than in blackberry. Similar, the accumulation of the three gene transcripts encoding 4-coumarate: CoA ligase (*4cl1*, *4cl2* and *4cl3*) was higher in raspberry than in blackberry. The importance of qRT-PCR in studies of secondary metabolism in raspberry and blackberry is discussed.

**Key words:** *rubus*, phenylpropanoids, gene expression, real-time quantitative PCR

**Rezumat.** Genul *Rubus* cuprinde specii cu importanță economică recunoscută, care produc și acumulează compuși cu valoare terapeutică. La aceste specii calitatea fructelor este strict dependentă de acumularea flavonoizilor. În plus, acești compuși sunt adesea implicați în mecanismele de rezistență la stresul biotic și abiotic. Pentru o mai bună cunoaștere și înțelegere a căii de sinteză a flavonoizilor este necesară identificarea strategiilor de stimulare a acumulării produșilor și calității în condiții specifice de mediu. În studiul de față, metoda qRT-PCR a fost aplicată în vederea determinării acumulării produșilor de transcripție ai unor gene implicate în calea de biosinteză a (*pal1*, *pal2*, *chs*, *4cl1*, *4cl2*, *4cl3*). Analiza comparativă a profilului transcriptomic a fost realizată pe plante de zmeur (*Rubi* cv.) și mur (*Lochness* cv.) crescute în condiții controlate de seră. Determinarea nivelelor relative de expresie ale genelor *pal1* și *chs* a aratat că la zmeur, acumularea acestor produși de transcripție este de 3 și 150 ori mai abundentă decât la mur. Nivele mai crescute de

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

*expresie la zmeur decât la mur, au fost detectate și în cazul celor trei gene ce codifică 4 cumarat- CoA ligaza (4cl1, 4cl2 și 4cl3). Importanța tehnicii qRT-PCR în studiul metabolismului secundar la specii de zmeur și mur este discutată.*

**Cuvinte cheie:** *Rubus, fenilpropani, expresie genică, analiza cantitativă qRT-PCR.*

## INTRODUCTION

Phenylpropanoids occur widely in plants and represent a major group of secondary metabolites. *Rubus* spp. is a very rich source of these healthy-related compounds with biological and pharmacological properties (Rao and Snyder, 2010). Phenolics play an important role in plant growth and development and are also involved in resistance against environmental stresses (Treutter D, 2006). In berries, phenylpropanoids-derived metabolites are significant determinants for plant quality (Weisshaar and Jenkins 1998, Kumar and Elis, 2003a). Moreover, the bioactive content of fruits varies among different genotypes (Pantelidis et al., 2007). To gain insights of the phenylpropanoids biosynthetic pathway and its regulation, the changes in the transcript level of several key genes involved in their biosynthesis was assessed by a qRT-PCR approach. For this purpose, raspberry (Rubi cv.) and blackberry (Lochness cv.) plants were grown under controlled, greenhouse environment, and gene expression in their leaf tissues was investigated. Total RNA was isolated from harvested samples and subjected to reverse transcription and real-time quantitative PCR. Comparative transcriptomic analysis was performed using relative quantification method, the most adequate approach to investigate physiological changes in gene expression level (Pfaffl, 2004). To eliminate non-biological variation, optimization and accurate normalization of qRT-PCR reaction was required. The importance of qRT-PCR method for assessing expression of target genes and ultimately for gathering significant information of their role in the metabolites biosynthesis and accumulation is also discussed.

## MATERIAL AND METHOD

**Plant tissues preparation.** Plant leaves were collected from three biological replicates ground to a fine powder in liquid nitrogen and stored at -80°C to preserve full-length RNA.

**RNA isolation and quantification.** Aliquots of 100 mg grounded plant material were subjected to total RNA extraction and purification according to previously described protocol (Salzman et al., 1999), or by using Spectrum Plant Total RNA kit. RNA quality was electrophoretically verified by ethidium bromide staining of RNA samples in 1% agarose gel, by spectrophotometer analysis at 230, 260 and 280 nm and by Agilent Bioanalyzer analysis using an RNA 6000 Nano kit. Total RNA samples were digested with RQ1 DNaseI (Promega) to remove any trace of contaminating genomic DNA.

**cDNA synthesis and RT-PCR.** Two µg of purified DNase-treated RNA was reverse transcribed with SuperScript II Reverse Transcriptase kit (Invitrogen) according to the manufacturer's protocols. The resulted first-strand cDNA was diluted to a final volume of 100ng, and target cDNAs were amplified using gene-specific primers (table1). The oligonucleotides were designed from the transcribed region of

*Rubus idaeus* specific - target genes using Primer Express 1.5 software (Applied Biosystems, Darmstadt, DE).

**qRT-PCR analysis.** Quantitative real-time PCR analysis was performed on the Rotor-Gene 6000 (Corbette) using MyTaq™RedMix (Bioline), gene specific primers at a final concentration of 0.25uM and 2 ul of the cDNA as template. The temperature cycle used comprised 40 cycles at 95°C for 15 sec and 60°C for 1 min. To monitor PCR specificity a dissociation curve was performed. For the relative quantification of transcript levels, a modification of the comparative threshold cycle method was used. Relative transcript levels of the gene of interest (X) were calculated as a ratio to the histone H3 gene transcripts (U), as  $(1 + E)^{-\Delta Ct}$ , where  $\Delta Ct$  was calculated as  $(Ct_x - Ct_U)$ . PCR efficiency (E) for each amplicon was calculated employing the linear regression method (Ramakers et al. 2003). All real-time qPCR reactions for relative quantification were performed as triplicates.

## RESULTS AND DISCUSSIONS

In *Rubus spp.*, the accumulation of metabolites, essential for plant growth and development, requires integrated expression of genes encoding enzymes in the phenylpropanoid pathway, such as phenylalanine ammonia-lyase (PAL), 4-coumarate CoA ligase (4CL), and chalcone synthase (CHS). The employed real-time quantitative PCR (qRT-PCR) method has become a very powerful tool for the quantification of gene expression, for its high sensitivity and reliability (Pfaffl M.W., 2004). The reliability and efficiency of gene expression evaluation is affected by several critical factors including RNA and cDNA quality and PCR optimization. Moreover, a successful qRT-PCR assay strongly depends on the accurate transcript normalization using an appropriate reference gene.

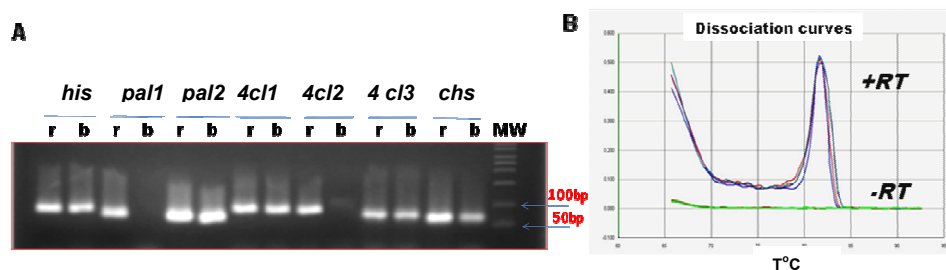
**Assessment of RNA quality.** *Rubus* plants contain many secondary metabolites that can interfere with RNA preparation and its use in downstream PCR-based applications. Therefore, different protocols for RNA extraction and purification from difficult tissues were employed in order to obtain excellent quality RNA, highly suitable for qRT-PCR approach. Sigma's Spectrum Plant total RNA kit provided the most simple, rapid and efficient procedure for total RNA extraction. Total RNA isolated samples were quantified by spectrophotometry, ( $A_{260}/A_{280} > 1.8$  and  $A_{260}/A_{230} > 1.95$ ) and agarose gel electrophoresis by ethidium bromide staining (data not shown). Moreover, RNA integrity and quality was verified using Agilent 2100 Bioanalyzer. This analysis showed clear and well defined 28S and 18S peaks, low noise between the peaks and minimal to moderate low molecular weight contamination and good RNA integrity numbers,  $RIN > 7$ , for all investigated samples.

**Evaluation of primer specificity.** The primer specificity of target genes was monitored by agarose gel electrophoresis and dissociation curve analysis (Fig.1). Our data confirmed that for each investigated gene, only a unique PCR product of the expected size and melting temperature was obtained (see table 1). Moreover, no primer-dimers and non-specific products formations were detected.

Table 1

| Primers used for qRT-PCR assay      |                       |                              |                   |
|-------------------------------------|-----------------------|------------------------------|-------------------|
| Target genes                        | Gene-specific primers |                              | Amplicon size(bp) |
| Phenylalanine ammonia-lyase1 (pal1) | pal1F                 | 5'-TCGACAATGCCAGGATCGA-3'    | 79                |
|                                     | pal1R                 | 5'-CAACGGATAAGACCTGCATTCC-3' |                   |
| Phenylalanine ammonia-lyase2 (pal2) | pal2F                 | 5'-ACCTCTTCCGATCTGCTAGCC-3'  | 70                |
|                                     | pal2R                 | 5'-CGAAGTGGAATGGAATGACACA-3' |                   |
| 4-coumarate:coA ligase1 (4cl1)      | 4cl1F                 | 5'-TGCTCGTCACCCATCCTAACA-3'  | 89                |
|                                     | 4cl1R                 | 5'-TCACGACAAATGCAACCGG-3'    |                   |
| 4-coumarate:coA ligase2 (4cl2)      | 4cl2F                 | 5'-CGGCTACTTTCCCAAATCGATA-3' | 85                |
|                                     | 4cl2R                 | 5'-TCACCCCGGCCATTATAGAA-3'   |                   |
| 4-coumarate:coA ligase3 (4cl3)      | 4cl3F                 | 5'-TCCGCAAAAAGATGATGCTG-3'   | 70                |
|                                     | 4cl3R                 | 5'-GCTCATTGCCGCCATTAGAT-3'   |                   |
| chalcone synthase (chs)             | chsF                  | 5'-TCACAGTGTGGCAGCTTCAAC-3'  | 62                |
|                                     | chsR                  | 5'-ACTGATCAAGGAGATCACCCAA-3' |                   |
| histoneH3 (his)                     | hisF                  | 5'-TTCCAGAGCCATGCAGTTTTG-3'  | 93                |
|                                     | hisR                  | 5'-TGGCATGAATGGCACAGAGA-3'   |                   |
| Actin (act)                         | actF                  | 5'-ATTGCAGACCGTATGAGCAAAG3'  | 62                |
|                                     | actR                  | 5'-GGTGCCACAACCTTGATCTTC-3'  |                   |

Furthermore, the PCR conditions were optimized with respect to *Taq* DNA polymerase, primers concentrations, various annealing temperature, MgCl<sub>2</sub> and dNTP concentrations.

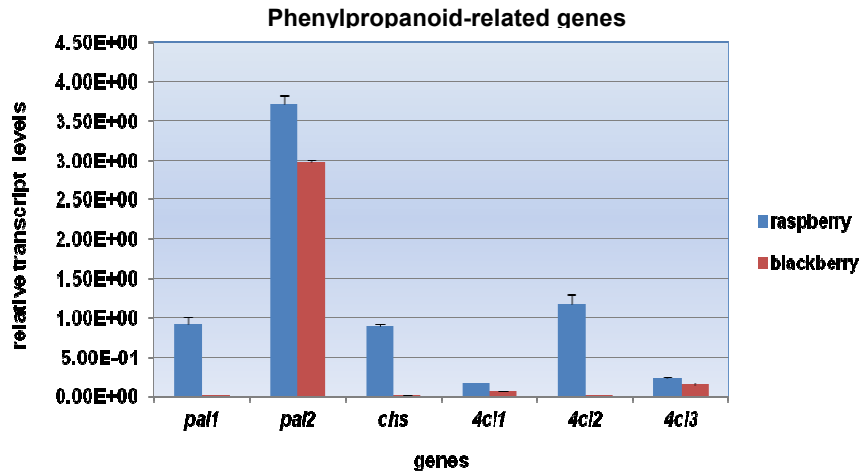


**Fig.1** - Validation of primer specificity of target genes. **A.** Visualization of RT-PCR specific products of the expected length on a 4% (w/v) agarose gel. **B.** Confirmation of primers specificity by qRT-PCR analysis using specific primers for the reference gene histone H3. No amplicon was detected within 40 cycles without RT to confirm absence of genomic DNA. Similar results were obtained for all investigated genes (data not-shown).

**Validation of the reference gene.** Prior to qRT-PCR analysis of target gene expression it is essential to identify and validates the reference gene, to accurately compare mRNA transcript among different samples. For this purpose, two potential reference genes, actin and histone H3, were chosen for a preliminary qRT-PCR assay. The frequently used reference gene in plant, actin, was the least

stable (data not-shown). Therefore, histone H3 gene was selected as reference gene based on its performance data such as earlier Ct values, no primer-dimers and non-specific products artifacts, clean melt curves in all replicates and stable expression across all investigated samples.

**Comparative transcriptional analysis of key genes involved in phenylpropanoid metabolism.** Transcriptional profiling was performed using relative quantification of target gene transcripts in comparison to the previously selected reference gene. The expression levels of the control gene, was used as internal standards to normalize small variations in cDNA template amounts. The relative transcript levels of the gene of interest were calculated as a ratio to the histone H3 gene transcripts (Fig.2).



**Fig. 2** - Accumulation of key gene transcripts involved in phenylpropanoid pathways in two *Rubus spp.* Total RNA was isolated from leaves, reverse transcribed to cDNA, and subjected to real-time quantitative PCR. Transcript levels in the different samples were normalized to those of the reference gene, histone H3. Relative mRNA level was calculated with respect to the level of histone H3 transcripts. Bars show means +SD (n = 3).

Although expression of both *pall* and *chs* genes was detected in examined tissues, determination of the relative expression levels of the two transcripts showed that in raspberry, *pall* and *chs* transcripts were 3- and 150-fold, respectively more abundant than those in blackberry, while *pal2* was detected only in raspberry. Similar, the transcript accumulation of the three genes encoding 4-coumarate: CoA ligase (*4cl1*, *4cl2* and *4cl3*) in raspberry was higher than the expression levels of genes in blackberry. Previously published data showed a differential pattern of expression for the investigated genes in various vegetative tissues as well as during fruit development which suggest their involvement in the formation of various phenylpropanoid-derived metabolites (Kumar and Ellis, 2001, 2003a, b). Furthermore, we are going to investigate whether the changes in gene expression due to various stress condition or genotypes are also reflected on the metabolite level. Transcriptional profiling data obtained by the extremely

sensitive and reliable qRT-PCR approach, corroborated with further metabolic results will facilitate a better understanding of plant growth and development and will contribute to the optimization of productivity in specific environments.

## CONCLUSIONS

1. RNA samples with identical high quality were investigated using a transcript profiling assay and processed with the same efficiency in every step of the analysis.

2. Dissociation curve analysis and agarose gel electrophoresis of the RT-PCR products amplified using gene-specific primers, confirmed reaction specificity.

3. To assess gene expression in *Rubus* plants, histone H3 housekeeping gene has been validated as reference gene for qRT-PCR normalization.

4. Relative quantification approach revealed that several key genes involved in phenylpropanoid metabolism are differentially expressed in the two investigated cultivars, their transcript levels being more abundant in raspberry (Ruvi cv.) than in blackberry plants (Lochness cv.)

5. Furthermore, a combined transcriptomic and metabolomic approach will allow the direct estimation of the plant nutritional and physiological state in various environmental conditions.

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**PHYSIOLOGICAL RESPONSE OF SUNFLOWER HYBRIDS  
TO *SCLEROTINIA SCLEROTIORUM* ARTIFICIAL  
INFECTION IN CLIMATES CONDITIONS IN 2011 AT  
EZĂRENI IAȘI**

**REAȚIA FIZIOLOGICĂ A UNOR HIBRIZI DE FLOAREA-SOARELUI  
LA INFECȚIA ARTIFICIALĂ CU *SCLEROTINIA SCLEROTIORUM* ÎN  
CONDIȚIILE CLIMATICE 2011, LA EZĂRENI IAȘI**

**LAZĂR (NECHITA) Adriana<sup>1</sup>, LEONTE C.<sup>1</sup>, LUPU Elena-Ancuța<sup>1</sup>, SPÂNU  
Oana<sup>1</sup>, SLABU Cristina<sup>1</sup>  
e-mail: adryana\_lzr@yahoo.com**

**Abstract.** *Sunflower adapts to large swings of temperature, developing normally at high temperature of 25-30 degrees and at bellow temperatures of 13-17 degrees, and in this case delaying flowering and ripening (Vrânceanu V., 2000). The negative influence of excessive temperatures, manifest differently depending on the further development of plants. Sunflowers tolerance to high temperatures was associated by Țerbea (1979), with the stability of chlorophyll. Chlorophyll stability index was defined as the difference between light transmission through chlorophyll sample taken from leaves exposed or not to heat stress of 65<sup>0</sup>C. The aim of research was to study the physiological response of sunflower hybrids to pathogen Sclerotinia sclerotiorum, in the climatic conditions of 2011, grown on the farm Ezăreni of University of Agricultural Science and Veterinary Medicine Iasi. It was intend, in particular, photosynthetic activity assessed by foliar pigments chlorophyll content and resilience to stress, assessed by the content of flavonoid pigments.*

**Key words:** sunflower, photosynthesis, foliar pigments

**Rezumat.** *Floarea soarelui se adaptează la oscilații însemnate de temperatură, dezvoltându-se normal atât la temperaturi ridicate de 25-30 de grade, cât și la temperaturi mai joase de 13-17 grade, întârziindu-și în acest caz înflorirea și coacerea (Vrânceanu, 2000). Influența negativă a temperaturilor excesive, se manifestă diferit în funcție de dezvoltarea ulterioară a plantelor. Toleranța florii soarelui la temperaturi ridicate a fost asociată de Țerbea (1979), cu stabilitatea clorofilei. Indicele de stabilitate a clorofilei a fost definit ca diferența dintre transmisia lumină prin probele de clorofilă prelevate de la frunzele expuse sau nu la stresul termic de 65<sup>0</sup> C. Scopul cercetărilor l-a constituit studiul reacției fiziologice a unor hibrizi de floarea soarelui la agentul patogen Sclerotinia sclerotiorum, în condițiile climatice ale anului 2011, cultivați în cadrul fermei Ezăreni a USAMV Iași. S-a urmărit, în special, activitatea fotosintetică apreciată prin conținutul de pigmenți foliari clorofilieni, precum și capacitatea de rezistență la stres, apreciată prin conținutul pigmenților flavonoizi.*

**Cuvinte cheie:** floarea soarelui, fotosinteză, pigmenți asimilatori

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

## INTRODUCTION

Global expansion of areas planted with sunflower energy gives a special place, by reconsidering the culture. Ecological response of sunflower, in various forms of abiotic stress has been highlighted for some hybrids (Andrei Elena, 2000, Jitäreanu Doina et al., 2007), the climatic conditions of NE Moldova. White mould (*Sclerotinia sclerotiorum*) is known as a disease of rainy summers, infection with this pathogen requires a period of humidity (Melvin D. et al 2006). The fungus grows in a very wide temperature range from 1- 2<sup>0</sup>C at 30-35<sup>0</sup>C with an optimum of 18-25<sup>0</sup>C and a relative humidity of 60-80% (Willetts and Wong, 1980).

## MATERIAL AND METHOD

Researches have focused 12 sunflowers hybrids grown under Ezăreni farm in 2011. Plant material was harvested from plants infected with *Sclerotinia sclerotiorum* in May and July. Plants were infected at head root (Table 1). To determine the photosynthetic capacity of the hybrids was analyzed photosynthetic pigments content by spectrophometric method, they are assessed by the ability of light absorption by acetone extract of pigments 1%, the read areas (663nm) and blue (432 and 453) in the visible spectrum characteristic of the main components of photosynthetic system.(Jitäreanu et al., 2009). Were determined: chlorophyll a 663 to assess the photosynthetic reaction centre, chlorophyll a and b (432-453) components of absorption centre. To appreciate the plants resistance of to various forms of stress especially in *Sclerotinia sclerotiorum*, we analyzed the content of flavonoid pigments with near ultraviolet absorption.

Table 1.

Hybrids take in study

| Nr. crt. | Hybrid  | Plants infected/uninfected |
|----------|---------|----------------------------|
| 1        | PR63A86 | uninfected                 |
| 2        | PR64G46 | infected                   |
| 3        | PR64A15 | uninfected                 |
| 4        | PR63A90 | uninfected                 |
| 5        | PR64E83 | uninfected                 |
| 6        | PR64E71 | infected                   |
| 7        | PR64A71 | uninfected                 |
| 8        | PR64A83 | uninfected                 |
| 9        | PR64F50 | uninfected                 |
| 10       | PR64J80 | uninfected                 |
| 11       | PR63A62 | infected                   |
| 12       | PR64A89 | infected                   |

## RESULTS AND DISCUSSION

Changing climate conditions, in year 2011, were analyzed monthly throughout the vegetation. Air temperature and sunshine duration were calculated averages, respectively decades, the average monthly and annual

average (table 2). Temperature presented less importance for pathogen development, provided decisive as humidity and light, but these factors did not influence significantly content foliar pigments chlorophyll and phlavonoid pigments in plants infected with *Sclerotinia sclerotiorum*.

Table 2

**Evolution of condition climatics, in year 2011**

| Specification              | April 2011 | May 2011 | June 2011 | July 2011 | August 2011 | Sept 2011 | Veg. per. |
|----------------------------|------------|----------|-----------|-----------|-------------|-----------|-----------|
| TEMPERATURE <sup>o</sup> C |            |          |           |           |             |           |           |
| Dec. I                     | 8,9        | 11,4     | 22,0      | 20,0      | 21,2        | 18,9      | 17,0      |
| Dec. II                    | 7,8        | 17,0     | 20,3      | 25,7      | 21,1        | 18,3      | 18,3      |
| Dec. III                   | 14,2       | 20,7     | 18,4      | 22,0      | 22,2        | 17,8      | 19,2      |
| Monthly average            | 10,3       | 16,5     | 20,2      | 22,6      | 21,5        | 18,3      | 18,2      |
| Normal                     | 10,1       | 16,1     | 19,4      | 21,3      | 20,6        | 16,3      | 17,3      |
| Deviation                  | 0,2        | 0,4      | 0,8       | 1,3       | 0,9         | 2         | 0,93      |
| Min. monthly               | 1,1        | 3,1      | 10,5      | 10,8      | 10,0        | 9,3       | 7,46      |
| Max. monthly               | 24,9       | 29,5     | 33,0      | 34,3      | 31,9        | 22,5      | 29,3      |
| RELATIVE HUMIDITY (%)      |            |          |           |           |             |           |           |
| Dec. I                     | 69         | 68       | 60        | 70        | 63          | 65        | 65,8      |
| Dec. II                    | 70         | 65       | 63        | 61        | 61          | 67        | 64,5      |
| Dec. III                   | 44         | 53       | 71        | 67        | 47          | 72        | 59        |
| Monthly average            | 61         | 62       | 64        | 66        | 57          | 68        | 63        |
| Normal                     | 62         | 62       | 63        | 62        | 63          | 66        | 63        |
| Deviation                  | 1          | 0        | 1         | 4         | 6           | 2         | 2,3       |
| PRECIPITATION (mm)         |            |          |           |           |             |           |           |
| Dec. I                     | 16,8       | 25,8     | 22,6      | 6,4       | 27,6        | 25,5      | 20,7      |
| Dec. II                    | 64,0       | 7,6      | 11,0      | 14,4      | 0,6         | 26,3      | 20,6      |
| Dec. III                   | 0,6        | 7,6      | 80,2      | 45,2      | 0,0         | 16,7      | 25,0      |
| Monthly average            | 27,1       | 13,2     | 37,9      | 22        | 9,4         | 22,9      | 22,0      |
| Normal                     | 40,3       | 52,5     | 75,1      | 69,2      | 57,6        | 40,8      | 55,9      |
| Deviation                  | 13,2       | 39,3     | 37,2      | 47,2      | 48,2        | 17,9      | 33,8      |

**The content of photosynthetic pigments**

The spectrophotometric absorbance measured with acetone extract of pigments at wavelength of 663 nm shows a high photosynthetic activity in the reaction center where hybrids *PR64A83-n*, *PR63A90-n*, *PR64A71-n*, *PR64F50-n*, *PR64A89-i* and lower *PR63A62-i* and *PR64J80-n* (fig. 1).

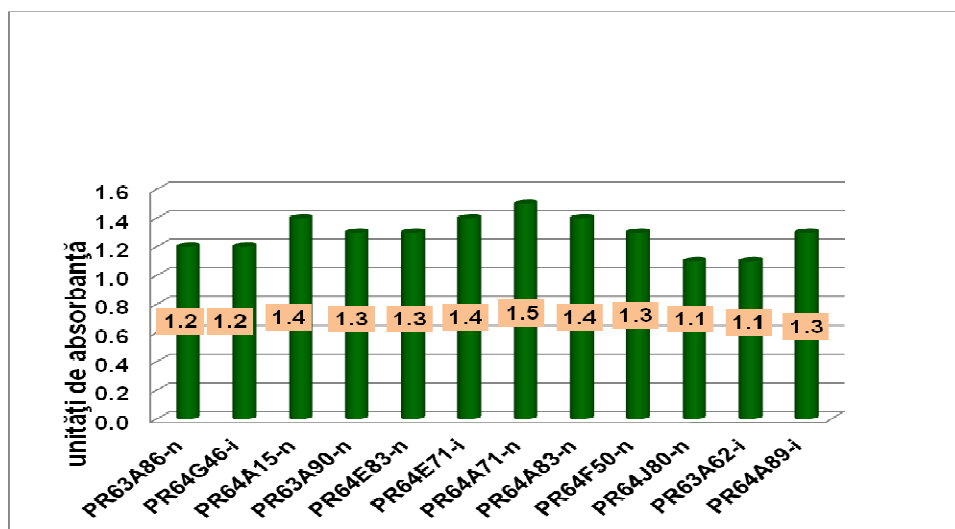


Fig. 1. -Effect of climatic conditions of year 2011 on the chlorophyll a 663 content

Researchers conducted by Jităreanu Carmenica Doina et al. (2004) showed that photosynthetic pigments with maximum absorption in the red radiation (663 nm) and blue (435 și 453 nm) of spectrum, has a lower content of others early hybrids throughout the growing cycle. The most obvious differences are found during the growth period, which demonstrates that the hybrids studied have effective biochemical strategy to ensure the resistance to stress conditions. Activity in the absorption centre has also proved superior to hybrid *PR64A71-n*, and lower in hybrids *PR63A62-i* and *PR64J80-n* (fig. 2).

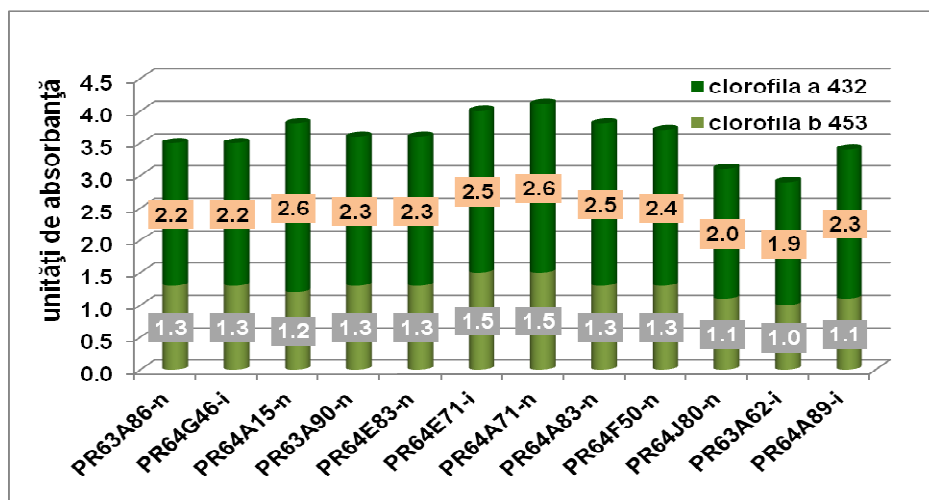


Fig. 2. - Effect of climatic conditions of year 2011 on the chlorophyll 432 a and chlorophyll b 453 content

### Flavonoids pigments

Analysis of pigment content flavonoid, with a role in a resistance to various forms of stress demonstrating that biosynthesis depends more on the hybrids natura and less on infection with *Sclerotinia sclerotiorum*, being comprised between 0,2 and 2,5 absorbance units (fig.3).

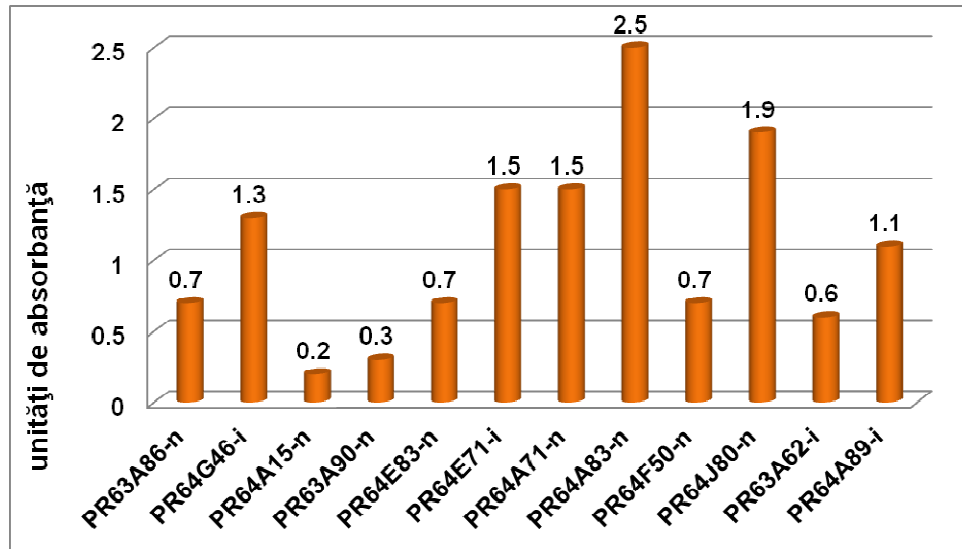


Fig.3 - Effect of climatic conditions of year 2011 on the flavonoid pigment content

### CONCLUSIONS

1. Following results were not found significant differences between sunflower hybrids tested as hybrids exposure to high temperatures and water stress did not stop the development of pathogen infection with *Sclerotinia sclerotiorum*.

2. The content of chlorophyll pigments not significantly different, being comprised in the range approximately equal to the infected plants and those infected with the pathogen.

3. Flavonoid pigments content showed a maximum biosynthesis at PR64A83-n and minimum PR63A1-n.

4. Infection of head root didn't affect the foliar chlorophyll content and flavonoid pigments.

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# OPTIMAL CONDITIONS FOR ANDROGENESIS INDUCTION IN PLANTS

## CONDIȚII OPTIME NECESARE PENTRU INDUCEREA ANDROGENEZEI LA PLANTE

LUCA M. A.<sup>1</sup>, LEONTE C.<sup>1</sup>

e-mail: luca\_mihai\_alexandru@yahoo.com

**Abstract.** *The totipotency of the male reproductive cells was revealed by Guha and Maheshvari in 1964 (Aionesei, 2005), allowing the development and improvement of haploid plants breeding methods and their use in fundamental and applied research. Among the methods for haploid production, the androgenetic ones have the advantage of chromosome doubling during the first mitotic division and producing fertile plants with complete doubled chromosomes, compared with the ginogenetic ones, where the egg cell is difficult to access, the embryo representing often the first stage in which a treatment for chromosome doubling may be applied (Kasha, 2005). The literature review made by us shows general aspects of androgenesis and the genetic, physiological, and physical factors that influence plant androgenesis. The following aspect was investigated: different androgenetic methods for haploid production, the processes that the microspore undergoes during sporophytic development and the optimal conditions for androgenesis induction.*

**Key words:** androgenesis, haploids, microspores

**Rezumat.** *Demonstrarea totipotenței celulelor reproductive masculine de către Guha și Maheshvari în 1964 (Aionesei, 2005) a permis dezvoltarea și îmbunătățirea metodelor de obținere a plantelor haploide și utilizarea acestora în cercetarea fundamentală și aplicată. Dintre metodele pentru producerea haploizilor, cele androgenetice prezintă avantajul posibilității dublării cromozomilor în prima diviziune mitotică, cu obținere de plante cu garnitura cromozomică complet dublată și fertile, față de metodele ginogenetice, în cazul cărora celula ou este dificil de accesat, deseori embrionul reprezentând primul stadiu în care poate fi efectuat un tratament pentru dublarea cromozomilor (Kasha, 2005). Studiul literaturii de specialitate efectuat de noi, expune aspecte generale asupra androgenezei, și factorilor genetici, fiziologici și fizici care influențează androgeneza la plante. Au fost investigate următoarele aspecte: semnificația termenilor, diferitele metode androgenetice pentru producerea haploizilor, procesele suferite de microspor în timpul schimbării dezvoltării normale cu dezvoltarea sporofitică și condițiile optime necesare pentru inducerea androgenezei.*

**Cuvinte cheie:** androgeneza, haploizi, microspor

### INTRODUCTION

The first natural haploid in flower plants was reported in 1922 by Belling and Blakeslee (Palmer and Keller, 2005), but only after the demonstration of the totipotency

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

of the male reproductive cells by Guha and Maheshvari in 1964 (Aionesei, 2005), the methodology of haploid plants production takes a step forward to the utilization of this type of plants in big number in different plant breeding programs. The genetic importance of haploid plants results from the full genetic constitution in phenotype (Leonte, 2003), recessive alleles expression (Raicu, 1980), time reduction in obtaining isogenic lines (Munteanu and Falticeanu, 2008; Gonzalez and Jouve, 2005), valuable lines particularly for heterogametic species (Radojevic, 2000). However, because of the monoploid condition, such plants are subject of the lethal and sub lethal genes action (Jacobsen and Ramanna, 1994 cited by Chani et. al., 2000).

Different methods are developed for haploid plants production at high frequencies. The literature review made by us presents general aspects of androgenesis.

## MATERIAL AND METHOD

The study was based on literature analysis. We have investigated the significance of the terms, different androgenic methods for haploid plants production, the processes suffered by the microspore during the switch from the normal development to sporophytic development and the optimal conditions for androgenesis induction.

## RESULTS AND DISCUSSIONS

Vegetal cells may express almost any part of their genome, having the ability to return to the embryogenic condition and regenerate whole plants (Reynolds, 1997, cited by Aionesei et. al., 2005), capacity called totipotency. Embryogenesis can be initiated not only from a zygote but also from the haploid generation cells, such as microspores (Oleszczuk et. al., 2005), a phenomenon called androgenesis.

Initially, the original significance of the term “androgenesis” described the fertilization of the egg cell and then the inactivation of the female nucleus, but today it refers to the microspore embryogenesis (Segui-Simarro, 2010), although the haploid sporophyte appears without the gametophyte formation and the microsporogenesis that precedes the male gametophyte is not completed (Vagera and Havranek, 1983).

In some conditions, *in vitro*, the normal gametophytic development of the microspores can be changed to the sporophytic one (Iqbal and Wijesekara, 2007), structural and biochemical changes being identified at the microspore level. However, the “critical point” which designates the transition to the sporophytic organization is unknown (Babbar, 2004), the haploidy representing a problem in plant cell culture domain, under theoretical and practical aspect (Zagorska and Dimitrov, 1995).

Depending on the events during the division of *in vitro* cultured pollen, androgenesis is classified into two types: direct androgenesis – by modifying the normal development program of the haploid nucleus and its mitotic division with the formation of an embryo-like structure and then a haploid plant – and indirect



androgenesis, by repeated divisions of the haploid nucleus with callus formation, tissue differentiation and haploid plants formation (Leonte, 2003).

The methods for *in vitro* androgenesis induction are anther culture and isolated microspore culture. Babbar et. al. (2004) identified five possible paths for androgenesis: the repeated division of the vegetative cell, the repeated division of the generative cell, the repeated division of both cells, and the symmetric division of the uninucleate microspore and the fusion products of the generative respectively vegetative cells.

For an optimal development of the *in vitro* androgenesis it is necessary that the androgenetic program to be designed considering the factors that influence androgenesis in plants.

**Genetic factors.** The androgenetic potential is high in few cultured plants, so that embryo production through this path, for double haploid lines use in plant breeding programs, is limited.

This limitation effect, manifested by the incapacity of morphogenesis in anther culture (Garcia et. al., 2009), determines the approach of those genotypes that manifests androgenetic potential (Yermishina et. al., 2004) and breeding for this character, as it is suggested by Beckert (1998) and Rudolf et. al. (1999) (cited by Segui-Simarro, 2010) because this androgenetic competence can be inherited by the descendants.

The androgenetic potential is genetically controlled by interactions of genes with non-additive effects, as it is the case of some triticales and secalotriticum species (Yermishina et. al., 2004), the three independent components involved in the anther culture response: callus induction, regeneration and plant development being under a polygenic control and inherited independently (Ekiz and Konzak, 1994, cited by Chaudhary et. al., 2003) However, Kasha et. al. (2001a, cited by Oleszczuk et. al., 2006) suggests that a correct treatment may be the main solution for passing over the genotypic dependence.

**Physiological factors.** Even though the effects of donor plants physiological state are not studied as much as the effects due to the genotype, choosing the anther donor plants must be performed with discernment. Reed (2005) indicates good results if the anthers were collected from healthy plants with vigorous growth.

Ghiorghita and Nicuta (2005) states that are to be avoided those plants which suffered a form of stress and that the humidity, light and temperature the donor plants were grown are factors that influence the embryogenic potential of anthers about to be cultivated.

Donor plants age represents an important factor in designing an androgenesis induction program, the best androgenetic response being achieved with the anthers of the first set of flowers appeared on a plant (Leonte, 2011), even though in the case of *Nicotiana tabacum* var. *Havanna* there are no observable differences between the first and the last floral buds, regarding the androgenetic response (Johansson and Erikson, 1977, cited by Ghiorghita et. al, 2005).

The developmental stage of microspores is critical for androgenesis induction. There is a moment when the microspore can change the normal

gametophytic evolution to a sporophytic development. This moment differs within a species and even from one variety to another, but the general rule is that the sensitive period of microspores is between the uninucleate stage and bicellular pollen around the first mitosis, a moment in which a multitude of external stimuli can be applied on microspores to mask the gametophytic program and induce the sporophytic development (Babbar et. al., 2004).

Although Touraev et. al. (1997, cited by Babbar et. al., 2004) reveals that the microspores ability to switch the development program is present in a relatively large time window in *Brassica napus*, *Nicotiana tabacum* and *Triticum aestivum*, in the case of some recalcitrant species, time windows are small (Seggui-Simarro, 2010).

Anther wall. The anther wall function in androgenesis is still discussed, some of the authors reporting that it has a stimulating effect, others that it is inhibitory. Ghiorghita and Nicuta (2005) remember Nitsch and Norrel (1973) that support the stimulatory effect of the anther wall because the aqueous extract of anthers induces the growth of isolated pollen. According to Suderland et.al. (1994, cited by Wang et. al., 1999) for androgenesis induction the tapetum and the anther wall properties are critical, much like the properties of anthers undergoing maturation, close to anthesis.

Culture medium. The culture medium composition is another factor that influences callus formation and *in vitro* organogenesis induction (Zagorska and Dimitrov, 1995) or embryogenesis. A series of species that were considered as recalcitrant came to be intense utilized in androgenesis programs due to the optimization of culture conditions. It is possible that the proliferation of the callus is not a genotype dependent alternative in embryogenesis induction, but a result of suboptimal culture conditions (Segui-Simarro, 2010).

According to Gonzalez and Jouve (2005) the culture medium and the genotype affect: the rate of survival of microspores during *in vitro* androgenesis, the percentage of symmetrical divisions, the number of division per 100 viable microspores and the numbers of microspores with four or more nuclei.

The multitude of culture mediums and different utilized concentrations of phytohormones make it impossible to create a single culture media for all plant species in order to promote androgenesis.

The aggregation state of the culture medium influence the androgenic response, the liquid ones providing better conditions than the solid ones, though there is the disadvantage that those liquid media do not ensure anther floatability (Ghiorghita et. al., 2005). To overcome this impediment two phase medias were created.

**Physical factors.** Pretreatments became an essential condition for improving androgenetic response in many species (tab. 1), stress or suboptimal conditions creating the possibility of a such a response.

For many plants, low temperatures represent the factor that disturbs the inner stability of the anther, with changes in microspore development and embryo formation (Oleszczuk et. al., 2006), but there can't be indicated an universal level of stress with positive effects on androgenesis and plant regeneration.

Table 1

## Pretreatments used for androgenesis induction

| Pretreatment                             | Species                                      | Cited bibliography          |
|--|--|-----------------------------|
| 4°C for 4 days and gamma irradiation 1Gy | Medicago sativa L.                           | Zagorska and Dimitrov, 2005 |
| 35°C for 12 hours                        | Solanum phuraja Juz. X S.<br>Chacoense Bitt. | Chani et. al., 2000         |
| Manytol                                  | Hordeum vulgare L.                           | Li et. al., 2001            |

Temperature. In general, anther cultures are incubated at 24-25 °C (Reed, 2005), but there are cases when higher temperatures or lower ones with initial heat shock give better responses.

The climatic conditions in which the donor plants were grown influence the production of floral bud production. In the case of higher temperatures during donor plant growth, in potato, the temperature brings benefits on to the embryo production in a higher manner than photoperiod (Chani et. al., 2004). Low androgenesis frequencies are recorded due to the long day conditions in which the anther donor plants are cultivated (Vagera and Havranek, 1983) in the case of the species *Nicotiana tabacum* L. and *Datura inoxia* Mill.

## CONCLUSIONS

1. Even if the androgenic potential is high for few cultured plants, adequate treatments represent a solution to overcome the genotypic dependence;
2. Choosing anther donor plants must be effectuated considering their health and development, avoiding plants that are exposed to different forms of stress;
3. In general, the sensitive period for androgenesis induction in microspores is between the uninucleat stage and the bicellular pollen around first mitosis;
4. The correct election of culture media for anthers or isolated microspores, supplementation with optimal concentration of phytohormones and pretreatments are essential for the androgenesis programs.

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# THE EFFECT OF CHEMICAL MUTAGEN AGENTS ON SOME MORPHOLOGICAL CHARACTERS TO *CORIANDRUM SATIVUM* L.

## EFFECTUL AGENȚILOR MUTAGENI CHIMICI ASUPRA CARACTERELOR MORFOLOGICE LA CORIANDRU (*CORIANDRUM SATIVUM* L.)

LUPU Elena-Ancuța<sup>1</sup>, LEONTE C.<sup>1</sup>, CREȚU L.<sup>1</sup>, LAZĂR (NECHITA) Adriana<sup>1</sup>, SPĂNU Oana<sup>1</sup>  
e-mail: elenaancutalupu@yahoo.com

**Abstract.** *Coriandrum sativum* L. is an annual herb of Near East origine, belongs to the Apiaceae family, and has genom  $2n = 22$  chromosomes (Diederichsen, 1996). In this paper we present some biometrical data obtained during vegetation period of coriander, for seeing the effect of some mutagen agents on the coriander plants. The  $M_1$  generation of plants was obtained from treated seeds with three types of chemical mutagen agents, like: ethidium bromide, colchicine and dimethyl sulfate, in concentration of 0,02%, 0,04%, 0,06%, 0,08%, each concentration having four and six hours for action time. Thus, it was determined: plant height, number of branches and number of umbels. Thus, the wealth of new biological material can be isolated useful forms for use in the process of creating new varieties (Leonte, 2011).

**Key words:** colchicine, ethidium bromide, dimethyl sulfate, *Coriandrum sativum* L.

**Rezumat.** *Coriandrum sativum* L. este o plantă erbacee, anuală, din familia Apiaceae, originară din Orientul Apropiat ( $2n = 22$ ) (Diederichsen A., 1996). În această lucrare sunt prezentate unele observații biometrice realizate în timpul perioadei de vegetație a coriandrului în scopul determinării acțiunii agenților mutageni asupra plantelor de coriandru în generația  $M_1$ . Această generație a fost obținută din semințe tratate cu trei tipuri de agenți mutageni chimici și anume bromură de etidium, colchicină și sulfat de dimetil, în concentrații de 0,02%, 0,04%, 0,06% și 0,08%, fiecare concentrație având ca timp de acțiune patru și șase ore. Astfel s-a determinat: înălțimea plantelor, numărul de ramificații și numărul de umbele. În felul acesta, din bogăția de material biologic nou se pot izola formele utile în vederea folosirii acestora în procesul de creare a noi soiuri (Leonte, 2011).

**Cuvinte cheie:** colchicină, bromură de etidium, sulfat de dimetil, *Coriandrum sativum* L.

## INTRODUCTION

Coriander (*Coriandrum sativum* L.) is an annual herb, belongs to the Umbelliferae family, and has genom  $2n=22$  chromosomes. The plant's name comes from the Greek word "koris", which means bug, immature plants and seeds having an intense smell of bug (Roman et al., 2008). The plant is originally of the

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

Near East, but has spread from the Mediterranean to Central Europe (Rausch Andrea, 2008). In Romania it can be grow with good results in all areas with fertile lands, but the highest yields are obtained in Bărăgan, Dobrogea, Burnazului, Olteniei and Covurlului plains.

The coriander (*Coriandrum sativum* L.) is an aromatic herb that is used either fresh leaves or fruits (seeds) which have a sweet-bitter, with a slight tint of pungency.

Complex chemical composition of fruit, give them the stimulant properties of gastro-intestinal secretions, soothing and carminative, helping the body to eliminate gas (Borcean Eugenia, 2005).

## MATERIAL AND METHOD

Biological material was represented by plants of *Coriandrum sativum* L. in generation M<sub>1</sub>, varieties: *Sandra* and *Omagiu*. The M<sub>1</sub> generation of plants was obtained from treated seeds with three types of chemical mutagen agents, like: ethidium bromide, colchicine and dimethyl sulfate, in concentration of 0.02%, 0.04%, 0.06%, 0.08%, each concentration having four and six hours for action time.

Observations on the three quantitative characters (plant height, number of umbels and number of branches) were made in the experimental field of the farm Ezăreni in Science Teaching Station in Iasi, in 2011. Biometric analysis was performed by protocols described by Axel Diederichsen in 1996 and Axel Diederichsen and Hammer in 2003.

The results obtained were processed using mathematical and statistical methods: analysis of variance and differences limit.

## RESULTS AND DISCUSSIONS

After treatment with mutagenic agents are obtained numerous mutations that show changes in morphological characters. Under the influence of chemical mutagens, mitotic cell division changes, induces the morphological changes of plants.

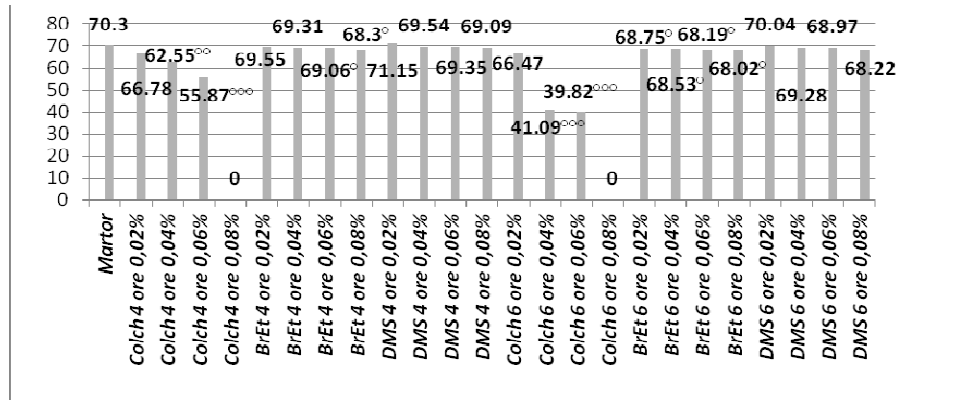
The height of the control was between 66,93 and 76,82 cm with an average of 70.30 cm from *Sandra* variety and between 69.82 and 72.94 cm with an average of 71.44 cm from *Omagiu* variety (fig. 1 și 2). Treatments with colchicine, to both varieties had a pronounced effect on reducing both plant height and number of branches and umbels per plant.

The *Sandra* variety, reduction effects on plant height were evident after treatment with the solution concentration of 0.04% and 0.06%, the values ranging between 62.55 and 55.87 cm at the time of exposure of 4 hours and between 41.09 and 39.82 cm to 6 h of treatment, the difference is very significant from the control (fig. 1).

On *Omagiu* variety the height was reduced at treatment by 6 h at all four concentrations of colchicine: 0.02%, 0.04%, 0.06%, 0.08%, the difference from the control was very significant (fig. 2).

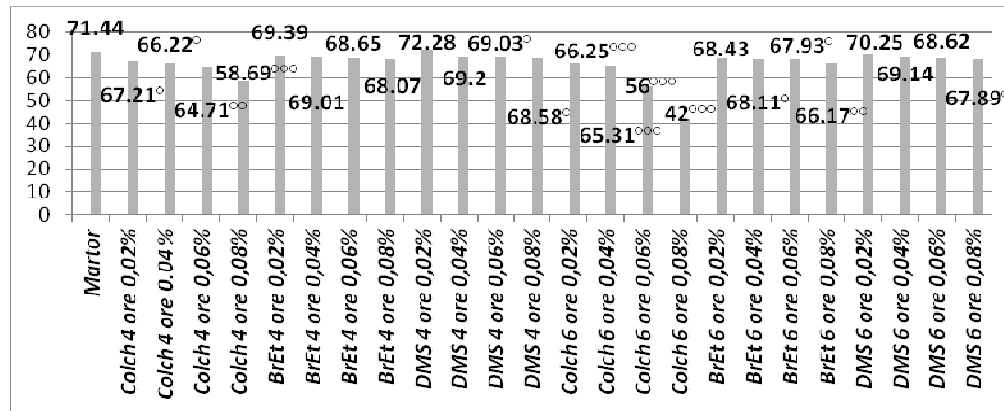
Dimethyl sulfate produced an insignificant reduction of plant to *Sandra* variety but in *Omagiu* variety this treatment produced significant differences compared with the control at concentrations of 0.06% and 0.08% in treatment 4 hours and the concentration of 0.08% at treatment for 6 hours. Thus, at treatment

of 4 hours the average plant height was 69.03 cm at concentration of 0.06% and 68.58 cm at concentration of 0.08% while at treatment of 6 hours the average was 67.89 cm (fig. 2).



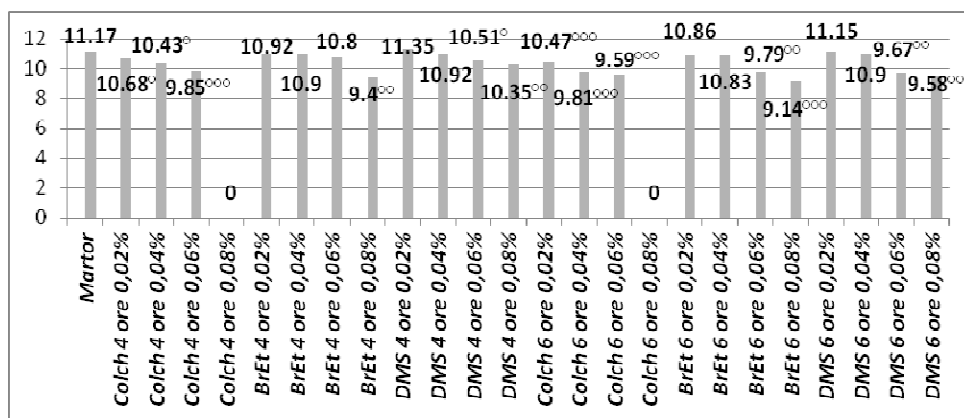
**Fig. 1** – Influences of mutagen agents on plant height and its significance in M<sub>1</sub> generation to Sandra variety

On *Sandra* variety the treatment with ethidium bromide, produced significant reductions in plant height compared with control. Thus, significant differences, at treatment the 4 hours occurred at concentrations of 0.06% and 0.08% and at treatment the 6 hours, differences became significant at all four concentrations (fig. 1).



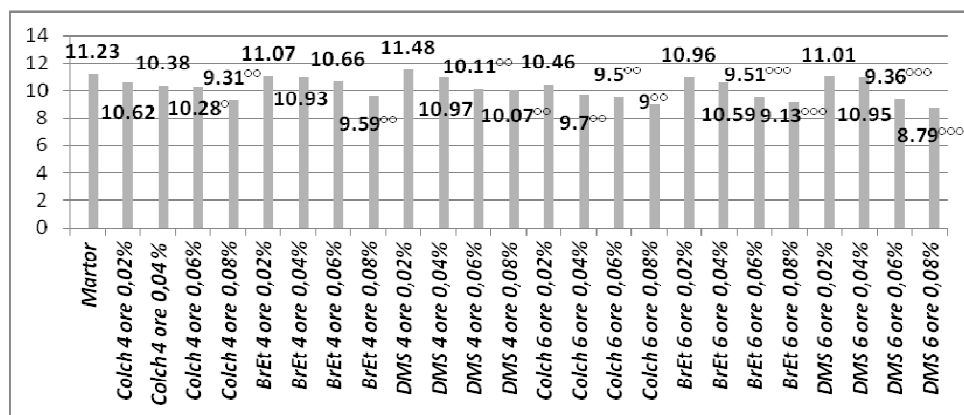
**Fig. 2** - Influences of mutagen agents on plant height and its significance in M<sub>1</sub> generation to Omagiu variety

On *Omagiu* variety, ethidium bromide treatment resulted in significant distinct differences from the control concentration of 0.08% with an average height of 66.17 cm (fig. 2).



**Fig. 3 - Influences of mutagen agents on the number of branches and its significance in M<sub>1</sub> generation to Sandra variety**

As the number of branches per plant, control variant had an average value of 11.17 to *Sandra* variety and an average value of 11.23 to *Omagiu* variety (fig. 3 and fig. 4). On *Sandra* variety, colchicine has produced significant differences in concentration of 0.06% in treatment 4 hours (average 9.85) and at concentrations of 0.02% (average 10.47), 0.04% (average 9.81) and 0.06% (average 9.59) to treatment for 6 hours (fig. 3).



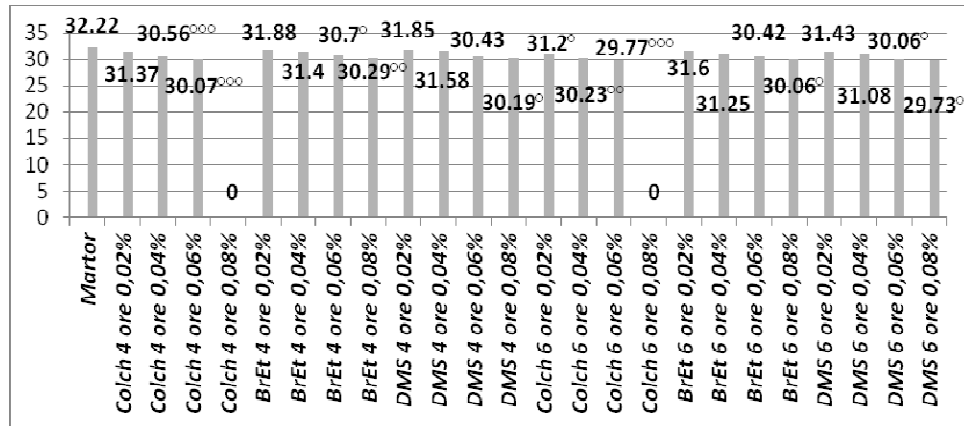
**Fig. 4 - Influences of mutagen agents on the number of branches and its significance in M<sub>1</sub> generation to Omagiu variety**

At *Omagiu* variety, variants showed significant differences in concentration of 0.08% to treatment with colchicine for 4 hours (average 9.31) and at concentrations of 0.04% (average 9.70), 0.06% (mean 9.50), 0.08% (average 9.00) to treatment for 6 hours (fig. 4).



Treatment with ethidium bromide, to *Sandra* variety, caused significant differences in concentration of 0.08% during exposure to 6 hours with an average of 9.14 (fig. 3). On *Omagiu* variety, ethidium bromide showed significant differences at concentrations of 0.06% (average 9.51) and 0.08% (average 9.13) to treatment time of 6 hours (fig. 4).

Dimethyl sulfate had a strong effect on the number of branches per plant to *Sandra* variety for concentrations of 0.08% (average 10.35) - 4 hours and 0.06% (average 9.67) and 0.08% (average 9.58) - 6 hours (fig. 3). On *Omagiu* variety was very significant differences to exposure time of 6 hours at concentrations of 0.06% (average 9.36) and 0.08% (average 8.79) (fig. 4).



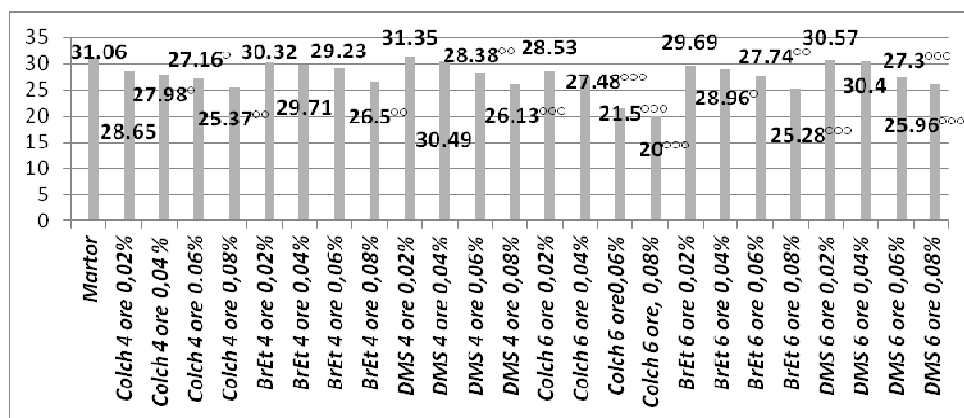
**Fig. 5** - Influences of mutagen agents on the number of umbels and its significance in  $M_1$  generation to *Sandra* variety

If umbels number per plant, to the control variants of *Sandra* variety have averaged 32.22, to *Omagiu* variety the control have an average value of 31.06 (fig. 5 and fig 6).

On *Sandra* variety, differences from the control were highly significant for treatment with colchicines at a concentration of 0.06% (average 30.07 respectively 29.77), with exposure time of 4 to 6 hours (fig. 5).

Significant differences were recorded and at *Omagiu* variety to concentrations of 0.04% (average 27.48), 0.06% (average 21.50) and 0.08% (20.00) on during the treatment with 6 h (fig. 6).

Ethidium bromide to *Sandra* variety had a pronounced effect on the variants at treatment for 4 hours with concentrations of 0.08% with a mean of 30.29 (fig. 5). On the variety *Omagiu* differences from control were highly significant to concentration of 0.08% during treatment with 6 hours recording an average of 22.58 (fig. 6).



**Fig. 6** - Influences of mutagen agents on the number of umbels and its significance in M<sub>1</sub> generation to Omagiu variety

Dimethyl sulfate led to a different decrease in the number of umbels per plants between the two varieties.

On *Sandra* variety the differences compared with control were significant at concentrations of 0.06% (average 30.06) and 0.08% (average 29.73) (fig. 5).

## CONCLUSIONS

1. The results led to the conclusion that reducing plant height, number of branches and number of umbels is achieved with increasing mutagen dose used, recording both distinct and significant differences compared with untreated control.

2. After treatments with colchicines (0.06% and 0.08%), it was found that plant height and number of umbels is in decrease as concentrations of colchicines is increases, the differences were significant.

3. Treatment with ethidium bromide and dimethyl sulfate led to sharp decreases to the number of branches and umbels, especially to *Omagiu* variety at concentrations of 0.06% and 0.08%.

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# ASPECTS OF BIOLOGICAL CHARACTERS AND THE BENEFITS OF SOME VARIETIES OF GROUNDNUT (*Arachis hypogaea* L.) CULTIVATED IN ROMANIA

## ASPECTE PRIVIND CARACTERELE BIOLOGICE ȘI BENEFICIILE UNOR VARIETĂȚI DE ARAHIDE (*ARACHIS HYPOGAEA* L.) CULTIVATE ÎN ROMÂNIA

SPÂNU Oana<sup>1</sup>, LEONTE C<sup>1</sup>., CREȚU L<sup>1</sup>., LAZĂR (NECHITA) Adriana<sup>1</sup>, POMOHACI T.I<sup>1</sup>.  
e-mail: spanuoana@yahoo.com

**Abstract.** *Arachis hypogaea* L. is an annual herb belongs to the Fabaceae family, native to South America origine and has  $2n = 40$  chromosomes genom. Seeds and groundnut butter are high in protein, fat, vitamins and minerals (Marin, 2011). Unsaturated fats contained are mainly beneficial for the human body by reducing the blood cholesterol levels and also the risk of cardiovascular disease. Unrefined groundnut oil and the refined one can be used in culinary techniques that require high temperatures of cooking (Pîrșan, 1998). In this study are exposed the general aspects on the biological characters of groundnut varieties grown in Romania, namely: *Arachis hypogaea* L. ssp *fastigiata* var. *fastigiata*, Valencia type and *Arachis hypogaea* L. ssp *fastigiata* var. *vulgaris*, Spanish type.

**Key words:** varieties, oil content, biological characters

**Rezumat.** *Arachis hypogaea* L. este o plantă anuală, din familia Fabaceae, originară din America de Sud ( $2n=40$ ). Semințele și untul de arahide au un conținut ridicat de proteine, grăsimi, vitamine și minerale (Marin, 2011). Grăsimile conținute sunt în principal nesaturate, benefice pentru organism prin efectul de reducere a nivelului de colesterol din sânge și reducerea riscului de boli cardiovasculare. Uleiul nerafinat de arahide și cel rafinat poate fi folosit în tehnici culinare care necesită temperaturi înalte de preparare (Pîrșan., 1998). În lucrarea de față se expun aspecte generale privind caracterele biologice ale unor varietăți de arahide cultivate în România și anume: *Arachis hypogaea* L. ssp. *fastigiata* var. *fastigiata*, tipul Valencia și *Arachis hypogaea* L. ssp *fastigiata* var. *vulgaris*, tipul Spaniol.

**Cuvinte cheie:** varietăți de arahide, conținutul de ulei, caractere biologice

### INTRODUCTION

Peanuts are particularly important because of high seed protein content (25-34%) and fat (45-60%) (Marin, 2011). The oil world production, peanuts ranked third (over 3 million tons annually), being as soybean, sunflower and before cotton (Marin, 2011). Oil (good quality with a high content of vitamin B1) is used in food, canning industry, margarine and butter, and fresh and whole grains are consumed fried or different dishes to get bread, biscuits and chocolate. Shells

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

resulting from peeling peanut pods and after grinding are used in the preparation of feed fodder, and the leaves and stems can be valuable for animal feed, but can be used as green manure (Marin, 2011).

Besides these uses, culture of peanuts are important because these require a low consumption of fertilizers, gives the possibility of a high sandy soil and plant is a very good run for other cultures (Marin, 2011). The oil from peanutsseeds can be extracted from cold or hot, the cold extraction being of high quality. Debris remaining after cold oil extraction contains 8% oil, 47.6% protein, 23% not nitrogen extractive substances, 48% minerals (Muntean et al., 2008).

In Romania, two varieties of peanuts are grown, namely: *Arachis hypogaea* L. ssp. *fastigiata* var. *fastigiata*, *Valencia* type and *Arachis hypogaea* L. ssp. *fastigiata* var. *vulgaris*, *Spanish* type. *Spanish* type has small seeds, covered with a brown shell, grouped two seeds in pods, and *Valencia* type has large seeds, covered with a red shell, grouped four seeds in pods.

## MATERIAL AND METHOD

The study was conducted based on analysis of existing information in the literature. Were analyzed two peanut varieties grown in our country. As a method has been used the comparative analysis.

## RESULTS AND DISCUSSIONS

Peanuts are annual plants with root systems of type II amounting to 150 cm depth, after 15-20 days from emergence appear nodule due fixing nitrogen-bacteria (Pîrșan, 1998). The stem is erect, the leaves have two pairs of sessile leaflets, hairy on the underside and leatherback on the top skin (Fig. 1). Flowers appear at 25-30 days after emergence, are small, yellow or orange, solitary or grouped in 2-4 inflorescences. The plant has two types of flowers: some open, with the visible corolla to pollination, these are located to the top of the stem, usually sterile and others do not open, located at the base of the stem and the underground portion, where the pollination is made with a closed flower, also called cleistogame flowers (fig. 2).

Fertilization is pollinating. After fecundation the ovary has a rapid increase (5-20 cm), forming an extension called ginophor. It bears the ovary and enters the ground (about 10 cm), which grows fruits (Fig. 3). The flowers from the top of the stem, which ginofor not reach the ovary to the ground, the fruit does not develop. The fruit formed in soil cover with a mycorrhizal ecototrof, which protects it from dryness (Fig. 4).

The fruit is an indehiscent pod, the shape of silkworm cocoons, with 1-6 seeds (representing 65-75% of the fruit). A plant form, in conditions of our country 250 flowers and 25-30 fruits (representing 5-15% of flowers).

Variety *fastigiata*, *Valencia* type are sweet peanuts, with three to six seeds in a pod covered with a bright red shell (Fig. 5 and 6). Mature plants reach up to 50 cm high, with most pods grouped based on strain. Entire growing season ranges from 95-110 days. The peanut is eaten roasted or boiled.

Variety *vulgaris*, *Spanish* type has two seeds in a pod, covered with a brown shell. Outer texture of the pod is woody than *Valencia* type (Fig. 7 and 8). Whole growing season lasts 120 days, slightly more delayed than *Valencia* type. This is used to obtain peanut oil and butter.



**Fig. 1.** Peanut leaf

([www.gerry0212.wordpress.com](http://www.gerry0212.wordpress.com))



**Fig. 2.** Peanut flower

([www.bloominthyme.wordpress.com](http://www.bloominthyme.wordpress.com))



**Fig.3.** Peanut ginofor

([www.waynesword.palomar.edu](http://www.waynesword.palomar.edu))



**Fig. 4.** Mature pods

([www.blog.peaceworks.net](http://www.blog.peaceworks.net))



**Fig. 5.** Red peanuts

([www.purcellmountainfarms.com](http://www.purcellmountainfarms.com))



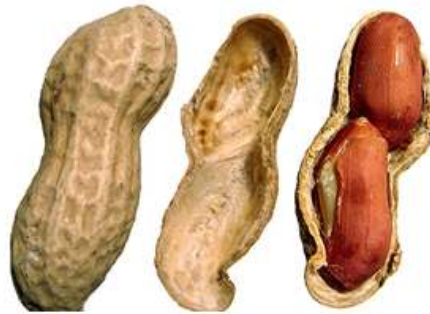
**Fig. 6.** Valencia type

([www.informedfarmers.com](http://www.informedfarmers.com))



**Fig. 7.** Brown peanuts

([www.bnrglobal.com](http://www.bnrglobal.com))



**Fig. 8.** Spaniol type

([www.waynesword.palomar.edu](http://www.waynesword.palomar.edu))

Peanut seeds contain between 44-56% fat and 20-30% protein, which are also a rich source of minerals (phosphorus, calcium, magnesium and potassium) and vitamins (group E, K, and B) (Savage and Keenan, 1994).

Oleic and linoleic acids are 75-80% of total fatty acids in peanuts (Treadwell et al., 1983; Dwivendi et al., 1993). Nutritional quality and storage of peanuts depends on the proportion of saturated and unsaturated fatty acids. Is desirable a high percentage of polyunsaturated fatty acids because it decreases plasma cholesterol and low density lipoproteins to reduce the risk of cardiovascular disease (Jackson et al., 1978).

Iodine value, providing a measure of the degree of unsaturation of the oil and the ratio of oleic / linoleic acid was commonly used to predict the validity and stability of oil (Worthington et al., 1972, Young and Walter, 1972). Nutritional quality of peanut seeds is strongly influenced by location, variety and season, especially soil moisture and temperature during growth and maturation of seeds (Dwivedi et al., 1990).

Peanuts are an excellent source of resveratrol, a fitoalexin stilbene with a role in protection against cancer, heart disease, degenerative nerve disease and Alzheimer's disease ([www.nutrition-and-you.com](http://www.nutrition-and-you.com)). Peanut butter not only awakens the taste for sweet, but also a considerable source of antioxidants. Resveratrol is an antioxidant found in peanut butter, in fact, found in black grapes.

Peanut butter also contains vitamin E, vitamin B3, copper, iron, calcium, potassium, etc. Thanks to fiber and healthy fats speeds up the feeling of fullness and regulates appetite. Remineralised body and is a beneficial food for those who neglect nutrition. Maintain strong bones and reduces hypertension, action is attributed to the presence of potassium. It is a nourishing food by the presence of protein, and a cure to prevent infections. In addition, all antioxidants reduce the risk of colorectal cancer, inflammation and specific signs of aging ([www.bucataras.ro](http://www.bucataras.ro)).

## CONCLUSIONS

1. Spanish type has small seeds, covered with a brown shell, grouped two seeds in pods, and Valencia type has large seeds, covered with a red shell, grouped four seeds in pods.
2. Peanut oil reduces cholesterol, thus contributing to the prevention of cardiovascular diseases.
3. Peanuts are used frequently and with good results in cases of intellectual exhaustion, physical fatigue increased in facilitating the bowel.
4. Peanut butter contains many proteins and fiber, the latter having an important contribution to the smooth digestive process.

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# EFFECT OF A PHYTOTHERAPY OIL PREPARATION UPON THE CUTANEOUS ERYTHEMA

## EFFECTUL UNUI PREPARAT FITOTERAPIC ULEIOS ASUPRA ERITEMULUI CUTANAT

ANDRÎTOIU C.V.<sup>1</sup>, PRISĂCARU Anca Irina<sup>1</sup>, ANDRIESCU Corina<sup>2</sup>, POPA I.M.<sup>1</sup>  
e-mail: dr\_calin\_andritoiu@yahoo.com

**Abstract.** Cutaneous erythema appears frequently as a reaction of immunosensitization due to industrialization and pollution, use of chemical substances for housekeeping, use of body care products. The present experiment evaluates the efficiency of a topical treatment of cutaneous chemically induced erythema in Wistar rats, using a phytotherapy oil preparation. The experimental model was achieved with 85% aqueous solution of lactic acid. The preparation used to treat the cutaneous lesions for 7 days included: olive oil (*Oleum olivarum*), linseed oil (*Oleum lini*), corn oil (*Oleum maydis*), sea buckthorn oil (*Oleum Hippophaë*), grape seed oil. Macroscopic, clinical and histopathological evaluations were performed. The results emphasize the efficiency of oil preparation in cutaneous erythema, with evident improvements from the third day of treatment. In conclusion, the oil preparation may be used with good results in the treatment of cutaneous erythema, as it is biocompatible, and can be included in dermatology and cosmetic products.

**Key words:** *Oleum olivarum*, *Oleum lini*, *Oleum maydis*, *Oleum Hippophaë*, grape seed oil.

**Rezumat.** Eritemul cutanat apare frecvent ca reacție de imunosensibilizare, datorită industrializării și poluării, folosirii de substanțe chimice în gospodărie, utilizarea de produse de îngrijire corporală ce conțin compuși chimici de sinteză. Prezentul articol își propune evaluarea eficienței tratamentului topic a eritemului cutanat indus chimic la șobolani Wistar, utilizând un preparat fitoterapic uleios. Modelul experimental s-a realizat cu ajutorul unei soluții apoase de acid lactic 85%. Preparatul uleios folosit pentru tratarea leziunilor timp de șapte zile conține: ulei de măsline (*Oleum olivarum*), ulei de in (*Oleum lini*), ulei de porumb (*Oleum maydis*), ulei de cătină (*Oleum Hippophaë*), ulei de sămburi de struguri. S-au urmărit evaluarea macroscopică, clinică și histopatologică. Rezultatele evidențiază eficiența preparatului asupra eritemului cu rezultate clinice evidente încă din a treia zi de utilizare topică. În concluzie, preparatul fitoterapic uleios se poate folosi cu bune rezultate în tratamentul eritemului cutanat, fiind biocompatibil și se poate include în produse cosmetoterapice.

**Cuvinte cheie:** *Oleum olivarum*, *Oleum lini*, *Oleum maydis*, *Oleum Hippophaë*, grape seed oil.

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<sup>1</sup> "Gheorghe Asachi" Technical University of Iași, Romania

<sup>2</sup> "Sf. Parascheva" Clinical Hospital of Infectious Diseases, Iași, Romania

## INTRODUCTION

Skin is a complex organ, having as major role the protection against fluid loss (Merică, 2003) and represents a way of administration for dermatology and cosmetic products, the highest rate of absorption being held by the oil formulations (Cosmovici et al., 1980).

Olive oil has antioxidant properties due to phenols, protective effects against UV skin injuries (Ichihashi et al., 2003), antitumor activity due to the property of 3,4-dihydroxyphenyl-ethanol (3,4-DHPEA) to inhibit the cell proliferation by blocking the cell cycle in G0/G1 phase and to induce the apoptosis of the tumor cell line (HL60) (Fabiani et al., 2002).

The grape seed oil contains compounds with antioxidant activity and unsaponifiable lipids ( $\beta$ - sitosterol, campesterol, and stigmasterol), offering resistance against peroxidation. Thus, it is suitable as a cosmetic ingredient, in different formulations used in treating dry and aged skin, helps to regulate the skin pH, and has the attribute of being hypoallergenic (Sineiro et al., 1995).

Furthermore, the plant and seed oils contain phytohormones that stimulate the blood flow, accelerate the cell regeneration, and improve tonicity, acting with success upon aged skin. Corn oil is one of these oils, with a high content of vitamins E and F (Braha, 1998). The essential fatty acids maintain the hydration of the skin and prevent from atopic dermatitis, psoriasis, acne and eczema. More over, the active principles from the linseed oil act upon open wounds and skin burns (Ardelean et Mohan, 2008), also possessing emollient effect (Istudor, 1998). The sea buckthorn oil contains glycerides of fatty acids, carotenoid compounds and other lipid substances that offer this oil benefic effects upon cutaneous lesions (Grigorescu et al., 2001).

The present study aims to evaluate the efficiency of a topical oil preparation that contains a mixture of natural oils (olive oil - *Oleum olivarium*, linseed oil - *Oleum lini*, corn oil - *Oleum maydis*, sea buckthorn oil - *Oleum Hippophaë*, grape seed oil) in the treatment of cutaneous chemically induced erythema in Wistar rats.

## MATERIAL AND METHOD

In order to demonstrate the reepithelization effect of the above mentioned oil preparation, the following experimental model of chemically induced erythema was achieved:

- female rats, Wistar strain, with a body weight comprised between 220-250 g, have been divided into 2 groups: non-treated group (group 1, with chemically induced erythema and non-treated), oil preparation group (group 2, with chemically induced erythema and treated with the natural oil preparation);
- the dorsal surface of the animals was shaved by mechanical and chemical methods (first, the hair was cut with a scissor; then an epilation cream was applied, according to the indications on the label of the product; consequently, the epilated area was rinsed with normal saline solution);
- the chemical erythema was induced by washing the two paravertebral areas of each rat with a 85% aqueous solution of lactic acid; the affected areas were left for 24 hours, without rinsing them with normal saline solution;

- after 24 hours, the laboratory animals were topically treated with the tested oil preparation for 7 days, until complete healing of the wound was ascertained; during these 7 days, macroscopic and clinical examinations were achieved;
- in the end, a specimen sample of tissue removed from the healed skin of all rats was taken with a 3 mm biopsy punch in order to be analyzed by histopathological examination. The collected samples were fixed in 10% buffered formalin for at least 24 h, progressively dehydrated in solutions containing an increasing percentage of ethanol (70, 80, 95, and 100%, v/v), embedded in paraffin under vacuum, sectioned at 5 µm thickness, de-paraffinized, and stained with hematoxylin-eosin (HE) and Szekely (Sz).

The tested topical oil preparation was formulated as a mixture of natural oils (olive oil - *Oleum olivarum*, linseed oil - *Oleum lini*, corn oil - *Oleum maydis*, sea buckthorn oil - *Oleum Hippophaë*, grape seed oil).

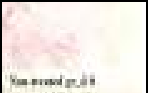
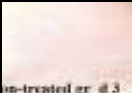
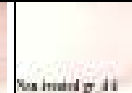
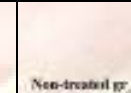




## RESULTS AND DISCUSSIONS

### *Clinical and macroscopic results*

The macroscopic evaluation of the cutaneous erythema for the 2 experimental groups (non-treated group and oil preparation group) demonstrates the efficacy of the topical oil preparation treatment. The complete healing occurred after 7 days of treatment, and evident clinical improvements were obtained even from the third day of treatment (table 1, table 2).

Table 1

Macroscopic evaluation of the cutaneous erythema

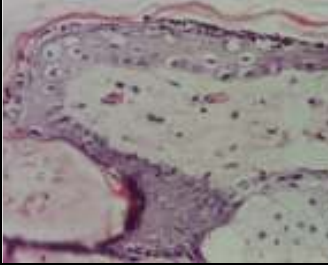
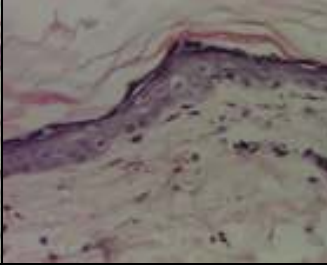
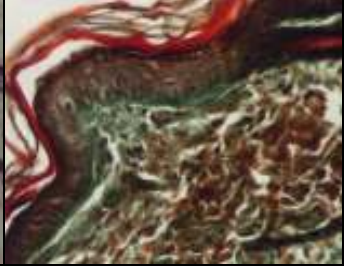
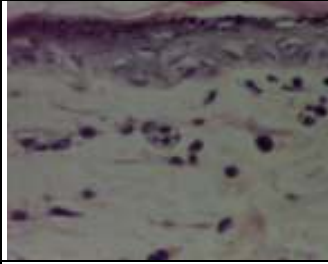
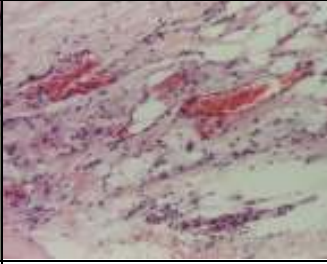
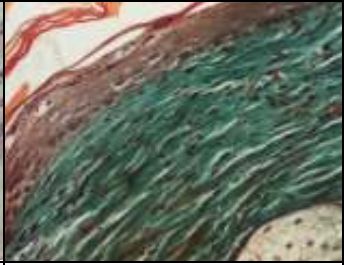
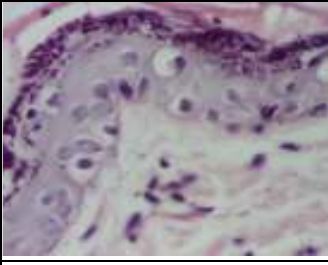
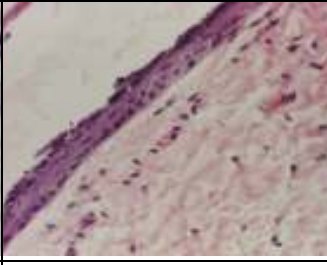
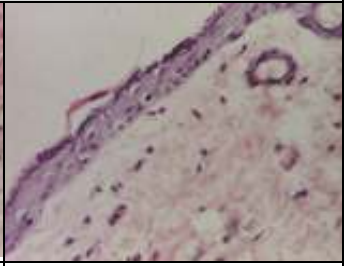
| Experimental groups   | Day 0   | Day 2   | Day 3   | Day 4  | Day 5   | Day 7   |
|-----------------------|---|---|---|--|---|---|
| Non-treated group     |   |   |   |   |   |   |
| Oil preparation group |  |  |  |  |  |  |

### *Histopathological results*

The epidermis is intact, rectilinear, with orthokeratosis, without dermal papillae. No exocytosis or spongiosis is present. The epidermis is represented by 1-2 cell layers (non-treated group) or 2-3 cell layers: basal layer, spinous layer, granular or cornified layer (oil preparation group). In areas with 1-2 layers, the cells are small, basophilic, with hyperchrome nuclei having a slightly increased volume and frequently with an irregular contour – aspects that certify the recent regeneration of the epithelium. In areas with 2-3 layers, there can be seen vacuolar degeneration, aspects of “koilocytes” (large cells with small nuclei, moderately hyperchrome, with perinuclear halo) or aspects of large pale vesiculous nuclei, with finely dispersed chromatin and abundant cytoplasm. These cytonuclear aspects are remarked in oil preparation group, suggesting recent regeneration (table 2). For the non-treated group the basal membrane of the epidermis shows rarely and isolated a slight accentuation (table 2), and the papillary dermis shows a severe densification by collagenization, with a discrete

expression in the reticular dermis (table 3). For the oil preparation group, the aspects of collagenization are very discrete in the papillary dermis, while in the reticular dermis no signs of collagenization are seen (table 3). For the oil preparation group, the normal aspect of all the 3 layers of the dermis prevails, and the lymphocytes are minimal, in normal range. Discrete edema is remarked in the papillary dermis, with the absence of edema in the reticular dermis for the oil preparation group, while for the non-treated group, edema is present in both layers of the dermis (tables 2 and 3). The aspects of congestion are minimal to moderate, mainly for the non-treated group (table 2). The small capillaries are dilated, filled with normal erythrocytes. Haemorrhage through erythro diapedesis is present only isolated, only for the non-treated group (table 2).

Table 2

| <b>Histopathological examination</b>  |   |  |
|---|---|--|
| <b>MICROGRAPHS</b>  |   |  |
| <b>Non-treated group</b>  |   |  |
|    |    |    |
| vacuolar degeneration in epidermis (HEX200)   | moderate edema at the level of dermis (HEX200)                                      | slight accentuation of the basement membrane (Sz x 200)                              |
|   |   |   |
| inflammatory elements in papillary dermis (HEX400)                                  | stasis and discrete inflammation in hypodermis (HEX100)                             | fibrosis in papillary dermis (Sz x200)   |
| <b>Oil preparation group</b>  |   |  |
|  |  |  |
| regenerated epithelium (HEX400)   | recently regenerated rectilinear epidermis (HEX200)                                 | regenerated epidermis; dermis with discrete edema (HEX200)                           |

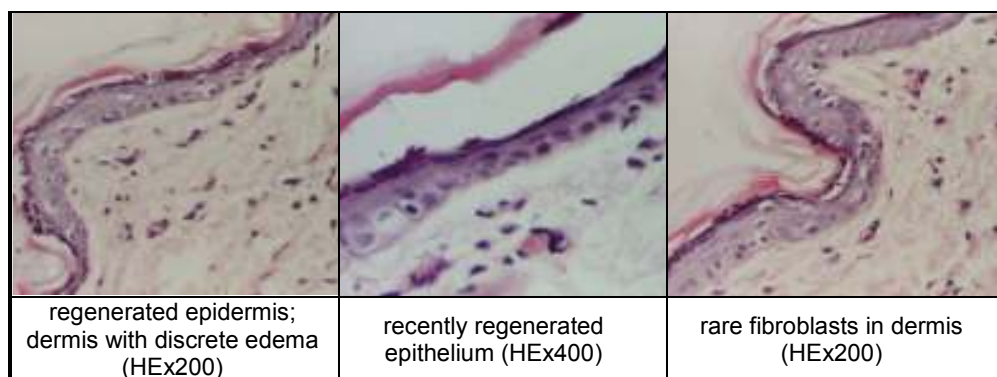


Table 3

**Collagenization and intensity of edema for the 2 experimental groups**

| Groups                | Collagenization  |                  | Edema            |                  |
|-----------------------|------------------|------------------|------------------|------------------|
|                       | Papillary dermis | Reticular dermis | Papillary dermis | Reticular dermis |
| <b>Non-treated gr</b> | ++               | +/-              | +                | +                |
| <b>Oil prep gr</b>    | +/-              | -                | +                | -                |

(evaluation scale of collagenization/edema: - absent, +/-discrete, + moderate, ++ severe)

Fats are indispensable for life not only as energy sources, but also for their structural role at the level of the skin, retina, nervous system, lipoproteins and biological membranes (Viola et Viola, 2009).

The oils used in this study were chosen taking into consideration the biocompatibility concept, by this understanding not only the tolerance, but also the affinity for the biological compounds at the level of the skin. The selection of olive oil was done due to the presence of 3 compounds: squalene,  $\beta$ -sitosterol, and  $\alpha$ -tocopherol. The squalene exerts antioxidant effects at the cutaneous level against the damage of the solar radiations, behaving as a biological filter of the singlet oxygen (Kohnno et al., 1995). Squalene is found in the unsaponifiable fraction of the olive oil, having a composition similar to that of the sebum. Squalene acts as a powerful scavenger of the singlet oxygen by inhibiting the UVA induced lipid peroxidation (Kohnno et al., 1995).  $\beta$ -Sitosterol is a phytosterol with a sebum regulation effect (Castellani et Zumiani, 2000). Regarding the third compound, it has been observed that, after 30 minutes of UV exposure, the level of  $\alpha$ -tocopherol in the skin is reduced to 50-60%. The topical application of  $\alpha$ -tocopherol significantly reduces the loss (Tavakkol et al., 2004). Vitamin E from the corn oil improves the blood flow in the peripheral tissues, thus influencing positively the skin regeneration (Cosmovici et al., 1980). The sea buckthorn oil exerts antibacterial and anti-inflammatory effects (Grigorescu et al., 2001), while linseed oil possesses antibacterial activity (Kaithwas et al., 2011) and the capacity to form a protective film. The grapeseed oil, through its content of linoleic acid higher than in any other oil (Ghisalberti, 2001), determines the rapid absorption through the skin and reduces the water loss from the epidermis (Trans Epidermal

Water Loss – TEWL), thus having as effect the re-establishment of the skin elasticity (Merică, 2003). Furthermore, the conjugated linoleic acid (CLA) is an efficient agent that inhibits the skin cancer (Ghisalberti, 2001).

In the present study there have been obtained important results from the clinical point of view, with evident improvements starting from the third day of treatment, and with the complete remission of the cutaneous erythema after 7 days of treatment. Moreover, the histopathological results emphasize the efficacy of the tested oil preparation upon the chemically induced erythema.

## CONCLUSIONS

The oil preparation tested in the present study proves to be efficient in the treatment of the cutaneous erythema, and the positive clinical and histopathological results open the way to new natural, biocompatible formulations based on the tested mixture of oils.

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# IMPLICATIONS OF APITHERAPY REGARDING THE EVOLUTION OF MINERALS IN ACETAMINOPHEN INDUCED INTOXICATION

## IMPLICAȚIILE APITERAPIEI PRIVIND EVOLUȚIA MINERALELOR ÎN INTOXICAȚIA MEDICAMENTOASĂ CU ACETAMINOFEN

ANDRIȚOIU C.V.<sup>1</sup>, PRISĂCARU Anca Irina<sup>1</sup>,  
e-mail: dr\_calin\_andritoiu@yahoo.com

**Abstract.** *Acetaminophen is one of the most used analgesic and antipyretic substances in Europe and United States. Found in over 100 pharmaceutical products, it is one of the most common drugs with liver and kidney toxicity potential. The drug-induced affection was achieved by gavage administration of acetaminophen (100 mg/100 g), for 2 weeks. Administration of apitherapy preparations to the group with drug-induced toxicity, in comparison with the unprotected group, determined: decrease of iron ( $242 \pm 48.23$  versus  $73.6 \pm 6.29$ ), decrease of potassium ( $6.24 \pm 0.43$  versus  $5.41 \pm 0.15$ ), increase of serum calcium ( $7.96 \pm 0.4$  versus  $9.68 \pm 0.47$ ), and increase of ionized calcium ( $3.86 \pm 0.24$  versus  $4.35 \pm 0.18$ ). Supplementation of apitherapy diet with Royal Jelly determined a less important decrease of iron and a more significant increase of serum and ionized calcium compared to the group treated only with apitherapy diet. In conclusion, administration of apitherapy products proved to be efficient in improving the levels of iron, potassium, serum and ionized calcium.*

**Key words:** apitherapy, minerals, acetaminophen

**Rezumat.** *Acetaminofenul este unul dintre cei mai folosiți agenți analgezici și antipiretici în Europa și Statele Unite. Acesta se găsește în peste 100 de produse farmaceutice, fiind unul dintre cele mai comune medicamente cu potențial toxic hepatic și renal. Afectarea medicamentoasă a fost indusă prin administrarea prin gavaj a acetaminofenului în doză de 100 mg/100 g, timp de 2 săptămâni. Administrarea preparatelor apiterapice la lotul cu afectare medicamentoasă, comparativ cu lotul neprotejat, a determinat: scăderea sideremiei ( $242 \pm 48.23$  versus  $73.6 \pm 6.29$ ), scăderea potasiului ( $6.24 \pm 0.43$  versus  $5.41 \pm 0.15$ ), creșterea calciului seric ( $7.96 \pm 0.4$  versus  $9.68 \pm 0.47$ ), creșterea calciului ionic ( $3.86 \pm 0.24$  versus  $4.35 \pm 0.18$ ). Suplimentarea apidietei cu lăptișor de matcă a determinat reducerea mai puțin importantă a fierului și creșterea mai semnificativă a calciului seric și ionic, comparativ cu lotul tratat doar cu apidietă. În concluzie, administrarea apidietei și lăptișorului de matcă loturilor cu afectare medicamentoasă s-a dovedit eficientă în normalizarea valorilor fierului, potasiului, calciului seric și ionic.*

**Cuvinte cheie:** apiterapie, minerale, acetaminofen

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<sup>1</sup> "Gheorghe Asachi" Technical University of Iași, Romania

## INTRODUCTION

Acetaminophen is one of the most used analgesic and antipyretic substances in Europe and United States, being included in over 100 pharmaceutical products. Therefore, acetaminophen is one of the most common drugs associated with accidental or voluntary intoxication. Acetaminophen may induce liver and kidney toxicity (Perry et al., 1998). Alcoholics develop acute hepatitis even with usual doses of acetaminophen (2.6-3.9 g/day) (Liaw et al., 1993). The prevalence of the drug induced liver lesions is not exactly known, as their diagnosis is achieved with difficulty and frequently belays (Zimmerman et al., 1994).

Liver represents the main place for the metabolism of the foreign substances (Lotterer et al., 1999). The metabolism of acetaminophen implies several ways of biotransformation. The most important among these (almost 95%) is achieved by the conjugation with glucuronic acid and sulfate. Only 5% is metabolized by the hepatic microsomal system of oxidation with mixed function, mainly P<sub>450</sub> 2E1 cytochrome (Brent et al., 1993; Hinson et al., 1994). The substances for these enzymatic systems include endogenous compounds, such as steroids, fatty acids (including prostaglandins and leukotrienes), and compounds such as drugs, food additives or industrial products, that enter the organism at the same time with foods, injections, or by inhalation (Nelson et al., 1996; Vessey, 2003; Murray, 2000; Bohan et al., 2002; Vickers et al., 1999).

In the present experiment, the influence of apitherapy diet, single or in combination with Royal Jelly (RJ), in acetaminophen induced hepatotoxicity was studied by evaluating some parameters of the mineral profile such as iron (Fe), potassium (K), ionized and serum calcium.

## MATERIAL AND METHOD

The experimental model included 6 groups of Wistar rats: control group standard food (group I - normal status, standard food), control group apitherapy diet (group II - normal status, apitherapy diet), control group apitherapy diet + RJ (group III - normal status, apitherapy diet and RJ), acetaminophen group (group IV - acetaminophen-induced toxicity, standard food), acetaminophen group + apitherapy diet (group V - acetaminophen-induced toxicity, treated with apitherapy diet), acetaminophen group + apitherapy diet + RJ (group VI - acetaminophen-induced toxicity, protected with apitherapy diet and RJ).

The drug-induced hepatotoxicity was achieved by gavage administration of acetaminophen (100 mg/100 g), for 2 weeks. The treatment consisted of apitherapy diet, single or in combination with RJ.

After 3 weeks of experiment, the laboratory animals were anesthetized with thiopental (dose of 1 ml/100 g from a 0.01% thiopental solution), blood samples were collected by the puncture of the cord with a Vacuette<sup>®</sup> system and submitted to the investigation of the parameters of the mineral profile: iron, potassium, serum and ionized calcium.

The statistical interpretation of the results was performed with One-Way ANOVA test and Tukey's post-hoc test. The results were given as mean ± standard deviation. The value of  $p < 0.05$  was considered significant.

## RESULTS AND DISCUSSIONS

In animals with acetaminophen induced toxicity (group IV) there can be noticed, in comparison to the other experimental groups:

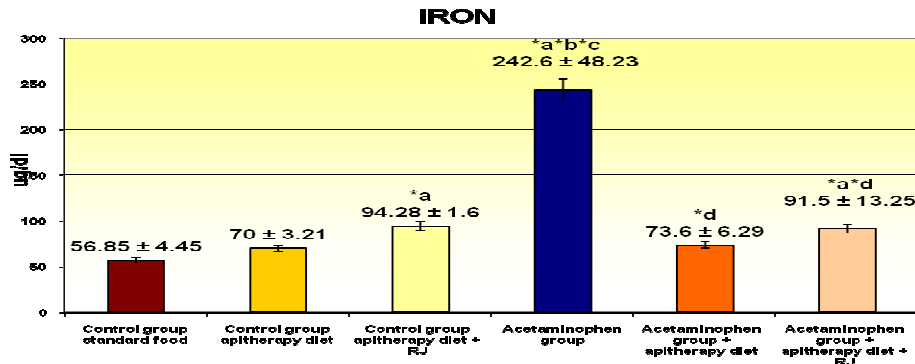


a) the increase of the following parameters for: i) control group standard food (group I) - Fe ( $56.85 \pm 4.45$  versus  $242.6 \pm 48.23$ ) (fig.1), K ( $5.1 \pm 0.16$  versus  $6.24 \pm 0.43$ ) (fig.2); ii) control group apitherapy diet (group II) - Fe ( $70 \pm 3.21$  versus  $242.6 \pm 48.23$ ) (fig.1), K ( $5.34 \pm 0.19$  versus  $6.24 \pm 0.43$ ) (fig.2); iii) control group apitherapy diet + RJ (group III) - Fe ( $94.28 \pm 1.6$  versus  $242.6 \pm 48.23$ ) (fig.1), K ( $5.37 \pm 0.18$  versus  $6.24 \pm 0.43$ ) (fig.2);

b) the decrease of the following parameters for: i) control group standard food (group I) – ionized calcium ( $4.19 \pm 0.07$  versus  $3.861 \pm 0.24$ ) (fig.4), serum calcium ( $8.81 \pm 0.5$  versus  $7.9625 \pm 0.4$ ) (fig.3); ii) control group apitherapy diet (group II) – ionized calcium ( $4.43 \pm 0.14$  versus  $3.861 \pm 0.24$ ) (fig.4), serum calcium ( $9.84 \pm 0.09$  versus  $7.9625 \pm 0.4$ ) (fig.3); iii) control group apitherapy diet + RJ (group III) – ionized calcium ( $4.58 \pm 0.06$  versus  $3.861 \pm 0.24$ ) (fig.4), serum calcium ( $10.51 \pm 0.3$  versus  $7.9625 \pm 0.4$ ) (fig.3).

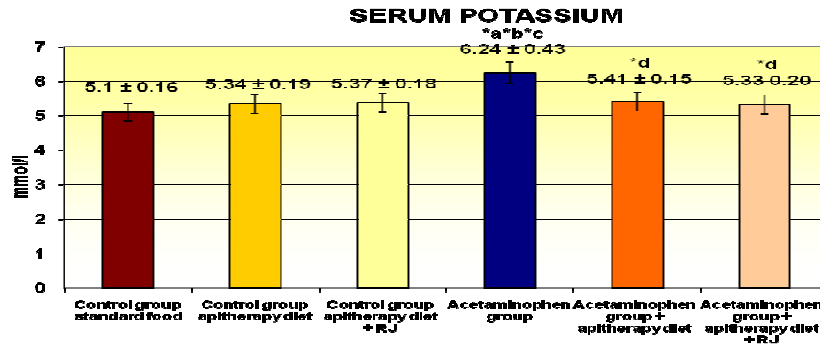
Administration of apitherapy diet to laboratory animals with acetaminophen induced toxicity (group V) determines, in comparison with the acetaminophen group (group IV), the following modifications: i) decrease of Fe ( $242.6 \pm 48.23$  versus  $73.6 \pm 6.29$ ) (fig.1) and K ( $6.24 \pm 0.43$  versus  $5.41 \pm 0.15$ ) (fig.2); ii) increase of ionized calcium ( $3.861 \pm 0.24$  versus  $4.358 \pm 0.18$ ) (fig.4) and serum calcium ( $7.9625 \pm 0.4$  versus  $9.68 \pm 0.47$ ) (fig.3).

Administration of apitherapy diet in combination with RJ to laboratory animals with acetaminophen induced toxicity (group VI) determines, in comparison with the acetaminophen group (group IV), the following modifications: i) decrease of Fe ( $242.6 \pm 48.23$  versus  $91.5 \pm 13.25$ ) (fig.1); ii) decrease of K ( $6.24 \pm 0.43$  versus  $5.33 \pm 0.2$ ), the values being comparable with the values of the other experimental groups (group I -  $5.1 \pm 0.16$ ; group II –  $5.34 \pm 0.19$ ; group III -  $5.37 \pm 0.18$ ) (fig.2); iii) increase of ionized calcium ( $3.861 \pm 0.24$  versus  $4.426 \pm 0.14$ ), and serum calcium ( $7.9625 \pm 0.4$  versus  $10.25 \pm 0.58$ ) (figs. 3 and 4).

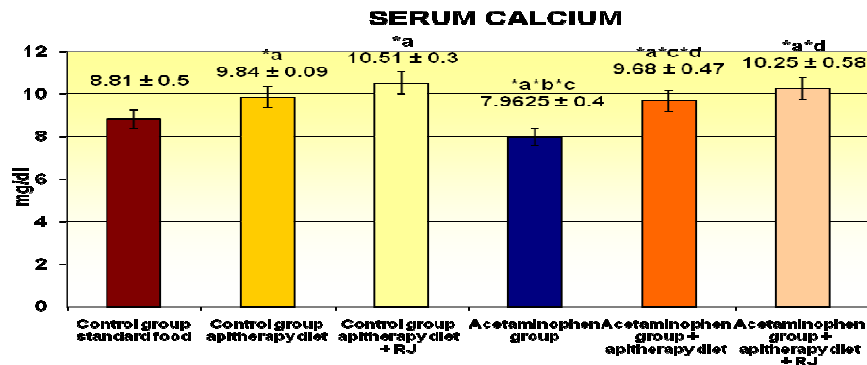


**Fig. 1 - Mean values of iron levels and standard deviation**

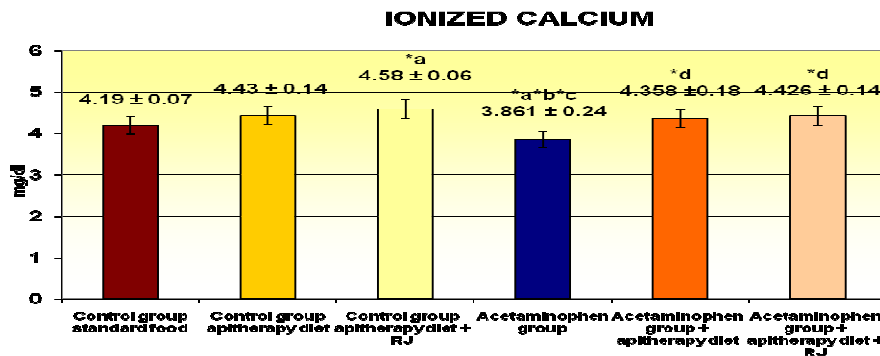
(\* a  $p < 0.05$  vs. control group standard food; \* b  $p < 0.0001$  vs. control group apitherapy diet; \* c  $p < 0.0001$  vs. control group apitherapy diet + RJ; \* d  $p < 0.0001$  vs. acetaminophen group)



**Fig. 2** - Mean values of serum potassium levels and standard deviation (\* a p<0.0001 vs. control group standard food; \* b p<0.0001 vs. control group apitherapy diet; \* c p<0.0001 vs. control group apitherapy diet + RJ; \* d p<0.0001 vs. acetaminophen group).



**Fig. 3** - Mean values of serum calcium levels and standard deviation (\* a p<0.05 vs. control group standard food; \* b p<0.0001 vs. control group apitherapy diet; \* c p<0.05 vs. control group apitherapy diet + RJ; \* d p<0.0001 vs. acetaminophen group)



**Fig. 4** - Mean values of ionized calcium levels and standard deviation (\* a p<0.05 vs. control group standard food; \* b p<0.0001 vs. control group apitherapy diet; \* c p<0.0001 vs. control group apitherapy diet + RJ; \* d p<0.0001 vs. acetaminophen group)

The increase of iron levels simultaneous to the presence of serum siderophilin, cellular protein that fixes the storing iron and which is normally absent in the blood, pleads for the cellular origin of iron. The factors responsible for the increase of iron levels are: the release of iron from the liver in conditions of hypoxia, excessive haemolysis, increased duodenal absorption of the food iron.

In subjects with chronic hepatopathies, it has been proven that the intestinal absorption of iron is 10 times higher than in healthy subjects. As the iron enters in higher amounts in the liver, it circulates under its unoxidized and free form through the portal system, favorizing its deposition as hemosiderin. Hemosiderin is a complex of heterogeneous iron achieved at the level of hepatocyte and reticulohistiocytic cells (Buligescu et al., 1999). At the level of liver, the deposition of iron as hemosiderin is preceded by the multiplication of lisosomes that elaborate a PAS-positive glycoprotein with a high affinity for iron. The lisosomes become siderosomes consequently to the incorporation of iron. By breaking their membrane, the iron is able to exert the negative impact upon the liver: interruption of oxidative phosphorylation, decrease of ATP and enzyme content, lactate accumulation, diminution of NADH<sub>2</sub> oxidation (Buligescu et al, 1999).

Acetaminophen is metabolically activated to produce reactive oxygen species that get fixed by a covalent bond to the macromolecules of the hepatocyte. The extension of these bonds is correlated to the incidence and severity of the liver necrosis. Accumulation of activated ionized calcium from the liver membrane decreases with 60-75% after a hepatotoxic dose of acetaminophen (Lewis et al., 1991).

In the case of therapeutical doses, 75% of the plasmatic acetaminophen undergoes sulfation or glucuronidation, but 5-10% is oxidized by the P<sub>450</sub> cytochrome into toxic metabolites such as N-acetyl-p- benzoquinone imine (Jerca et al., 2007).

The modifications of iron levels in chronic hepatitis interfere with multiple mechanisms, respectively with factors that increase or decrease the iron values. The present study reveals that chronic drug administration (acetaminophen), interferes with the levels of iron, potassium, ionized and serum calcium. Furthermore, there can be concluded from this experiment that the apitherapy treatment has benefic results that lead to the improvement of the above mentioned parameters to normal levels.

## CONCLUSIONS

1. Administration of apitherapy diet to the group with acetaminophen induced toxicity proved to be efficient by improving the iron levels to normal values.

2. Supplementation of the apitherapy diet with Royal Jelly for the group with acetaminophen induced toxicity led to a decrease of the iron levels, but not as important as in the case of the group treated only with apitherapy products.

3. Administration of apitherapy diet led to a significant decrease of serum potassium in comparison with the group with induced toxicity. Supplementation of

the apitherapy diet with Royal Jelly determined a more significant decrease of the potassium levels when compared to the group treated only with apitherapy diet.

4. Supplementation of the apitherapy diet with Royal Jelly determined a more significant increase of the ionized and serum calcium levels when compared to the group treated only with apitherapy products.

***Acknowledgments:**This paper was supported by the project PERFORM-ERA "Postdoctoral Performance for Integration in the European Research Area" (ID-57649), financed by the European Social Fund and the Romanian Government.*

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# EVALUATION OF THE OXIDATIVE STRESS STATUS AND HEPATIC DETOXIFICATION FUNCTION AFTER THE INTAKE OF FOODS CONTAINING HIGH ACRYLAMIDE LEVELS

## EVALUAREA STRESULUI OXIDATIV ȘI A FUNCȚIEI DE DETOXIFIERE HEPATICĂ ULTERIOR INGESTIEI DE ALIMENTE CU UN CONȚINUT BOGAT ÎN ACRILAMIDĂ

*PRISĂCARU Anca Irina*<sup>1</sup>, *ANDRIȚOIU C.V.*<sup>1</sup>, *HURDUC N.*<sup>1</sup>  
e-mail: irina.prisacaru@yahoo.com

**Abstract.** *The present experiment evaluates the pathological changes in oxidative stress and hepatic detoxification function after the intake of foods containing high acrylamide levels, by the means of biochemical analysis (superoxide dismutase, catalase, free sulfhydryl groups). The experimental model included 4 groups of Wistar rats: reference group (standard food), control group (the drinking water was replaced by an acrylamide solution), FP group (fried potatoes and standard food), T group (toast and standard food). The results reveal that the intake of foods containing high acrylamide levels leads to the following modifications: decrease of CAT for control group, FP group, and T group; decrease of SOD for control group, FP group, and T group; decrease of free sulfhydryl groups for control group, FP group, and T group. In conclusion, the intake of foods with high acrylamide levels has an important negative impact upon the oxidative stress status and hepatic detoxification function.*

**Key words:** acrylamide, fried potatoes, toast, oxidative stress, hepatic detoxification function.

**Rezumat.** *Prezentul experiment evaluează modificările patologice ale stresului oxidativ și funcției de detoxifiere hepatică, prin determinarea biochimică a superoxid dismutazei, catalazei și grupărilor sulfhidril libere. Modelul experimental a inclus 4 loturi de șobolani Wistar: lotul de referință (hrană standard), lotul de control (apa de băut a fost înlocuită cu soluție de acrilamidă), lotul FP (cartofi prăjiți și hrană standard), lotul T (hrană standard și pâine prăjită). Rezultatele analizelor biochimice relevă faptul că ingestia alimentelor cu un conținut bogat în acrilamidă determină: scăderea activității CAT pentru lotul de control, lotul FP și lotul T; scăderea activității SOD pentru lotul de control, lotul FP și lotul T; scăderea concentrației grupărilor sulfhidril libere pentru lotul de control, lotul FP și lotul T. În concluzie, ingestia alimentelor cu un conținut bogat în acrilamidă are un important impact negativ asupra statusului stresului oxidativ și a funcției de detoxifiere hepatică.*

**Cuvinte cheie:** acrilamidă, cartofi prăjiți, pâine prăjită, stres oxidativ, funcție de detoxifiere hepatică.

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<sup>1</sup> "Gheorghe Asachi" Technical University of Iași, Romania

## INTRODUCTION

The preferences of human beings for a better taste, colour and texture of every day food persuaded them to transform cooking into culinary art, by developing new food processing technologies, that allowed the formation of different chemical compounds that improve the organoleptic qualities of foods. On the other hand, the rapid rhythm of this century's living determined man to prefer semi-prepared foods, which implied the use of different food additives, many of them with harmful effects upon health. In both cases, the living food and its benefits have been neglected, unfortunately with undesirable effects upon human health.

Acrylamide, a process-induced food toxicant with an alarming toxicological profile, was found in fried, roasted, baked and grilled foods by the Swedish researchers in April 2002 (Eriksson, 2005; Friedman, 2003). During the first years after its discovery, acrylamide received considerable attention, due to the significant exposure of consumers to foods containing high acrylamide levels. The toxicity of acrylamide has been extensively investigated, indicating its neurotoxic and genotoxic potentials, its reproductive and developmental toxicity, and also the carcinogenic potential in rodents. Acrylamide has also been classified as a "probable human carcinogen" (Burlacu et al., 2008; Dybing et Sanner, 2003). The „acrylamide" subject has lately been abandoned, due to the lack of evidence of the epidemiological studies.

The present experiment evaluates the pathological changes in the oxidative stress status and the hepatic detoxification function after the intake of foods containing high acrylamide levels (fried potatoes and toast), by the means of biochemical analysis (superoxide dismutase, catalase, free sulfhydryl groups).

## MATERIAL AND METHOD

### Analysis instruments

The biochemical analysis was achieved with an open system EOS 880 PLUS semi-automatic analyzer.

### Experimental model

All the experimental proceedings in this experiment were achieved according to the international ethic regulations and were approved by the Ethics Commission of the University of Medicine and Pharmacy "Gr. T. Popa" Iași.

A total number of 24 male rats, Wistar strain, having body weights comprised between 180 and 220 g, were divided into 4 groups, as follows:

- reference group - fed with standard food and drinking water;
- control group - received the same diet as the reference group, except for the drinking water, which was replaced by an acrylamide solution 0.1 µg/L (the maximum allowed limit regarding the presence of acrylamide in the drinking water, according to the European Union legislation);
- FP group - the food supply was made up of French fries (given in the morning, around 8.00 o'clock), and the reference group's diet (at 14.00 o'clock);
- T group – the diet consisted of sliced white bread, toasted for 3 minutes into the electric toaster (8.00 o'clock) and the reference group's diet (at 14.00 o'clock).

The animals were housed in separate cages, in order to control their diets. The experiment was conducted over a period of 90 days.

**Biochemical investigation**

At the end of the experiment the animals were anesthetized with ketamine i.p. (75mg/kg) and blood samples were collected by the puncture of the cord with a Vacuette ® system and submitted to biochemical analysis, which aimed to evaluate the oxidative stress status (by determining superoxide dismutase - SOD, catalase – CAT) and the hepatic detoxification function (by determining free sulfhydryl groups).

**Statistical interpretation**

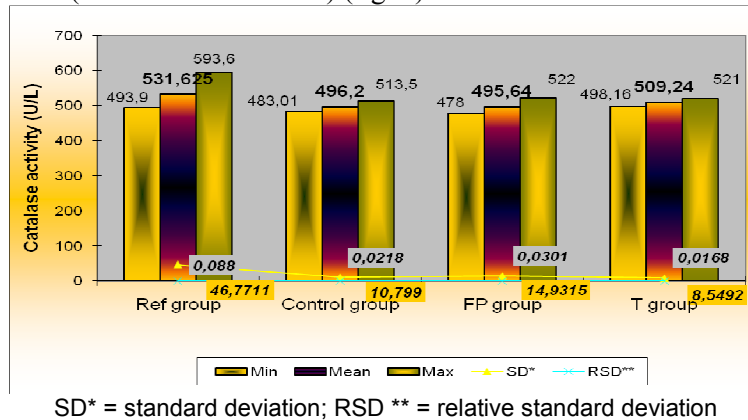
Statistical data were processed by program StatsDirect version 2.7.2 (2008). The accepted significance threshold is 95%, i.e.  $p < 0.05$ . The value of  $p$  is inversely proportional to the statistical significance. Statistical interpretation of data considered the differences corresponding to a significant threshold as follows:  $p > 0.05$  statistically insignificant;  $p < 0.05$  statistically significant;  $p < 0.01$  highly statistically significant;  $p < 0.001$  very highly statistically significant.

**RESULTS AND DISCUSSIONS**

**Evaluation of the oxidative stress**

**a. Determination of serum catalase**

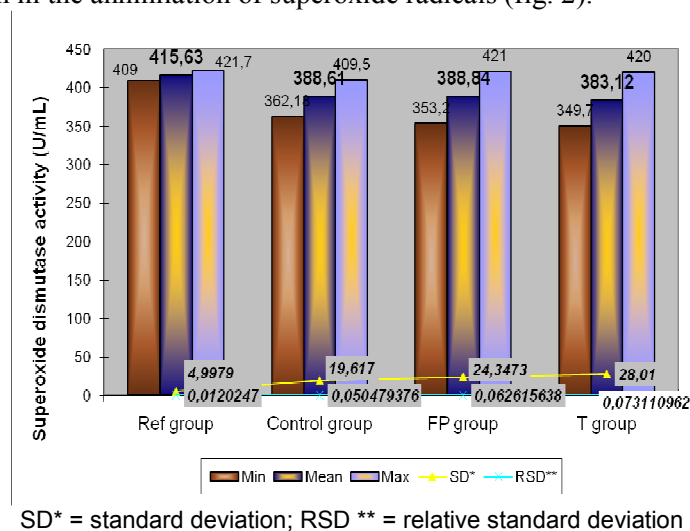
The first parameter indicating the presence of reactive oxygen species with peroxide structure is catalase, enzyme with mitochondrial and peroxisome localization. The analysis of the activity for this enzyme, indicates the involvement of oxygen free radicals in the annihilation of this enzyme in the serum of the animals exposed to the chronic intake of acrylamide (fig. 1). If for the reference group the catalase activity amounts to  $531.625 \pm 46.771$  U/L, values decrease to  $496.2 \pm 10.799$  U/L for the control group, highlighting the interference of the enzyme in the inhibition of free radicals (fig.1). Catalase activity returns to levels close to those of the reference group for the animals exposed to chronic intake of acrylamide through consumption of toast ( $509.24 \pm 8.549$  U/L), while the lowest enzyme activity is recorded for the animals fed with fried potatoes ( $495.64 \pm 14.931$  U/L) (fig. 1).



**Fig. 1 - The activity of catalase**

### ***b. Determination of superoxide dismutase***

The second parameter of oxidative stress is superoxide dismutase, metalloenzyme discovered by Irwin Fridovich, which uses as substrate the superoxide radical (Temneanu *et al.*, 2011). The variations of this enzyme reveal its implications in the eradication of free radicals for all experimental groups (fig. 2). Therefore, if for the reference group the SOD activity amounts to 415.63 U/mL  $\pm$  4.9979, for the control group (the group exposed to chronic administration of the acrylamide aqueous solution) the value significantly decreases to the amount of 388.61 U/mL  $\pm$  19.617, demonstrating the intervention in the annihilation of superoxide radicals (fig. 2).



**Fig. 2 - The activity of superoxide dismutase**

### **Evaluation of the liver detoxifying function**

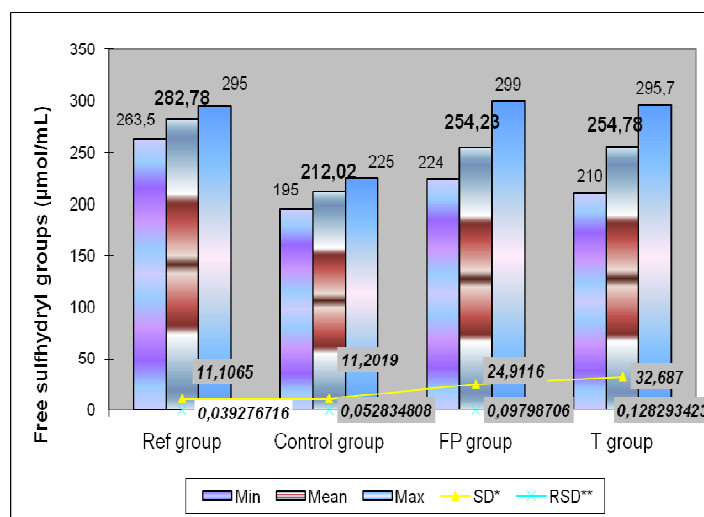
#### ***c. Determination of free sulfhydryl groups***

Glutathione ( $\gamma$ -glutamyl-cysteinyl-glycine) is a nucleophilic tripeptide, which has a critical role both in combating reactive oxygen species, but also in the process of detoxification, removing harmful metabolites after conjugation reactions, reactions conducted under guardianship enzyme glutathione-S transferase (Coleman *et al.*, 1997).

Results of the quantitative determination of free sulfhydryl groups in the serum of the laboratory animals are given in fig. 3. The analysis of the obtained values supports the antioxidant and detoxifying roles of glutathione. If for the reference group, the concentration of free sulfhydryl groups has a value of 282.78  $\pm$  11.106  $\mu$ mol/mL, the control group reflects a very significant reduction of 70 units. This negative variation shows either the intervention of glutathione in combating overload cellular free radicals of oxygen, or the involvement in the mechanisms of metabolism and conjugation of toxic amide. For the animals of the



groups fed with fries and toast an uniform increase compared to the control group values can be seen, the values still being below those of the reference group ( $254.23 \pm 24.911 \mu\text{mol/mL}$  for the animals fed with fried potatoes and  $254.78 \pm 32.687 \mu\text{mol/mL}$  for the animals fed with toasted bread) (fig.3).



SD\* = standard deviation; RSD \*\* = relative standard deviation

**Fig. 3 - The activity of free sulfhydryl groups**

## CONCLUSIONS

1. The variations of the catalase activity emphasize its involvement in inhibiting the reactive oxygen species by reducing its concentrations in the serum of the groups exposed to the chronic acrylamide intake, either by the 0.1 g/L acrylamide solution or by fried potatoes and toast.

2. The second parameter relevant for the oxidative stress, superoxide dismutase, reveals statistically significant modifications of its serum levels (up to 27-30 units) that demonstrate its involvement in the eradication of the oxidative stress.

3. The analysis of the free sulfhydryl groups sustains the double role of glutathione (antioxidant and detoxifying agent) by the significant decrease of its serum values, mainly for the group exposed to the acrylamide aqueous solution.

**Acknowledgments:** This paper was supported by the project PERFORM-ERA "Postdoctoral Performance for Integration in the European Research Area" (ID-57649), financed by the European Social Fund and the Romanian Government.

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# THE INVOLVEMENT OF FOODS CONTAINING HIGH ACRYLAMIDE LEVELS IN THE PROTEOSYNTHETIC FUNCTION OF THE LIVER

## IMPLICAȚIILE ALIMENTELOR CU UN CONȚINUT BOGAT ÎN ACRILAMIDĂ ÎN FUNCȚIA DE PROTEOSINTEZĂ A HEPATOCITULUI

*PRISĂCARU Anca Irina<sup>1</sup>, ANDRIȚOIU C.V.<sup>1</sup>*  
email: irina.prisacaru@yahoo.com

**Abstract.** *Acrylamide is a process-induced food toxicant found in fried, roasted, baked and grilled foods. The present experiment evaluates the hepatic proteosynthesis function after the intake of foods containing high acrylamide levels (fried potatoes and toast), by determining total proteins, albumins, and cholinesterase. The experiment included 4 groups of Wistar rats: reference group (standard food), control group (standard food and acrylamide solution 0.1 µg/L), FP group (fried potatoes and standard food), T group (toast and standard food). The results reveal that the intake of foods containing high acrylamide levels leads to: decrease of total proteins for control group, FP group, and T group; increase of albumins and cholinesterase for control group, FP group, and T group. In conclusion, the intake of foods with high acrylamide levels has a negative impact upon total serum proteins, the results not being sustained by the albumin and cholinesterase activities, unless a nephrotic syndrome is present.*

**Keywords:** acrylamide, fried potatoes, toast, hepatic proteosynthesis.

**Rezumat.** *Acrilamida este un toxic alimentar prezent în alimentele prelucrate termic prin prăjire sau coacere. Prezentul experiment evaluează capacitatea de proteosinteză a ficatului după consumul de alimente cu un conținut bogat în acrilamidă (cartofi prăjiți și pâine prăjită), prin determinări biochimice (proteine totale, albumină, colinesterază). Experimentul include 4 loturi de șobolani Wistar: lotul referință (hrană standard), lotul control (hrană standard și soluție apoasă de acrilamidă 0.1 g/L), lotul FP (cartofi prăjiți și hrană standard), lotul T (pâine prăjită și hrană standard). Rezultatele analizelor biochimice relevă faptul că ingestia alimentelor cu un conținut bogat în acrilamidă determină: scăderea proteinelor totale pentru lotul control, lotul FP și lotul T; creșterea albuminelor și colinesterazei pentru lotul control, lotul FP și lotul T. În concluzie, ingestia alimentelor cu un conținut bogat în acrilamidă are o influență negativă asupra proteinemiei totale, rezultatele nefiind susținute de valorile albuminemiei și colinesterazei serice decât prin prisma unui sindrom nefrotic.*

**Cuvinte cheie:** acrilamidă, cartofi prăjiți, pâine prăjită, proteosinteză hepatică.

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<sup>1</sup> "Gheorghe Asachi" Technical University of Iași, Romania

## INTRODUCTION

Nowadays, nutrition has revealed its tremendous importance in our lives. Acrylamide is a process-induced food toxicant found in fried, roasted, baked and grilled foods (Mottram et al., 2002). The neurotoxic and carcinogenic potential of acrylamide has been known ever since the first years of production and use (McCollister et al., 1964). Later, the carcinogenic action was re-confirmed through experimental researches on laboratory animals (CERHR, 2005), but the epidemiologic studies did not reveal such conclusive results (Mucci et al., 2003). This is why the “acrylamide” subject has lately been discarded from the research field.

The present experiment brings the exposure to acrylamide into the forefront and evaluates the proteosynthetic function of the liver consequently to the intake of foods containing high acrylamide levels (fried potatoes and toast), by the means of biochemical analysis (total proteins, albumins, and cholinesterase).

## MATERIAL AND METHOD

### Analysis instruments

The biochemical analysis was achieved with an open system EOS 880 PLUS semi-automatic analyzer.

### Experimental model

All the experimental proceedings in this experiment were achieved according to the international ethic regulations and were approved by the Ethics Commission of the University of Medicine and Pharmacy “Gr. T. Popa” Iași.

A total number of 24 male rats, Wistar strain, having body weights comprised between 180 and 220 g, were divided into 4 groups, as follows:

- reference group - fed with standard food and drinking water;
- control group - received the same diet as the reference group, except for the drinking water, which was replaced by an acrylamide solution 0.1 µg/L (the maximum allowed limit regarding the presence of acrylamide in the drinking water, according to the European Union legislation);
- FP group - the food supply was made up of French fries (given in the morning, around 8.00 o'clock), and the reference group's diet (at 14.00 o'clock);
- T group – the diet consisted of sliced white bread, toasted for 3 minutes into the electric toaster (8.00 o'clock) and the reference group's diet (at 14.00 o'clock).

The animals were housed in separate cages, in order to control their diets. The experiment was conducted over a period of 90 days.

### Biochemical investigation

At the end of the experiment the animals were anesthetized with ketamine i.p. (75mg/kg) and blood samples were collected by the puncture of the cord with a Vacuette® system and submitted to biochemical analysis, which aimed to evaluate the proteosynthesis function of the liver (by determining the total proteins - TP, the serum concentration of albumins – ALB and the serum cholinesterase – ChE).

### Statistical interpretation

Statistical data were processed by program StatsDirect version 2.7.2 (2008). The accepted significance threshold is 95%, i.e.  $p < 0.05$ . The value of  $p$  is inversely proportional to the statistical significance. Statistical interpretation of data considered the differences corresponding to a significant threshold as follows:  $p > 0.05$  statistically

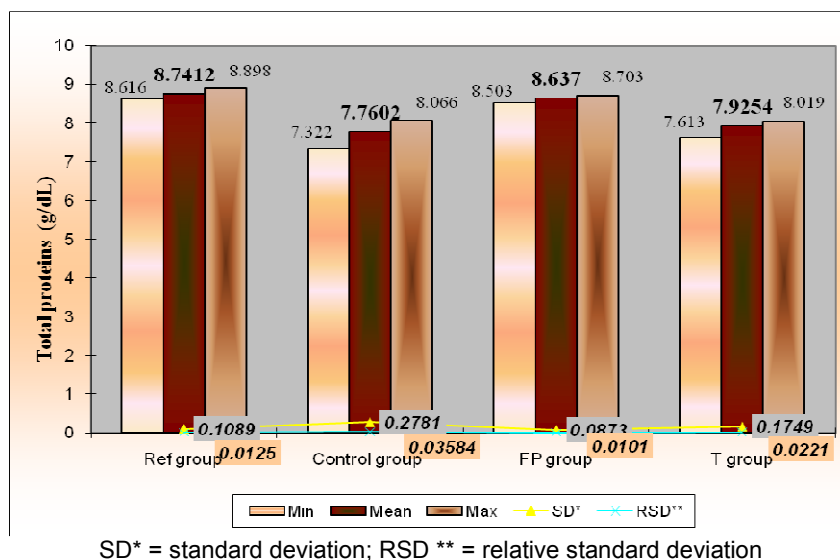
insignificant; p <0.05 statistically significant; p <0.01 highly statistically significant; p<0.001 very highly statistically significant.

## RESULTS AND DISCUSSIONS

### *Determination of total proteins*

Most of the proteins are synthesized in the liver and have additional functions as compared to the other proteins. Such proteins maintain the osmotic pressure of the plasma, participate into the coagulation and fibrinolysis processes and provide the protein reserve which is necessary for the tissue development and repair.

The results obtained from the quantitative determination of the total proteins in the serum of the animals from the present experiment are given in fig. 1. The study of the values shows a decrease of the total proteins from  $8.7412 \pm 0.1089$  g/dL, a concentration which corresponds to the reference group, to  $7.7602 \pm 0.2781$  g/dL, value which is characteristic for the control group that was given the acrylamide solution instead of the drinking water. This significant decrease of the proteinemia of the animals in the control group suggests the negative impact of acrylamide upon the proteosynthetic function of the liver. Carrying on the study on the third group, which was fed with Fried potatoes, one can ascertain a discretely diminished concentration in comparison with that of the reference group, and a significantly increased one as compared to that of the control group ( $8.6370 \pm 0.0873$  g/dL) (fig. 1). A damage of the proteosynthetic function at the level of liver may be translated from the decreased value of the proteinemia of the group which was fed with toast, value which ranges at  $7.9254 \pm 0.1749$  g/dL (fig. 1).

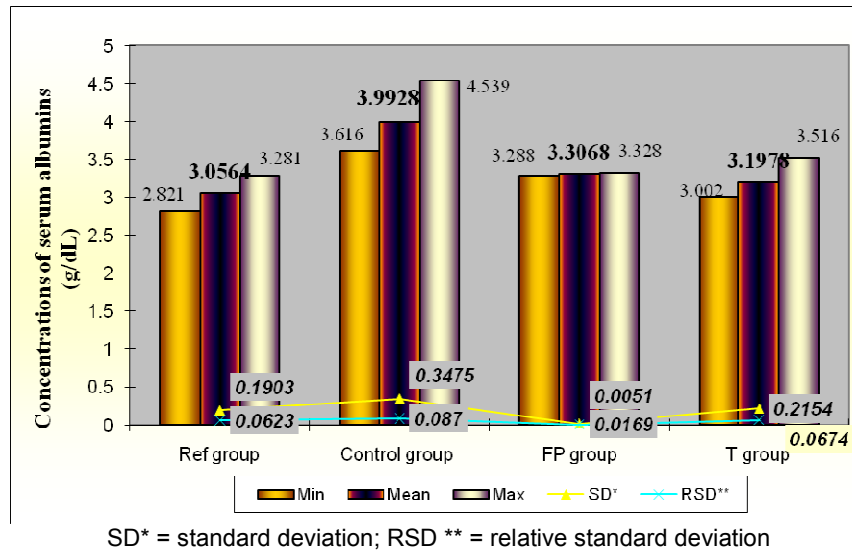


**Fig. 1** – Serum concentrations of total proteins (g/dL)

**Determination of serum albumins**

Another biochemical parameter relevant for the functional integrity of the liver is the serum concentration of albumins, the values of which are mentioned in fig. 2. The results reveal an increase of the albuminemia of the control group from  $3.0564 \pm 0.1903$  g/dL (reference group) to  $3.9928 \pm 0.3475$  g/dL. Lower values, however increased as compared to the reference group, can also be noticed in the serum of the animals fed with French fries ( $3.3068 \pm 0.0051$ g/dL) and toast ( $3.1978 \pm 0.2154$ g/dL). Such variations of the albuminemia, although they seem inconclusive for the assessment of the proteosynthetic function of the liver, may be correlated with the existence of a nephrotic syndrome.

The plasmatic level of albumin doesn't depend only on the hepatic synthesis, but also on the volume of distribution and its degradation. The synthesis of albumin is regulated by: the nutritional status, osmotic pressure, inflammatory status, and hormone balance, loss through urine or intestine (Buligescu et al., 1999; Szanto, 2004). The synthesis of albumin is stimulated by certain amino acids: tryptophan, phenylalanine, glutamine, and lysine (Buligescu et al., 1999). The albumin synthesis is also dependent on the availability of the amino acids, mainly tryptophan (Kirsch et al., 1968) and may be suppressed by the alcohol consumption and by the decrease of food protein intake (Friedman et al., 2003).

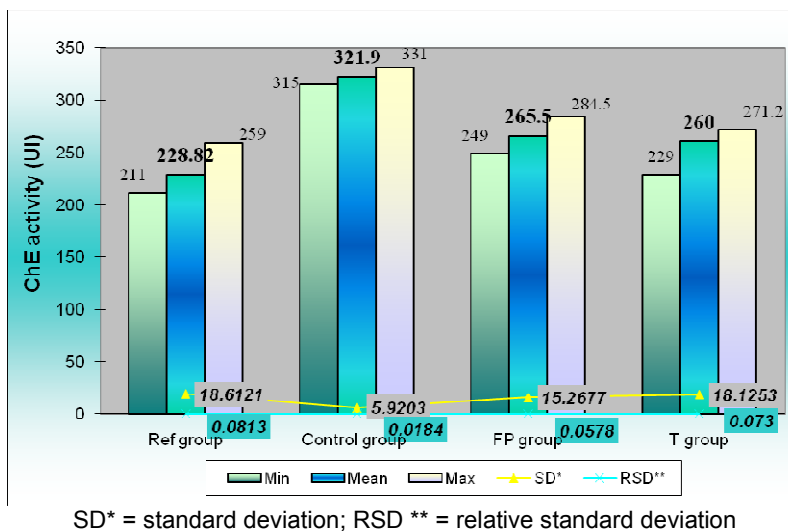


**Fig. 2 – Serum concentrations of albumins (g/dL)**

**Determination of serum cholinesterase**

Serum cholinesterase, an enzyme which is synthesized in the liver, offers accurate information in the toxicological investigations, due to its slow turnover (T1/2 is comprised between 14 and 20 days). The oscillation of this parameter (fig. 3) is surprising due to the fact that in the serum of the control group, the ChE's

activity increases from  $228.82 \pm 18.6121$  UI (the value of the reference group) to  $321.90 \pm 5.9203$  UI. The increase of ChE activity from  $228.82 \pm 18.6121$  UI up to  $265.50 \pm 5.9203$  UI (FP group), and  $260.00 \pm 18.1253$  UI (T group) does not suggest any affection of the hepatic proteosynthetic function. Even though the ChE evolution was surprising due to the increased values for all the experimental groups, this increase may be correlated to the existence of a nephrotic syndrome, a phenomenon which is present in the case of intoxication with acrylamide (Konings et al., 2003; Atanasiu et Mohora, 2004).



**Fig. 3** - Activity of the serum cholinesterase (UI)

## CONCLUSIONS

1. The study of the total proteins concentrations for the experimental groups shows a significant decrease for the control group and for the group which was fed with toast, suggesting the negative impact upon the hepatic proteosynthesis function.
2. The variation of the albuminemia of the experimental groups, although it seems inconclusive for the assessment of the proteosynthetic function of the liver, may be correlated with the existence of a nephrotic syndrome.
3. The evolution of cholinesterase was surprising due to the occurrence of increased values in the case of the animals treated with acrylamide, either as an aqueous solution, or as thermally-processed foods; this increase may not be correlated with the damage of the proteosynthetic function of the liver, however it may be correlated with the existence of a nephrotic syndrome, a phenomenon which is present in the case of intoxication with acrylamide.

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# RESEARCH ON WATER CONTENT OF JONATHAN APPLES BY CLASSICAL AND INNOVATIVE METHODS

## CERCETĂRI PRIVIND DETERMINAREA CONȚINUTULUI DE APĂ PENTRU MERELE DIN SOIUL JONATHAN PRIN METODE CLASICE ȘI INOVATIVE

TRINCĂ Lucia Carmen<sup>1</sup>, CĂPRARU Adina Mirela<sup>1</sup>, AROTĂRIȚEI D.<sup>2</sup>,  
VOLF Irina<sup>3</sup>, CĂLIN M.<sup>1</sup>, CHIRUȚĂ C.<sup>1</sup>  
e-mail: lctrinca@yahoo.com

**Abstract.** *This paper presents results on the moisture determination on the Jonathan apples by oven drying method and lyophilization. Statistical interpretation of experimental results allowed the comparison of the two methods, selection of the most convenient in terms of simplicity, speed and cost as well as achieving a hypothetical model on the percentage variation of water forms with applicability for the conservation of food substrates.*

**Key words:** water, Jonathan apples, lyophilization, FTIR spectroscopy.

**Rezumat.** *În lucrare sunt prezentate date privind determinarea umidității la merele din soiul Jonathan prin metoda uscării la etuvă și liofilizare. Interpretarea statistică a rezultatelor experimentale a permis compararea celor două metode, selectarea celei mai convenabile din punct de vedere al simplității, rapidității și a costurilor precum și realizarea unui model ipotetic privind variația procentuală a diverselor forme de apă cu aplicabilitate pentru conservarea substraturilor alimentare.*

**Cuvinte cheie:** apa, mere Jonathan, liofilizare, spectroscopie FTIR.

### INTRODUCTION

The scientific literature contains information on the likely forms of water from supply substrates.

Content of principal forms of water (or proportional ratio) provides practical information for agriculture, horticulture, biology, medicine.

Free water/bound water proportional ratio can be a useful index for assessment of physiological processes-organs specific, or for metabolic characterization of living organism (plant/ animal).

Currently scientific literature presents the characterization of water forms as a general concept level (Baucour et al., 2000, El Sayd et al., 2010) with limited focus depending on the specialization approached field (physiological, physical, chemical, biochemical, technology).

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

<sup>2</sup> "Gr T. Popa" University of Medicine and Pharmacy of Iasi, Romania

<sup>3</sup> "Gheorghe Asachi" Technical University of Iași, Romania

Development of analytical methods will provide characterization and determination of the principal forms of water from food supply with applicable results in practice (Derossi et al., 2007, Trincă, 2004).

It is accepted that by reducing the water content of fruits and vegetables it is possible to preserve them for a long time, high contents of water (90-98%) affecting their perishability.

In practice the drying processes are used to inhibit the growth of microbial flora and to limit the effects of degradation reactions (Rahman, 2006).

This paper aims to determine physico-chemical water content and to highlight the presence of chemically bound water from Jonathan apples structure by various methods and to carry out statistical correlations of experimental data.

## MATERIAL AND METHOD

In early November 2011 JONATHAN apples were purchased from the city market with weight ranging between 81-103 g and normal, healthy appearance.

Periodic measurements for various types of water monitoring were carried out for 60 apples (by considering the lot of ten apples stored under the same conditions of temperature and humidity).

Determination of free water was performed for twenty days by weighing (every five days) the apples, the difference in mass being attributed to the percentage of free water. Drying of the samples was carried out by two methods: the oven drying and lyophilization drying.

For oven drying 5 g sample of Jonathan apple (cutted into pieces, minced, and pressed to get the juice) have been subjected to drying at 90 °C temperature until constant mass ( $\Delta < 10^{-2}$  g).

Water content was determined according formula:

$$\text{H}_2\text{O} \% = \frac{m - m_1}{m_2} \times 100$$

$m$  = mass dish + mass sample before drying,  $m_1$  = mass dish + mass sample after drying,  $m_2$  = mass of the sample.

Lyophilisation is a process of dehydration by cold : water freezes faster than other components and is removed in the form of ice, without changing the structure of the food substrate.

Lyophilisation has occurred with Freeze Dryer ALPHA type 1-4 LD plus. 5 g sample of apple (cutted into pieces, minced, and pressed to get the juice) were subjected to freeze-dryer drying at a temperature of - 50 -60 C°, and 0.02-0.03 mbar pressure until constant mass.

Spectral analysis by FTIR spectroscopy highlights functional changes of the structure sample substrate in relation to the standard.

FTIR spectra were recorded in KBr pill using DIGILAB-EXCALIBUR SDS 2000 spectrometer fitted with a heating device.

Working parameters were: spectral range between 4000-400  $\text{cm}^{-1}$ , resolution 4  $\text{cm}^{-1}$  and the number of scans 24.

By MATLAB function the correlation coefficients have been identified for apples samples (day five and day twenty) for free water, physico-chemical water (determined

by drying in the oven and lyophilization for the Apple (cutted into pieces, minced, and pressed to get the juice).

For the statistical analysis of the recorded differences it was applied the method of analysis report variances ( $X$ ) and Pearson correlation coefficient, determination gradient has been calculated (by considering the case  $p < 0.05$  statistically significant). Statistical evaluation was performed using SYSTAT 13 (SYSTAT SOFTWARE, Inc. CHICAGO).

## RESULTS AND DISCUSSION

Our study provide that drying methods can be used in the determination of water content.

*Table 1*

**Free water content (%) dynamics variation during experimental period**

| Parameter   | Day five    | Day ten      | Day fifteen | Day twenty  |
|-------------|-------------|--------------|-------------|-------------|
| MEAN±ST DEV | 1.423±0.148 | 2.878± 0.546 | 3.671±0.668 | 5.363±0.944 |
| MINIMUM     | 1.199       | 2.051        | 2.833       | 4.234       |
| MAXIMUM     | 1.565       | 3.442        | 4.420       | 6.427       |
| VARIANCE    | 0.110       | 0.435        | 0.556       | 0.792       |

Free water content increased statistically insignificant on the day twenty compared to day five, which reveals a moderate loss of water in the food substrate for Jonathan apples variety compared to other varieties (Vesali et al., 2011, Gradinariu et al., 2002).

*Table 2*

**Dynamic variation of physico-chemical water content (%) determined by lyophilization during experimental period**

| Parameter   | Day five     | Day ten      | Day fifteen  | Day twenty   |
|-------------|--------------|--------------|--------------|--------------|
| MEAN±ST DEV | 78.340±3.248 | 78.025±1.211 | 77.923±0.960 | 77.528±1.185 |
| MINIMUM     | 75.432       | 77.020       | 77.202       | 75.457       |
| MAXIMUM     | 83.854       | 79.888       | 79.216       | 78.340       |
| VARIANCE    | 10.551       | 1.467        | 0.921        | 1.404        |

Water percentage of samples was statistically significant when compared drying by oven to drying by freeze.

Physico-chemical water content determined by lyophilization decreased by 1.04 % on day twenty compared to day five, the same sense of variation (1.17 %) being registered for the juice resulted by pressed apples.

Statistical processing highlighte a positive correlation between physico-chemical water-bound determined by lyophilization and physico-chemical water-bound determined by drying in oven the juice resulted by pressing the apples ( $r^2 = 0.757$ ).

Statistical processing of individual results for each of the ten apple revealed linear correlations for day twenty between free water and physico-chemical water determined by lyophilization ( $r^2 = 0.376$ ).

Table 3

**Dynamics of physico-chemical water-bound variation content (%) determined by drying in oven the juice resulted by pressing the apples**

| Parameter   | Day five      | Day ten       | Day fifteen  | Day twenty   |
|-------------|---------------|---------------|--------------|--------------|
| MEAN±ST DEV | 80.424 ±1.982 | 80.262 ±0.484 | 80.209±2.324 | 79.482±1.366 |
| MINIMUM     | 77.998        | 79.543        | 76.556       | 77.349       |
| MAXIMUM     | 83.372        | 80.655        | 82.965       | 80.871       |
| VARIANCE    | 3.931         | 0.234         | 5.403        | 1.866        |

Statistical processing of individual results for each of the 10 apple revealed for twenty day linear correlations between free water and to physico-chemical water determined by drying in the oven the juice resulted by pressing the apples ( $r^2 = 0.280$ ).

Statistical analysis of the results showed through statistical modelling interrelationships between the two forms of water investigated.

Table 4

**Dynamic variation of physico-chemical water content (%) determined by drying apple pieces in the oven**

| Parameter   | Day five      | Day ten       | Day fifteen  | Day twenty   |
|-------------|---------------|---------------|--------------|--------------|
| MEAN±ST DEV | 79.801 ±0.587 | 79.543 ±1.699 | 77.690±3.885 | 77.193±2.848 |
| MINIMUM     | 78.925        | 76.865        | 71.048       | 73.166       |
| MAXIMUM     | 80.578        | 80.655        | 80.580       | 81.208       |
| VARIANCE    | 0.345         | 2.887         | 15.097       | 8.116        |

Physico-chemical water content determined by drying minced apple in the oven decreased by 0.01 % on day five, and by 0.47 % on day twenty.

In day five hydration state of the food substrate ensured a good resistance of cellular walls compared to day twenty, in which the significantly elimination of free increased the amount of physico-chemical water pool (table 3).

In the same time the dehydration process stressed the destruction of cellular walls and release of intracellular water.

Table 5

**Dynamic variation of physico-chemical content (%) determined by drying minced apple in the oven**

| Parameter   | Day five      | Day ten       | Day fifteen  | Day twenty   |
|-------------|---------------|---------------|--------------|--------------|
| MEAN±ST DEV | 79.802 ±5.106 | 79.384 ±1.035 | 78.925±1.612 | 77.666±2.379 |
| MINIMUM     | 71.998        | 77.693        | 77.075       | 76.105       |
| MAXIMUM     | 86.264        | 80.274        | 80.133       | 81.653       |
| VARIANCE    | 2.608         | 1.073         | 2.599        | 5.662        |

Statistical processing of the results for the apple pieces and minced for day five does not revealed a linear correlation between the individual parameters.

This fact can be explained by consideration of crushing as a process of cellular destruction not so easy to quantify in terms of intracellular water released.

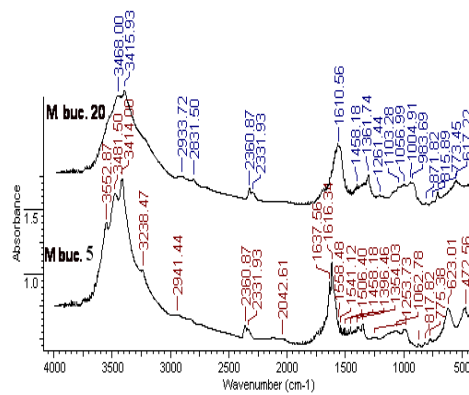
In the case of minced apple it was produced the destruction of cellular walls (process largely difficult to quantify) which caused the release of intracellular water.

Appropriate analyses of experimental data of twenty day highlighted positive correlation ( $r^2 = 0.354$ ) between physico-chemical water of apple pieces compared to minced which sustains the hypothesis that increased dehydration produce interconversion water forms.

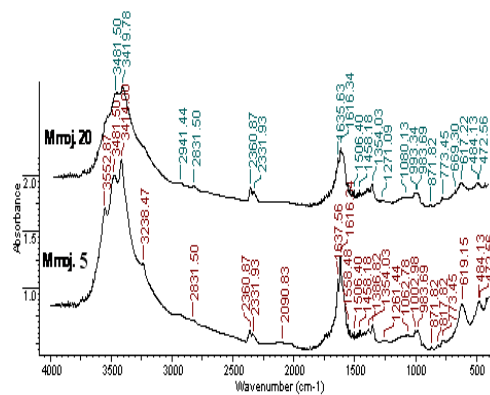
Comparative analysis of methods used in this trial highlighted the advantage of accessibility for drying oven method while freeze-drying method provides better results by cancelling the potential errors of drying oven such as evaporation of volatile compounds or destruction of the walls of the food substrate.

### Determination of chemical water related by FTIR spectroscopy

In figures 1 and 2 are presented FTIR spectra for apples (pieces and minced) in day five and day twenty.



**Fig. 1** - FT-IR Spectra of apple pieces samples in day five and day twenty



**Fig. 2** - FT-IR Spectra of minced apple samples in day five and day twenty

The corresponding signals of water in the food substrate are given by symmetric and asymmetric bands of excitation of phenolic groups -OH present in the area  $3552-3540\text{ cm}^{-1}$ . These signals are specific to chemical bound water from the substrate structure. Thus, from these spectra can be notice that intense signals appears in the area just in the case of the samples analysed in day five. Spectra recorded for samples of day twenty day presented low intensity signals in this area maybe because of the existence of a smaller percentage of chemical bound

water in raw samples. In this case the signals appear only in the area of 3480-3420  $\text{cm}^{-1}$  both for apple pieces and minced samples.

## CONCLUSIONS

1. Determination of apple moisture samples revealed that drying by lyophilization ensures a high content of physico-chemical water because this method does not cause the destruction of the substrate.

2. FTIR analyses confirmed the presence of chemical bound water (specific signals for are powerful in day five while in day twenty can be traced their absence in certain areas of the absorption).

3. Statistical data sustain for day five linear correlations only between free water and physico-chemical water determined by drying in oven of apples pieces fact that can be explained by corresponding hydration state in day five.

4. In day twenty the free water content is related to physico-chemical water-bound (both for apple pieces, minced apple and juice resulted from pressing the apples samples) determined by the oven drying and lyophilization.

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# PYRROLOPYRIDAZINE DERIVATIVES: SYNTHESIS AND FLUORESCENT PROPRIETIES

## DERIVAȚI PIROLOPIRIDAZINICI: SINTEZĂ ȘI STUDIUL PROPRIETĂȚILOR FLUORESCENTE

TUCALIUC Roxana<sup>1</sup>, COTEA V. V.<sup>1</sup>, NICULAUA .M<sup>2</sup>, MANGALAGIU I.<sup>3</sup>

e-mail: roxanatulaliuc@yahoo.com

**Abstract.** Recent studies proved that 1,2-diazines derivatives are invaluable materials in the fields of medicine (such as anti-HIV, antiviral and anticancer, antibacterial and antifungus medicines), opto-electronics (compounds with liquid crystal properties and highly fluorescent derivatives: sensors and biosensors, electroluminescent materials, lasers) and agriculture (herbicidal activity and the grow up factor for plants). 1,3-Dipolar cycloaddition is one the most important methods of constructing the pyrrolopyridazine. For some pyrrolopyridazine derivatives was studied the absorption and emission spectra, in ethanol, chloroform and cyclohexane solutions at room temperature.

**Key words:** pyrolopiridazine derivatives, fluorescence, 3+2 dipolar cycloadditions.

**Rezumat.** Studii recente au demonstrat faptul că derivații 1,2-diazinici sunt compuși cu proprietăți deosebite în medicină (cum ar fi anti-HIV, medicamente antivirale și împotriva cancerului, proprietăți antibacteriene și antifungice), proprietăți opto-electronice (compuși cu proprietăți de cristale lichide și produse derivate foarte fluorescente: senzori și biosenzori materiale electroluminiscente, lasere) și în agricultură (compuși cu activitate erbicidă și stimulatori în creșterea și dezvoltarea plantelor). Reacțiile de cicloadiție 1,3-dipolare sunt cea mai accesibilă metodă în sinteza derivaților pirolpiridazinici. Spectre de absorbție și emisie au fost înregistrate, pentru diferiți derivați piridazinici, în etanol, cloroform și ciclohexan la temperatura camerei.

**Cuvinte cheie:** derivați pirolpiridazinici, fluorescență, cicloadiții 3+2 dipolare.

### INTRODUCTION

Literature describes a large variety of pyridazine compounds with different biological activities: anticancer, antituberculosis, antimicrobial, antihypertensive etc (Mangalagiu, 2011).

1,2-diazines are also reviewed for their applications in opto-electronics, with a focus on highly fluorescent derivatives (with potential as sensors and biosensors, electroluminescent materials, lasers and other semiconductor devices) and compounds with liquid crystal properties. Herbicidal activity and grow up factor for plants are also reviewed (Mitsumori et al., 2005; Valeur, 2002).

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

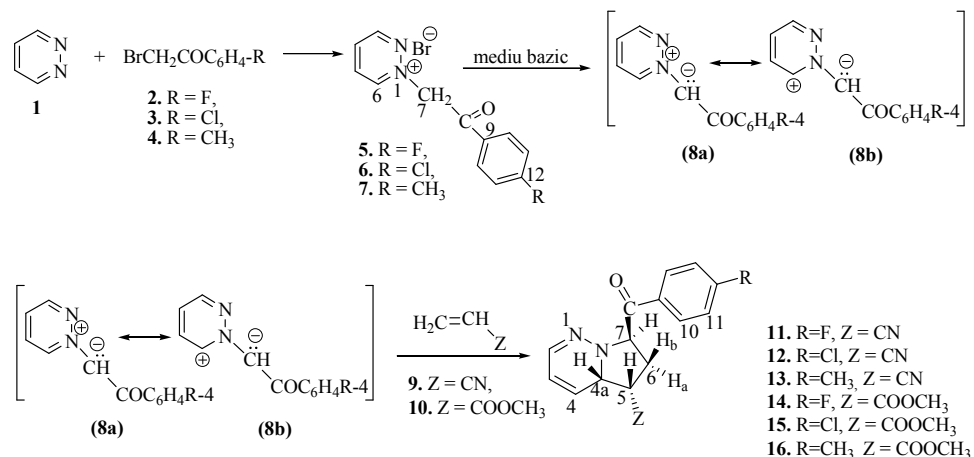
<sup>2</sup>Oenological Research Center – Romanian Academy, Iași, Romania

<sup>3</sup>“Alexandru Ioan Cuza” University of Iasi, Romania

In a preliminary communication (Zbancioc et al., 2006) is presented the synthesis and spectral analysis of pyrrolopyridazine derivatives. The aim of this work was to study the relationship between optical properties and structure (the effect of substituents and conjugation).

## MATERIAL AND METHOD

The strategies adopted for construction of fluorescent derivatives, are depicted in figure 1. The preparation of all derivatives (**11-16**) involves two steps: initially N-alkylation of the pyridazine, followed by a 3 +2 dipolar cycloaddition of diazinium ylides (**8a-8b**) (generated *in situ* from the corresponding salts) to the corresponding dipolarophiles (activated alkenes nonsymmetrical substituted: **9** and **10** - acrylonitrile and methyl propiolate).



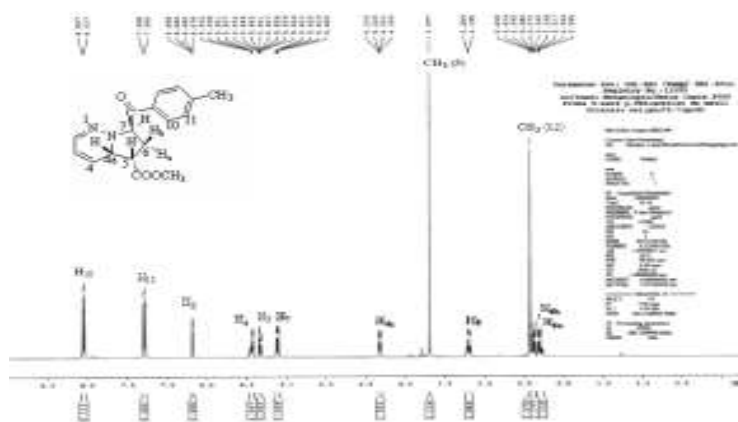
**Fig. 1** - Synthesis of pyridazine derivatives.

The 3+2 cycloaddition occurs with high stereospecificity and no formation of other isomers was observed (Butnariu et al., 2009).

All reagents and solvents employed were of the best grade available and were used without further purification.

The structure of the compounds was proved by spectral analysis: the <sup>1</sup>H NMR and <sup>13</sup>C NMR spectra and two-dimensional experiments 2D-COSY, 2D-HETCOR(HMQC), long range 2D-HETCOR (HMBC) were recorded on a Bruker Avance 400 DRX spectrometer at 400/100 MHz. Chemical shifts are given in parts per million (δ-scale), coupling constants (J) in hertz and downfield shift from internal tetramethylsilane (δ 0.00 ppm). The IR spectra were recorded on an FT-IR Shimadzu Prestige 8400s spectrophotometer in KBr. Melting points were determined using an electrothermal apparatus and are uncorrected. Flash chromatography was performed with Aldrich 230e400 mesh silica gel. TLC was carried out on Merck silica gel 60-F-254 plates.





**Fig. 2** - <sup>1</sup>H-NMR spectrum for compound **16**.

In the next stage of our work, we studied the absorption and emission spectra of the obtained compounds. The spectra of all the compounds were recorded in ethanol, chloroform and cyclohexane solutions at room temperature.

The fluorescence spectra were recorded with a Turner Bio Systems fluorimeter using FluoOpticalKitID PN: 9300-043 SN: F2000000BB5A4C2D SIG: UV with  $\lambda_{\text{ex}} = 365$  nm and  $\lambda_{\text{em}} = 410\text{--}460$  nm.

Relative quantum yields were determined by using anthracene in ethanol ( $\Phi = 0,27$  at 25° C) (Parker C. A., 1986). Although, compounds are relatively similar in molecular structure, exhibit clear differences in their experimental absorption and emission spectra, as summarised in table 1.

*Table 1*

**$\lambda_{\text{max}}$  (nm) of absorption spectra and relative quantum yields (%) of piridazine derivatives (11-16)**

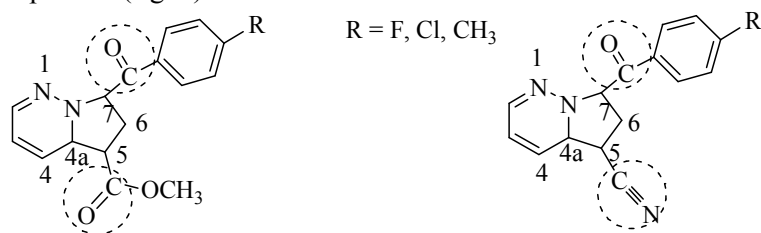
| Comp.     | Fluorescence ( $\lambda_{\text{max}}$ , nm)<br>(quantum yield %) |           |            | Absorption ( $\lambda_{\text{max}}$ , nm) |           |            |
|-----------|--|-----------|------------|---|-----------|------------|
|           | Etanol   | Cloroform | Ciclohexan | Etanol                                    | Cloroform | Ciclohexan |
| <b>11</b> | 425 (3)  | 421 (2)   | Insolubile | 320                                       | 318       | Insolubile |
| <b>12</b> | 423 (2)  | 420 (2)   | Insolubile | 320                                       | 319       | Insolubile |
| <b>13</b> | 438 (2)  | 429 (2)   | Insolubile | 323                                       | 320       | Insolubile |
| <b>14</b> | 456 (3)  | 453 (3)   | Insolubile | 335                                       | 330       | Insolubile |
| <b>15</b> | 458(4)   | 455 (3)   | Insolubile | 336                                       | 330       | Insolubile |
| <b>16</b> | 461(4)   | 459 (4)   | Insolubile | 338                                       | 335       | Insolubile |

## RESULTS AND DISCUSSIONS

As shown in Table 1, the compounds (**11-16**) are blue emitters ( $\lambda_{\text{max}}$  of fluorescence around 423-461 nm,  $\lambda_{\text{max}}$  of absorption around 320-338 nm) and have low quantum yield.

The comparative analysis of the obtained data (table 1) leads to the conclusion: the compounds (**14-16**) which possess a carbomethoxy group, in 5-position, have a higher fluorescence.

The effect of conjugation and the presence of double bonds in azaheterocycles compounds determine fluorescence and quantum yields of the analyzed compounds (fig. 3).



**Fig. 3** – The detailed structure for compounds **14-16**.

If pyrroloderivatides were fully aromatized, then the quantum yield was extremely high (Zbancioc et al., 2010).

## CONCLUSIONS

1. We report a fast, efficient and straightforward method for preparation of fluorescent derivatives containing the pyridazine ring.
2. The compounds obtained and tested possess fluorescent properties ( $\lambda_{\text{max}}$  of fluorescence is around 423-461 nm,  $\lambda_{\text{max}}$  of absorption is around 320-338 nm).
3. A certain influence of the substituents concerning absorption and fluorescent properties were observed: the substituent from the position 5 being important for fluorescence.

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# BILINGUALISM AND FAMILY LANGUAGE LEARNING

## BILINGVISMUL ȘI ÎNVĂȚAREA UNEI LIMBI ÎN FAMILIE

**GHEORGHIU Irina<sup>1</sup>, GHEORGHIU Elza<sup>2</sup>, COLIBABA Șt.<sup>3</sup>,  
GARDIKIOTIS Rodica<sup>4</sup>**

e-mail: irina\_gheorghiu16@yahoo.com

**Abstract.** *This article aims at describing an innovative educational model based on family language learning as applied in Romania by EuroEd Foundation, Iasi, inside a European funded project under the Lifelong Learning Programme: BILFAM (Bilingual Families). The model also gives bilingualism another dimension, which is in tune with EU main objective because it best serves work mobility. Scientific literature has found out that foreign language learning before the age of 8 in the context of informal education (learning by doing) can develop native speaker competence. Moreover, research on the Narrative Format methodology (done by the psycholinguistic faculty from the Sapiientia University in Rome) has shown that the affective relationship between the adult as model and children has a great contribution to the foreign language learning process. Grand/parents with no or little foreign language competences are involved in their children's foreign language acquisition, being provided with necessary tools and strategies. The process, showing a dramatic impact on everybody's foreign language acquisition (children and adults), works well due to the fact that grand/parents spend a lot of time with their children and can follow their development throughout the years, offering them input. They will also feel encouraged to learn the language they are teaching their children. The family foreign language learning will also create a positive attitude towards multilingualism, creating better contextual conditions for multilingual education.*

**Key words:** family language learning, bilingualism, the Narrative Format

**Rezumat.** *Acest articol își propune să descrie un model educațional inovator care se bazează pe învățarea în familie și care e aplicat în România de Fundația EuroEd Iași, prin proiectul BILFAM (Bilingual Families), finanțat de către Uniunea Europeană în cadrul programului Învățare pe tot parcursul vieții. Modelul propune o nouă dimensiune a bilingvismului, care se aliniază cu un obiectiv major al Uniunii Europene pentru că servește mobilitatea forței de muncă. Literatura științifică a subliniat că învățarea unei limbi străine înainte de vârsta de 8 ani în contextul unei învățări informale poate dezvolta competențe native. Mai mult, cercetarea, care s-a bazat pe metoda Formatului Narativ (propusă de Facultatea de psiholingvistică a Universității Sapiientia Roma), a arătat importanța legăturii afective dintre adulți ca model și copii în procesul de învățare a unei limbi străine. Bunicii și părinții fără sau cu foarte reduse cunoștințe de limbă au fost implicați în acest proces dându-li-se instrumentele și strategiile necesare. Procesul, care a evidențiat schimbări*

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<sup>1</sup> "Albert Ludwigs" Freiburg University, Germany

<sup>2</sup> "Vasile Alecsandri" Highschool Iasi, Romania

<sup>3</sup> „Alexandru Ioan Cuza" University of Iași, Romania

<sup>4</sup> "Grigore T. Popa" University of Medicine and Pharmacy of Iasi, Romania

*importante pentru toți participanții (copii și adulți), funcționează bine datorită faptului că bunicii și părinții petrec mult timp împreună cu copiii, le sunt alături de-a lungul anilor și pot să-i ajute ori de câte ori e nevoie. La rândul lor adulții se simt încurajați să învețe limba pe care o predau copiilor lor. Învățarea în familie va promova o atitudine pozitivă față de plurilingvism, creând condiții contextuale mai bune pentru o educație plurilingvistă.*

**Cuvinte cheie:** învățarea limbilor străine în familie, bilingvism, formatul narativ

## INTRODUCTION

The project BILFAM is based on the educational model of intergenerational learning. It provides a new approach and resources to grandparents and parents with no or little foreign language competences for their direct involvement in their children's foreign language acquisition. Grandparents and parents can leverage the emotional bond to make learning fun and engaging, creating the conditions for a really enriching learning experience. There is a strong desire among parents to help their children learn a foreign language (Pirchio, 2011) as they are well aware of the instrumental role it may play in the future of the child but they lack the confidence and tools to do so. By providing the adults with necessary tools and strategies and exploiting the affective relationships (Taeschner, Traute et al. 2004) existing within the families the project will encourage grandparents and parents to learn the language they are teaching their children.

There have been 25 families per country (Italy, The UK, Slovakia, Spain and Romania) involved in the project. Each family is made up of at least one parent or grandparent and one or more children of three or six years old. At an international level the group is made up of 500 parents and 500 children.

In Romania the group has chosen English and German as the target languages. The adults had a language test at the beginning of the project which showed a low level of knowledge in the foreign language. The adults were selected due to their interest in their children's linguistic performance and their own interest in language acquisition, which is considered to be essential in their future. The families are from different walks of life but all of them have basic computer skills, have a computer and internet connection and are at least high school graduates.

## MATERIAL AND METHOD

The Narrative Format methodology, the theoretical basis of the project, relies on the natural process of acquiring one or two languages within the family context. This method has been designed by Prof Traute Taeschner of the Università Sapienza in Rome and has shown that the use of gestures, intonation, facial expressions and the affective relationship between adults and pupils is of great contribution to the foreign language learning process (Taeschner, Traute & al. 2004- p 4). It involves a series of educational strategies, activities and materials whose main aim is to recreate the conditions a child has when learning his mother tongue. Families are provided with the necessary tools and materials to make learning efficient. In its attempt to facilitate

learning the Narrative Format also facilitates good communication between the participants.

There are three key concepts at the basis of this method:

A. *Learning a foreign language should follow a similar pattern to acquiring a first language.* The child learns his mother tongue by sharing repeated experiences with the adults around them. The formats, the repeated experiences are therefore real-life situations that both parent and child share every day in a very emotional context which favours the development of non-verbal and verbal communication.

B. *Using interactive teaching/learning techniques, which follow the natural language acquisition processes.* The principle of the method stipulates that the best incentive for communication and learning lies in the emotional relationship between the child and the adult. The child starts to speak and wants to speak because he wants to communicate with the person with whom he has established an emotional relationship. This happens in the first language and by analogy can happen in the second language too.

C. *Communicative skills are at the basis for the teaching/ learning of the new language.*

The project also sheds new light upon bilingualism, which is considered to be common world wide. The human being is able to learn more than one language; it is also known that children learn languages as easily as they learn to walk. The best period in one's life for learning languages would therefore be childhood when our brain is flexible. Children can become bilingual if they are given enough input in the language and they have enough fun. Bilingual children can recognise their two languages and differentiate them from a very young age.

Bilingualism brings cognitive advantages:

- bilingual children have an early awareness and knowledge of the words, structures and sounds of their languages;
- bilingual children often learn to read earlier than others;
- bilingual children are better at learning other languages;
- bilingual children are better at switching tasks;
- bilingual children are better able to focus attention.

The disadvantages of bilingualism are outnumbered by its advantages. Some bilingual children start to speak a bit later than children who have learned one language. Bilingual children may initially have a reduced vocabulary in each of their languages, although their total vocabulary may be larger than the vocabulary of a monolingual child. Bilingual children sometimes mix both languages together (it often occurs when the child knows that s/he will be understood by the speaker because s/he is also bilingual (Pirchio, S., Taeschner, T., Sorace, A., Francese, G., Passiatore, Y. 2011- p 3).

There are usually two types of bilingual families: the family speak and understand a language and live in a society where another language is spoken and understood; some members of the family speak and understand a language and other members of the family speak and understand another language.

In both cases the child's language learning is the responsibility of the family. There are also a few schools though which encourage bilingualism. The objective of our project is this: for the first time, parents will be given accredited tools to teach their children two languages at home and with friends and by doing so the family will become a bilingual family. Being bilingual does not necessarily mean having perfect knowledge of two languages. It means being able to communicate in two languages even at different levels of competence. Children who learn two languages from birth or who learn them in succession when they already speak one language are bilingual.

Adults who learn a foreign language well are also bilingual. A bilingual family is therefore one that has daily experiences in two languages, which is exactly what our project suggests.

## RESULTS AND DISCUSSIONS

### **Face-to-face meetings and online tutorials**

The first face-to-face meetings with the parents concentrated on familiarising them with the project: its method, objectives, principles, innovative teaching strategies, activities and materials. This project provides families with a model of language teaching and learning called “the Narrative Format”, designed by Prof Traute Taeschner of the Università Sapienza in Rome. This method has already been tried out with great success at Nursery level, and at both Pre- and Primary School levels.

The presentations also highlighted the adult’s great influence on the children’s language development and the new role that they will have: making learning possible and successful. Some psychological aspects of the foreign language learning process as well as of bilingualism were also underlined. Theoretical aspects go hand in hand with practical issues: parents gain hands on experience in how to make a video, how to upload it on the platform or how to make a Voki.

These meetings were followed by online tutorials via a web site by which parents and tutors communicate. Parents have access to a forum and activities to be carried out with their children at home, downloadable materials, instructions, *how-to* advice and the support of a dedicated tutor. The project encourages exchanges of ideas on the site among parents and their tutors with a view to creating a community. The site becomes an excellent means of communication by which everybody can share ideas or find support and help.

**The acting out of stories** with the support of gestures and facial expressions encourages language learning through active engagement. The story is experienced with actions, gestures, facial expressions, and words. The adult and the child act various characters together at the same time. Children feel safe as long as their emotional communication with the adult remains intact and strong. The parent’s task is therefore to maintain the quality of the emotional communication with the children through eye contact, facial expressions, through warm and open body language and through gestures. Repetition also helps children understand the story. There should be no need for translation or explanations. There is a wide range of variety when it comes to repetition itself, as role playing and acting out are strengthened by the illustrated books and mini-musicals.

**Singing the song** while using gestures creates a kind of mini-musical which allows the child to listen to the story again and experience the story in the foreign language. This new experience via singing reinforces learning the new language through music and rhythm.

**The animated cartoons of the adventures of Hocus and Lotus** make use of graphic and animation techniques and introduces other language learning principles such as the temporal sequencing of events and linguistic progression.

**The e-books** of the adventures of Hocus and Lotus provide another way to listen to the stories accompanied by pictures. The e-books reinforce the vocabulary that the children have started to learn.

The Hocus and Lotus **Puppet theatre** engages children to participate in different ways according to their particular stage of development. It offers a challenging opportunity to repeat the story.

**Voki activity** challenges children to create their own avatar (with an electronic or personal voice), which can be sent to a friend or to another family in the project that is learning the same language, or it can be uploaded onto the project website.

The families can produce **a new Hocus and Lotus story**. Children are asked to organise pictures in sequence to tell a new story, by using the vocabulary and grammatical structures of the language they know creatively.

**The dinogame of the goose** is one of the best known table games, which invariably engages both adults and little ones around the table. The game challenges children to use not only their language knowledge but also their knowledge of the characters and the stories.

Spending time together and playing with Hocus and Lotus in English, French, German, Italian or Spanish, daily or several times a week, has many positive effects on both children and adults. It leads to:

- good communication and excellent family relationships; both children and adults spend quality time together which enhances their relationship: they share a wide range of happy and positive experiences together in a new language;
- high motivation: the adult feels satisfied with his child who is learning a new language which is taught by him/her;
- happy and positive memories: the child will thus gain a series of social and affective reference points within the context of shared play;
- an increase in self-esteem: children and adults learn together.

## CONCLUSIONS

The project has a direct impact on the participating families: they spend much time together speaking a foreign language learnt together. The project encourages better communication as well as closer relationships between game partners and community generally. The adult feels satisfied because he learns together with the child and motivated to act as wished.

The project provides an innovative solution as regards bilingualism. For the first time parents are given a valid instrument to facilitate their children's acquisition of two languages.

The project also motivates the learning of a foreign language by adults and children, encouraging learning which involves different generations within a family. This objective is achieved by adapting the Narrative Format method, the

Hocus and Lotus materials, to the context of the family. These materials provide a very creative and varied support and background for foreign language learning activities (video, songs, printed material, DVDs).

The model also gives bilingualism another dimension, which is in tune with EU main objective because it best serves work mobility (Commission of the European Communities, 1995).

The project aims at two different target groups, which nonetheless have many things in common: children, who are at a critical time of language learning, and their parents, who need a good motivation to learn a foreign language, very often for instrumental reasons regarding work mobility, and who find it in their wish to support their children during the process of learning and practising a foreign language.

The collected data about their attitudes and behaviour as well as the results obtained in the learning process will constitute a measure of the project's impact on the families. Apart from this direct impact we expect changes in the social context through the dissemination activities suggested by the project.

The piloting of this model is used by the Psycholinguistics Department of the University of Rome as an example of the way bilingualism works not only as a spontaneous but also as a guided phenomenon. The model underlines the role of emotions and feelings in learning foreign languages both at a young age and by adults, as well as the role of motivation in learning foreign languages. The study highlights the effect of intergenerational learning and of the consolidation of learning through teaching and practice, the role of the narrative format and of play in learning. The prototype is classic but its application in the field of languages and particularly the implications of learning on both categories makes this model a remarkable and transferable one.

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# IMPROVING THE HUMAN RESOURCES QUALITY WITHIN APIA SUCEAVA

## ÎMBUNĂTĂȚIREA CALITĂȚII RESURSELOR UMANE DIN CADRUL APIA SUCEAVA

**BREZULEANU S.<sup>1</sup>, UNGUREANU G.<sup>1</sup>,  
BREZULEANU Carmen Olguța<sup>1</sup>, IGNAT Gabriela<sup>1</sup>**  
e-mail: stejarel@uaiasi.ro

**Abstract.** For any successful organization, human resources is the kernel around which revolve all other resources (material, financial, information) and issues concerning the recruitment, selection, training, development, evaluation, reward and motivation are the main directions of research of human resource management of any organization. Implementing an efficient management in the system of public administration acquires major values, since this will lead to professionalizing the developed activities in the public administration authorities, at changing the values and way of action of the public personnel, at developing some managerial conceptions characteristic of modern public administration. In this context in the paper was made an ample analysis in integrity way of the human resources management, as a result of which the problems have been highlighted and made concrete proposals, reasons for implementing and development of this in the public service. The financial aid granted to the farmers under the form of the unique payment scheme, both under the form of the historic model and of the regionalized one, is based in a greater or less measure on a past reference period (in present established to be between 2007 – 2013). Therefore is intended the update of this to take into account the changes produced meantime in the European agricultural landscape. For the new member states which applies the unique payment scheme on surface, (including Romania) is foreseen the possibility to apply this simplified system and after 2013.

**Key words:** improving, quality, management, human resources analysis

**Rezumat.** Pentru orice organizație de succes, resursele umane reprezintă nucleul în jurul căroră gravitează toate celelalte resurse (materiale, financiare, informaționale), iar problemele privind recrutarea, selecția, instruirea, perfecționarea, evaluarea, recompensarea și motivarea constituie principalele direcții de cercetare a managementului resurselor umane a oricărei organizații. Implementarea unui management eficient în sistemul administrației publice capătă valori majore, deoarece acest lucru va duce la profesionalizarea activităților desfășurate în autoritățile administrației publice, la schimbarea valorilor și modul de acțiune a personalului public, la dezvoltarea unor concepții manageriale moderne caracteristice administrației publice. În acest context, în lucrare a fost făcută o analiză amplă, în mod integritatea managementului resurselor umane, ca urmare a problemelor care au fost evidențiate și a făcut propuneri concrete, motivele pentru care punerea în aplicare și dezvoltarea de acest lucru în serviciul public. Ajutorul financiar

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

*acordat fermierilor sub forma de schema de plată unică, atât sub forma de modelul istoric și a regionalizat o, se bazează într-o măsură mai mare sau mai puțin pe o perioadă de referință trecut (în prezent stabilit să fie între 2007 - 2012). Prin urmare, va urma o actualizare a acesteia pentru a lua în considerare schimbările produse între timp, în peisajul agricol european. Pentru noile state membre care aplică schema de plată unică pe suprafață, (inclusiv România), se prevede posibilitatea de a aplica acest sistem simplificat și după 2013.*

**Cuvinte cheie:** punerea în aplicare, management, resurse umane, de analiză

## INTRODUCTION

**The purpose** of this paper is to highlight specific human resource management in public administration, its role in improving public administration activities in Romania and strategic directions basic foundation of human resource management in public administration.

To achieve its purpose the following objectives were established: the theoretical foundations of human resource management in the APIA; determination of specific human resource management in public administration, human resources management strategy in the APIA and proposals to improve the efficiency of public administration in Romania.

## MATERIAL AND METHOD

The research methodology was based on a systemic approach and use of different methods, the most common being: normative, statistical, graphical and dynamic. They also used other research methods, such as economic analysis, comparison, induction and deduction, and other methods and tools of scientific knowledge of the socio-economic processes.

The information base consists of information gathered from Local Centre Falticeni APIA and APIA sources Suceava, existing literature on human resource management strategy for 2011-2013.

## RESULTS AND DISCUSSIONS

**Payments and Intervention Agency for Agriculture (APIA)** is a public institution with legal personality, belonging to the Ministry of Agriculture and Rural Development, financed entirely by the state budget. APIA is the body that runs European funds by implementing support measures for farmers, financed both by the EU budget through the European Agricultural Guarantee Fund and the Romanian state budget, the amounts allocated annually by budget law.

The administrative structure of APIA provides separation of functions of authorization, execution and accounting. Each of these functions lies in the responsibility of separate administrative sections highlighted in agency organization and functions are specified in the rules of organization and functioning.

In the newly created context, the Romanian farmer has to adapt to current global economic reality, to take technical and economic methods to give him

stability and secure economic efficiency, while being forced to produce in line with the European market (Brezuleanu et. al. 2011).

The case study was conducted at City Center APIA Fălticeni, a center of special importance to the local economy, due to many farmers subordinated and high number of employees.

The Head Office-APIA Suceava, CL-Fălticeni is in charge of 20 employees, is responsible for achieving quality and deadlines assigned work center that coordinates local, authorized pay within the legal powers granted and responsible for processing applications and complaints of citizens and solves the specialized field of activity that is coordinating.

In human resource management, the head office prepares the monthly timesheet, the overtime work and is responsible for training subordinates.

Of the 20 employees at a number of six graduated from animal husbandry engineering and 5 from agricultural studies. This is explained by the fact that the APIA has something specific activity in this field agronomists and engineers working specialists in animal husbandry (tab. 1).

Table 1

Professional training of employees of CL-APIA Falticeni

| Specialization                  | Higher education      |                   |            |                 |         | Secondary education |
|---------------------------------|-----------------------|-------------------|------------|-----------------|---------|---------------------|
|                                 | Animal husbandry eng. | Agricultural eng. | Economists | Mechanical eng. | Lawyers | Technicians         |
| Employment in the category (N): | 6                     | 5                 | 2          | 2               | 4       | 1                   |

In the structure of staff with higher education an important share is held by the engineers (75%), while economists share is about 30% (Fig 1).

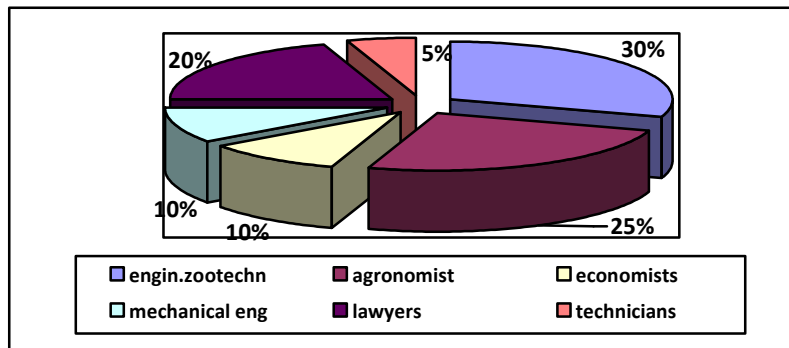


Fig. 1 - Structure of higher education staff at CL APIA Falticeni

**Structure of employees according to the employment contract** is as follows: 100% of staff is employed for an indefinite period. But in certain situations, for a specific work (eg checks) they make use of staff seconded from other institutions (Iordachescu, 2010).

Analyzing the structure of the personnel and skill level we can see the natural tendency of increasing skills (tab. 2).

Table 2

| Employment structure by type of study at APIA Suceava |                      |                |        |        |        |                                 |
|---|----------------------|----------------|--------|--------|--------|---------------------------------|
| Nr. crt.  | Qualification degree | Training level | 2009   | 2010   | 2011   | (+/- % 2010 in comp. with 2009) |
| 1   | University           | III            | 15     | 17     | 19     | 26.6                            |
| 2   | % of total           | -              | 75.0   | 85.0   | 95.0   | 20.0                            |
| 3   | Post- high school    | II             | -      | -      | -      | -                               |
| 4   | % of total           | -              | 0.00   | 0.00   | 0.00   | -                               |
| 5   | High school          | I              | 5      | 3      | 1      | -20.00                          |
| 6   | % of total           | -              | 8,45   | 8.05   | 6.16   | -27.05                          |
| 7   | Total                | -              | 20     | 20     | 20     | 0.00                            |
|   | % of total           | -              | 100.00 | 100.00 | 100.00 | -                               |

In the period under review there is a normal trend of increasing average level of qualification, which may be due to increased demands of education in the interest of this agency and employees to keep their jobs.

The specialists from the local centres have the following duties and responsibilities: provide information on rights and duties of farmers that have access to direct area payment schemes financed from the European or national records in the database farmers farm register, provide professional advice producers to fill requests for state aid for livestock, check applications to be accompanied by documents proving the conditions of eligibility for financial support.

Under the powers provided in the job description, employees of Fălticeni CL APIA are required to participate in training programs, seminars, courses held in Suceava APIA or by other institutions in specific areas of activity.

Training and raising skill levels of labour by organizing training courses when enrolling in classes led by remote sensing Control of a number of 3 employees, the Information Security courses according to ISO 17799 and ISO 27001 - 1 employee and Management at the public function in the context of specific legislation 2008-2011-1 employee.

Also, their sources, 10 people did and graduated ECDL courses.

The dynamics of the staff is related to the stability of the institution, which is an important condition for efficient use of labour.

Labour mobility is both as inputs and outputs, regardless of the cause generators (fig. 2).

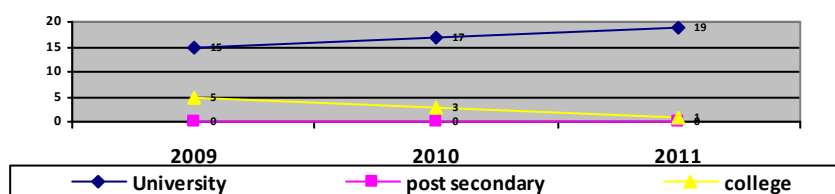


Fig.2. Evolution of staff according to the studies - CL APIA Falticeni

Movement of labour, is the movement of staff within a period (inputs and outputs) due to social causes - economic (retirement, deaths, transfers, restricting, etc.) (tab. 3).

Table 3

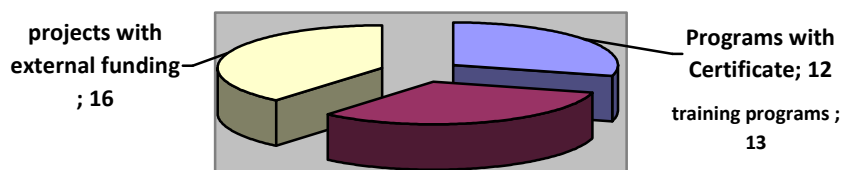
**Personal input and output stream. of the APIA-Suceava and CL-Falticeni**

| Centre       | Year | Total staff | Staff inputs | % of total | Staff outputs | % of total |
|--------------|------|-------------|--------------|------------|---------------|------------|
| CL-Fälticeni | 2007 | 12          | 5            | 41.66      | 1             | 8.33       |
|              | 2008 | 16          | 6            | 37.5       | 2             | 12.5       |
|              | 2009 | 20          | 1            | 5.0        | 1             | 5.0        |
|              | 2010 | 20          | 0            | 0.0        | 0             | 0.0        |
|              | 2011 | 20          | 0            | 0.0        | 0             | 0.0        |

Training activity for staff of APIA CL Fälticeni is made so as that, every civil must know the principles of European regulations, to acquire content translating these regulations into national law and procedure manuals to learn to be able to apply them and to form their own ideas as a basis to start editing a new version of the manual procedure above (in some areas has already reached version 7.0).

Training activity (Fig. 3) can be summarized as follows:

- training programs organized and conducted by training providers, completed certificate of attendance or, where appropriate diploma (12 people);
- training programs organized and conducted, or where appropriate, approved by the employers in the administrations and institutions (13 people);
- training programs organized and conducted in the implementation of projects with external funding (16 persons).



**Fig. 3 - Professional training at CL APIA Fälticeni**

Personal Training Service operates within the Human Resources Management, based Training Strategy 2007-2010 APIA personnel. In accordance with the Lisbon target and the Government Programme 2005 - 2008, the major objective of the strategy is to develop human capital, the most important resource of any organization (Ungureanu et. al., 2011).

This time, the training of employees APIA is based on two main pillars: PHARE projects and funding from its budget. Currently, the service works towards meeting the training needs of staff and long term aims to reinforce the skills acquired in the previous period and excellence.

CL-APIA Fälticeni will review the problems encountered in 2011, trying to turn them into targets solved in 2012, contributing and mechanisms available to improve the activity, and hence the service delivered to citizens.

In this regard, regular assessments are made by staff. We present in table 4 a summary of job evaluation assistant counsellor.

Job requires a high level skill, analytical and logical reasoning power of the owner. From this perspective, the post provides a sense of professional achievement for its owner. It has a great deal of freedom to perform their work, producing mainly situations and statistics for physical blocks of farmland eligible for payment, subject to administrative controls and other conditions required centrally (Iatco et. al. 2011).

Table 4

**Assessment of post of assistant counsellor**

| No. criteria | Evaluation criteria                           | Weight C % | Minimum score Pm | Maximum score PM | C*Pm | C*PM |
|--------------|---|------------|------------------|------------------|------|------|
| I            | Professional training                         | 20         | 3                | 4                | 0.6  | 0.8  |
| II           | Experience required                           | 25         | 4                | 5                | 1    | 1.25 |
| III          | Difficulty of operations specific to the post | 10         | 2                | 3                | 0.2  | 0.3  |
| IV           | Responsibility involved by the post           | 30         | 4                | 5                | 1.2  | 1.5  |
| V            | Scope of relations                            | 15         | 2                | 4                | 0.3  | 0.6  |
|              | TOTAL   | 100        |                  |                  | 3.3  | 4.45 |

## CONCLUSIONS

1. Implementation of effective management in the APIA Suceava City Center Fălticeni requires the following: increased transparency of decision through consultation and greater involvement of civil society in decision-making, increase access to APIA citizens via the internet, effective resolution, professional and timely of requests from farmers, civil servants adopted by a professional attitude, polite and efficient in dealing with citizens, updating of web page APIA Suceava with relevant and useful information for all local centres.

2. Staff training strategy should be integrated into APIA strategy, so to help eliminate vulnerabilities such as farmers dissatisfied, default penalties, inefficient activity of technical chapters.

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# STUDY ON PROSPECTS FOR THE DEVELOPMENT OF VAMA COMMUNE AS AGRITOURISTIC AREA OF BUCOVINA

## STUDIU PRIVIND PERSPECTIVELE DEZVOLTĂRII COMUNEI VAMA CA ZONĂ AGROTURISTICĂ DIN BUCOVINA

*CIORNEI Liliana<sup>1</sup>, BADALE Aurelia<sup>2</sup>*  
e-mail: liliana.ciornei@gmail.com

**Abstract.** *In recent years more and more talk about the development of agritourism in Bucovina. In this direction were initiated several projects and programs with national and international impact for the promotion of this region, their results are best experienced by the pension administrators. However, in relation to the real potential of the area, the exploitation of tourism resources is still quite at a low level, this being demonstrated by the small number of agritourism pensions approved, and by the agritouristic potential areas. In fact, agritourism is unequal developed through Bucovina focusing on major tourist attractions. Therefore, to support tourism development in the entire region should be analyzed and identified local tourism resources and finding the most optimal solutions in common use. Vama is located in the center of Bucovina, is an example for models and strategies for developing tourism, starting from the ideas mentioned above.*

**Key words:** agritourism, agritourism pensions, commune Vama, agritouristic potential

**Rezumat.** *În ultimii ani se vorbește tot mai des despre dezvoltarea agroturismului din Bucovina. În această direcție au fost inițiate mai multe proiecte și programe cu impact național și internațional de promovare a acestei regiuni, rezultatele acestora fiind resimțite cel mai bine de administratorii de pensiuni. Însă, în raport cu adevăratul potențial al zonei, valorificarea resurselor turistice este încă la un nivel scăzut, acest lucru fiind demonstrat de numărul mic de pensiuni agroturistice omologate, cât și a celor turistice rurale. De altfel, dezvoltarea agroturismului s-a realizat neuniform pe cuprinsul Bucovinei, concentrându-se în jurul marilor obiective turistice. De aceea, pentru sprijinirea dezvoltării agroturismului la nivelul întregii regiuni trebuie analizate și identificate resursele turistice locale și găsirea celor mai optime soluții de valorificare în comun a acestora. Comuna Vama, situată în partea centrală a Bucovinei, reprezintă un exemplu elocvent pentru alegerea unor modele și strategii de dezvoltare a agroturismului, plecând de la ideile enunțate mai sus.*

**Cuvinte cheie:** agroturism, pensiuni agroturistice, comuna Vama, potențial agroturistic.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

<sup>2</sup> Tourism Association Vama Bucovina, Romania

## **INTRODUCTION**

The Commune Vama which is located in the heart of Bucovina, is truly an agritouristic destination, hosting great in everything - many natural resources, cultural and historical, but not promoted enough. Situated at the crossroads of touristic areas of notoriety like Sucevita, Moldovita, Marginea, Vatra Dornei si Gura Humorului, Vama commune can provide both a starting point towards these areas as well as self-contained touristic zone. For these reasons, the prospects of socio-economic development of the Vama Commune can be found certainly in the sphere of agritouristic activities, as it is evidenced in this paper.

## **MATERIAL AND METHOD**

The present paper is based on results of a qualitative study on agritouristic activities performed by some of the Vama commune pensions conducted between October 2011 - March 2012, research made on land fund of the village, identifying the main tourist attractions of the area and conducting a comparative analysis of capitalization agritouristic potential in the main areas of Bucovina. The data collection was done mostly while driving in Vama commune area, to the agritouristic pensions and rural touristic, to the local institutions and the main attractions in the area, as well as consulting the bibliographic sources specific for the area and the strategy for economic and social development of the Suceava County established by the County Council.

## **RESULTS AND DISCUSSIONS**

The actual economic, political and social context requires the majority of the local communities of Bucovina area, to rethink the possibilities for developing, seeking more seriously solutions in the sphere of tourism. This happens both among families of residents who in the lack of jobs from the area, are looking to capitalize from the household or by arranging accommodation rooms or transforming the entire houses in the hostels or by exploiting animal and vegetal products to other hostels from the area, and the local authorities are forced to find and apply strategies to support and develop tourism and related activities. Vama commune, located in the heart of Bucovina territory, has become in recent years a special agritouristic destination, being the third resort in Bucovina by the number of structures of tourist accommodation in rural areas, as shown in Figure 1.

Documented in the year 1408, Vama area is located between Obcina Feredeului, Obcina Humorului and Masivul Rarău. With a population of over 6000 inhabitants, Vama commune consists of 4 villages spread across an area of 13,628 ha. The landscape is predominantly depression with a strong impact on visitors, forests occupying the largest area of the commune (70%), being one of the regions with the largest forest areas in the country. The main activities of the inhabitants of the area are related to livestock, cultivation of land, woodworking, tourism and related services. Residents even practice many traditional crafts keeping habits of ancient times, such as stone carving, weaving, woodworking, egg decoration, wool spinning. Vama is a landmark and a starting point to major tourist attractions in Bucovina, being at a distance of 14 km from the Câmpulung



Moldovenesc, 17 km from the Gura Humorului, 18 km from Moldovita, 46 km from Sucevita and 53 kilometers from the Suceava municipality.

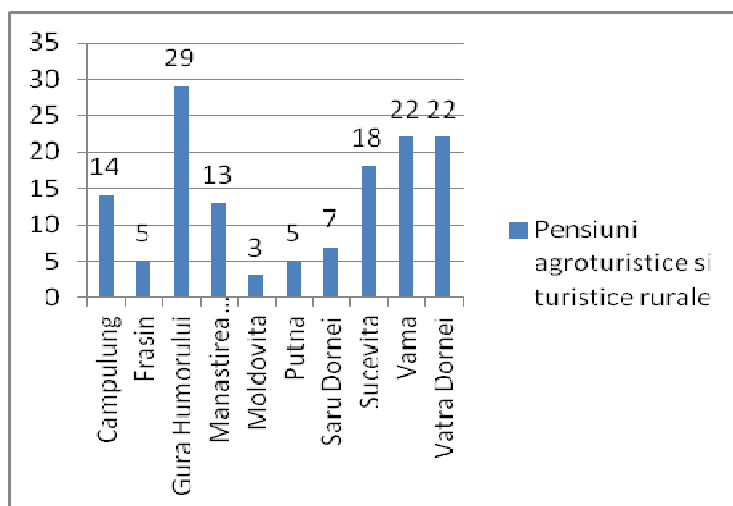


Fig. 1 – Representation of the number of agritouristic pensions and rural tourist facilities in the main localities from Bucovina

Types of tourism that can be developed in the area are: rural tourism, mountain tourism, ecumenical, scientific, rest and recreation, tourism, adventure tourism, transit, cultural tour organized for visiting monuments of art, culture and other achievements of human activity (handicraft), travel trade exhibition, whose practice is occasioned by high-profile events (fairs, exhibitions), which attract many visitors, festival tourism, cultural and artistic events occasioned by (ethnographic, folkloric) sports tourism (fishing, cycling).

Agritourism is a great potential in the area. It is characterized by several key factors, including: quality of the landscape and numerous religious sights, air and water quality; hospitality of the people as a defining element for Bucovina. The main tourist resources are related to folk art, traditional crafts and occupations - Ethnographic Museum - hosted in an old house (memorial house Iorgu Toma - the most representative figure raised in the middle farmers of Vama from 1871 to 1935), older than 200 years, where were the ancestors, are kept female and male traditional costumes, furniture, harness for animals, household items, rugs, carpets, towels, icons, carved frames, medals and old money, family photos and paintings, - Painted Eggs Museum "Letitia Orsivschi" that shelters the largest collection of decorated eggs in the region, local and international exhibits, made in various techniques - the wood Church of the Ascension of the Lord Vama built in 1783 (according to legend from the explanatory board of Bucovina Village Museum) Voda's pole (known as the Cross and the Tartars, or Tartars stone) is a column of stone which dates from 1717 and was built on the order moldavian voivode Mihai Racovita as proof of his victorious campaign against the Austrians from Transylvania, Heroes of World War Cemetery (Drăgușanul I.,

2011). Life in Bucovina is marked by a series of events, celebrations and festivals that take place annually (Wedding in Bucovina, Plugusorul Bumbierilor, Grace water at the crossing of rivers Moldova and Moldovita). A special attraction is the Hrib Festival organizer to Vama which includes a gastronomic exhibition and the housewives are competing in the preparation of delicious food, stuffed cabbage leaves, cooked pork, turkey, featured in a special way, trout, hribii, and sweets of the region.

The main arguments that determine the need for tourism development in the commune Vama, results from the analysis of some elements and indicators included in this research as:

- the existence of institutions and organizations in tourism (Tourism Association Vama Bucovina, OVR Association, Cultural and artistic association "Casa Bucovineana" ANTREC Romania - Branch Vama / Bucovina), public private partnership between them and local and county authorities;
- the existence of studies and market research, developing a monograph of Vama (600 years from the documentary attestation);
- attitude of local people - are hospitable residents, proud to show their port and popular customs, art objects and collections inherited from ancestors;
- jobs in tourism - small business administration in agritourism and rural tourism is the responsibility of family, there is a surplus of female labor;
- existing tourist potential - there are good opportunities to exploit the mountain throughout the year by hiking, riding, climbing, extreme sports, skiing;
- natural potential - landscape, mountains, forests, fast rivers, gorges, caves, natural reserves, flora and fauna specific the area;
- traditional architecture - Ethnographic Museum, Museum "La Gorita", "Jucan" house, farms and their annexes, inventory and conservation of all authentic old houses and huts of peasant;
- local cuisine - known the existence of local cuisine, menus and traditional natural products;
- traditions and local habits - the existence of a treasury ethnographic of great originality and folkloric with a rich calendar of fairs and traditional folk events throughout the year. There is a core of craftsmen and artisans;
- local crafts - egg painting, pottery, wood sculpture, carved in stone, icon painters, fabrics etc. The existence of thematic summer schools or camps where young people are initiated into the mysteries of ancient crafts;
- transport infrastructure - road transport is the E576, DN17, exist rail transport with railroad node and modernized station, air transport - Suceava Salcea airport;

- tourism infrastructure - the existence of 24 accommodation units classified and authorized but many structures and unauthorized (over 50%);
- tourist signs - is done properly to the main tourist objectives as well as to reception facilities;
- fairs with local manufacturers / goods produced in their own households - presence of a large number of farmers and local farmers;
- local Events /events ethno folk, religious, events organized by the Tourism Association Vama Bucovina, "Plugusorul Bumbierilor", "Pilgrim Road", "Green grass from home", "Race haymaker", "Hrib Festival" etc.
- souvenirs and additional products - local crafts (painted eggs, icons on wood, wood or stone carvings, weaving, stitching);
- Tourist Information Centre - existence of a tourist information point at the City hall;
- communication policy and promotion - existence of a website [www.vamabucovina.ro](http://www.vamabucovina.ro), printing of flyers, banners, roll-ups, local participation in local fairs, regional, national and international, organizing info tours for journalists and tour operators;
- policy distribution - existing partnerships with leading tour operators in Suceava, Iasi and Bacau, in the region and other European countries.

## **CONCLUSIONS**

1. Tourism resources are practically inexhaustible, and tourism is one of the few sectors of the economy with real prospects of long-term development.

2. The commune Vama is currently developing an agritouristic model for other areas of Bucovina, an important role has Vama Bucovina Tourism Association.

3. The increase of tourist traffic in Vama will determine the development of tourism economic related sectors: food, transportation, trade, garments, handicrafts, agriculture (producing "bio" products in particular), etc. Thus tourism development is contributing to a sustainable and balanced development of all sectors from this area.

4. Initiation of actions at local and regional unit to improve the legal framework in tourism by all professional associations authorized represent a solution of reviving the agrotouristic activity caused due to the effects of economic crisis in recent years.

5. There is real potential to develop agritourism in Vama which must be exploited through the implementation of coherent strategy to develop and promote tourism in the area.

6. Development of transport infrastructure, of accommodation and travel in the main cities of Suceava county as well as between them is very important for tourism activity across the region.

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## MARKET CAPITALIZATION OF FRESH HORTICULTURAL PRODUCTION ON IASI TRADE

### VALORIFICAREA ÎN STARE PROASPĂTĂ PE PIAȚA IEȘEANĂ A PRODUCȚIEI HORTICOLE

UNGUREANU G.<sup>1</sup>, BREZULEANU S.<sup>1</sup>, BREZULEANU Carmen Olguța<sup>1</sup>,  
IGNAT Gabriela<sup>1</sup>, BOGHITĂ E.<sup>1</sup>  
e-mail: unurgeo@yahoo.com

**Abstract.** Valorized agricultural products in general, and horticultural products, in particular, represent aspects from organizational activities, technical, economic and legal, through which the increased value of production and circulation of goods, from fruit farms as producers, representatives from various consumer direct beneficiaries (the stores), commercial companies, enterprises and industrial processing of fruits, etc. The purpose of this work is that for finality of fruit production must be planned character. In Iasi, exist 8 farmers' markets through which supplies over 400,000 customers, take advantage of the fresh horticultural products and fruits – vegetables processing and preserved. Depending on the number of places of sale, employment and the volumes traded, Iasi markets are divided into two categories: large markets: market Alexandru cel Bun, Nicolina Square, Central Market Hall, Independence Square.; Small Markets: Dacia, CUG, Tatarasi; Pacurari. From a functional perspective, markets include various facilities for traders, such as commercial areas, administrative areas, bathrooms, stalls for vegetables - fruit surfaces for wholesale directly from the machine. Based on the analysis of balance sheets of companies that sell fruits and vegetables (about 43 companies), 7 are companies that have more than 84% of total turnover. Most wholesalers in this category of transactions are the sale of bananas and citrus, as there Iasi market wholesalers to sell only vegetables and fruits indigenous kind.

**Key words:** market, horticultural production, organizational, technical, economic activities

**Rezumat.** Valorificarea produselor agricole, în general, și a produselor horticole, în special, reprezintă aspecte dintr-un ansamblu de activități organizatorice, tehnice economice și juridice, prin care se realizează creșterea valorii și circulația producției marfă, de la fermele pomicole în calitate de producători, la diverși beneficiari reprezentați de consumatori direcți (prin magazine proprii), firme comerciale, întreprinderi de prelucrare și industrializare a fructelor etc. În orașul Iași, cele 8 piețe agroalimentare prin intermediul cărora se aprovizionează peste 400.000 de consumatori, valorifică produse horticole atât în stare proaspătă precum și fructe – legume semiprosesate și conservate. În funcție de numărul de locuri de vânzare, gradul de ocupare și volumul de marfă tranzacționată, piețele orașului Iași sunt împărțite în 2 categorii: Piețe mari: Piața Alexandru cel Bun; Piața Nicolina; Piața Hala Centrală; Piața Independenței. Piețe mici: Dacia; C.U.G.; Tătărași; Păcurari. Din punct de vedere funcțional, piețele cuprind diferite facilități pentru comercianți, cum ar fi: spațiile comerciale, spații administrative,

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

*sanitare, tarabe pentru legume – fructe, suprafețe pentru vânzarea en-gros direct din mașini. Pe baza analizei bilanșurilor firmelor ce comercializează fructele și legumele proaspete (aproximativ 43 de societăți comerciale), 7 sunt firmele ce cumulează peste 84 % din cifra totală de afaceri. Cea mai mare parte a tranzacțiilor angrosiștilor din această categorie o reprezintă vânzarea de banane și citrice, deoarece pe piața orașului Iași nu există angrosiști care să vândă exclusiv legume și fructe de tipul celor indigene.*

**Cuvinte cheie:** piață, producție horticolă, activități organizatorice, tehnice, economice

## INTRODUCTION

In the period 1990-2012, numerous structural transformations occurred in the Romanian agriculture, that created imbalances on farm size, level of equipment with mechanical means, ensuring the production factors with direct effects on economic performance.

In the next stage, it is necessary to achieve a modern agro-industrial system of production by creating competitive organizations within EU agriculture.

One way to revive the Romanian agriculture from the productive and economic point of view is the organization of agricultural production in an integrated system. The effects that determine this process are many, both economically and socially. (Ciurea et al., 2001 & 2005).

Valorized agricultural products in general, and horticultural products, in particular, represent aspects from organizational activities, technical, economic and legal, through which the increased value of production and circulation of goods, from fruit farms as producers, representatives from various consumer direct beneficiaries (the stores), commercial companies, enterprises and industrial processing of fruits etc (Constantin et al., 1997).

The purpose of this work is that for finality of fruit production must be planned character. In Iasi, exist 8 farmers' markets through which supplies over 400,000 customers, take advantage of the fresh horticultural products and fruits – vegetables processing and preserved.

Depending on the number of places of sale, employment and the volumes traded, Iasi markets are divided into two categories: large markets: market Alexandru cel Bun, Nicolina Square, Central Market Hall, Independence Square; Small Markets: Dacia, CUG, Tatarasi; Pacurari.

From a functional perspective, markets include various facilities for traders, such as commercial areas, administrative areas, bathrooms, stalls for vegetables - fruit surfaces for wholesale directly from the machine.

Based on the analysis of balance sheets of companies that sell fruits and vegetables (about 43 companies), 7 are companies that have more than 84% of total turnover.

Most wholesalers in this category of transactions are the sale of bananas and citrus, as there Iasi market wholesalers to sell only vegetables and fruits indigenous kind.

## MATERIAL AND METHOD

A study make a diagnosis on the situation of vegetable area and secondly, the design of an integrated processing and trading of horticultural production in the Iasi county.

## RESULTS AND DISCUSSIONS

From a functional perspective, markets include various facilities for traders, such as commercial areas, administrative areas, bathrooms, stalls for vegetables - fruit surfaces for wholesale directly from the machine.

The main markets are: Alexandru cel Bun and Market Square Nicolina, which are the most popular and due to cheaper products and diversified supply of vegetables and fruit.

Scale and diversification of recovery define a complex organizational structure processing subsystems, packaging, storage and transport.

Table 1

Recovery of horticultural food markets in Iasi (characteristics)

| Market name       | Total area (square meters) | area (sqm) | Places of wholesale sales | Selling Seating (stalls) |           | Selling Seating (stalls) |           | Selling Seating (stalls) |
|-------------------|----------------------------|------------|---------------------------|--------------------------|-----------|--------------------------|-----------|--------------------------|
|                   |                            |            |                           | SC**                     | Producers | Covered                  | Uncovered | Vegetables/ fruits       |
| Alexandru cel Bun | 9860                       | 2233       | 40                        | 40                       | 253       | 210                      | 83        | 203                      |
| Nicolina          | 4778                       | 2000       | 20                        | 39                       | 223       | 222                      | 40        | 183                      |
| Central hall      | 2024                       | NA         | NA                        | NA                       | NA        | NA                       | NA        | NA                       |
| Independence      | 958                        | NA         | NA                        | NA                       | NA        | NA                       | NA        | NA                       |
| Dacia             | 3027                       | 2148,9     | 0                         | 0                        | 70        | NA                       | NA        | 64                       |
| CUG               | 4506                       | 3677,92    | NA                        | NA                       | NA        | 115                      | 0         | 115                      |
| Tătărași          | 5091                       | NA         | NA                        | NA                       | NA        | NA                       | NA        | NA                       |
| Pacurari          | 1430                       | 352        | 0                         | 0                        | 20        | 20                       | 0         | 20                       |

Selling vegetables in the food markets of the country is more profitable due to higher sales price per unit, which horticultural practice in mining areas which have smaller family members are more able to work and grow more diverse assortment of vegetables .

Selling in organized wholesale markets in each city ad hoc or on the main access roads and horticultural practice holding larger areas cultivated with vegetables, where the number of family members to participate in this process is less productive, resort to seasonal workers and sold the same day or next day harvesting, primary attention is given to crops and less recovery process.

As this is a problem of optimization of results, the manager initiates the decision process by listing the possible options. Thus, agricultural products can be sold through the following channels:

- distribution by ultra-short channel (directly to the final consumer), known in economic practice as "trade to stall", is a disorganized form of trading that farmers obtain immediate revenue from direct sales to market agricultural products, packaged properly transported in their vehicles;
- distribution by short channel runs between the farmer and retail stores - less common system in Romania - or between farmers and speculators in the market, buying agricultural products and farmers calls himself not to enter the category of traders and being forced thus pay taxes;
- long distribution channels is practiced by companies in the agriculture, manufacturers, wholesalers and retailers;
- the wholesale distribution of specific ongoing since 1998, when it was founded Wholesale of vegetables, fruit and other perishable agricultural products in Bucharest;
- choosing the optimal utilization of agricultural products obtained is a decision that depends on a variety of factors, most important being the nature and size of farm product;
- based on the analysis of companies that sell fruits and vegetables (about 43 companies), 7 are companies that have more than 84% of total turnover, as shown in the figure below.

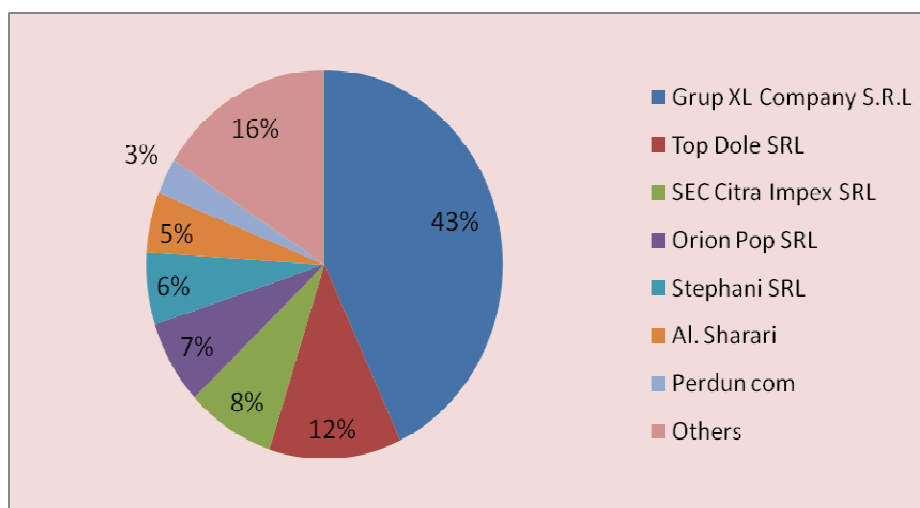


Fig. 1 - Wholesale of fruit and vegetables

Table 2

Direct costs for raw materials and packaging for cucumbers in vinegar  
- production of 600 t / year

| No | Name of products | U.M. | Quantity | Price (rol) | Value (rol) |
|----|------------------|------|----------|-------------|-------------|
| 1  | Cucumbers        | kg   | 230.000  | 0,6         | 138.000     |
| 2  | Vinegar 9o       | l    | 33.000   | 1,4         | 46.200      |
| 3  | Bay leaf         | kg   | 500      | 5,9         | 2.950       |



|    |                        |        |         |      |         |
|----|------------------------|--------|---------|------|---------|
| 4  | Pepper                 | kg     | 500     | 5,9  | 2.950   |
| 5  | Mustard seeds          | kg     | 500     | 1,5  | 750     |
| 6  | Dill                   | kg     | 600     | 2,0  | 1.200   |
| 7  | Onions                 | kg     | 1.000   | 1,2  | 1.200   |
| 8  | Salt                   | kg     | 8.000   | 0,26 | 2.080   |
| 9  | Twist-off 720 ml jars. | pieces | 583.000 | 0,53 | 308.990 |
| 10 | Twist-off caps         | pieces | 583.000 | 0,12 | 69.960  |
| 11 | Labels                 | pieces | 583.000 | 0,05 | 29.150  |
| 12 | Foil packaging         | kg     | 1.850   | 5,71 | 10.563  |
| 13 | Strechfoil             | kg     | 375     | 4,2  | 1.575   |
| 14 | Total                  |        |         |      | 615.568 |

The raw materials used to manufacture canned vegetables will come from their farms and of the six recipes for manufacturing emerges above the general structure of plant materials.

Table 3

**Need of vegetables used as raw materials for canned vegetables for 1700 tons proposed for a year**

| Name of products           | Cucumbers | Onions | Egg-plant | Pepper gras-njel | Parsley leaves | Dill | Cap-sicum | Courgettes | Carrot | Parsnip | To-matoes | Parsley Parsnips I |
|----------------------------|-----------|--------|-----------|------------------|----------------|------|-----------|------------|--------|---------|-----------|--------------------|
| Cucumbers in vinegar       | 230       | 1      |           |                  |                | 0,6  |           |            |        |         |           |                    |
| Red peppers in vinegar     |           |        |           |                  |                |      | 120       |            |        |         |           |                    |
| Courgettes in brine        |           |        |           |                  |                |      |           | 132        |        |         |           |                    |
| Root vegetables in water   |           |        |           |                  |                |      |           |            | 107    | 47      |           | 83                 |
| Vegetable stew of eggplant |           | 19     | 160       |                  |                |      | 80        |            |        |         | 60        |                    |
| Vegetable stew             |           | 30     |           | 115              | 2,5            |      |           |            | 22     |         | 100       |                    |
| Total general              | 230       | 50     | 160       | 115              | 2,5            | 0,6  | 200       | 132        | 129    | 47      | 160       | 83                 |

The analysis of table 3 for recipes and quantities of finished goods proposed to be made; there is an annual consumption of vegetables needed by 1309.1 tons production process, which represents 3% of annual production of vegetables in the village queen made in 2009 and 1.72% of the annual review conducted in the micro area.

To achieve the production of vegetables were determined labor costs for the period of a year. (Ungureanu et al., 2004).

## CONCLUSIONS

1. The small size and dispersion of farm vegetables and vegetable products that increase value through processing and recovery requires favorable conditions need to integrate the vegetable industry. Practice advanced countries the

advantages of gardening in vegetable production structure integrated within the pathways.

2. Branches of agriculture, vegetable growing is most suitable for integration. Variety of vegetable products, their timing during the growing season, the possibility in protected vegetables and processed products are diverse elements favoring integration.

3. Wholesale distributors and supermarkets in Romania imported a lot of vegetables directly, and the main reasons for significant import volumes are:

- local production cannot meet demand in the season;
- prices for imported vegetables are competitive with local production;
- imported products are homogeneous in terms of quantity, quality and punctual delivery.

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# THE INFLUENCE OF SOME NATURAL BIOREGULATORS SUBSTANCES APPLICATION ON THE GROWTH AND DEVELOPMENT OF TOMATO CROP CULTIVATED IN POLYTUNNELS INTO AN ECOLOGICAL SYSTEM

## INFLUENȚA APLICĂRII UNOR SUBSTANȚE BIOREGULATOARE NATURALE ASUPRA CREȘTERII ȘI DEZVOLTĂRII CULTURII DE TOMATE ÎN SOLAR ÎN SISTEM ECOLOGIC

AVASILOAIEI D.I.<sup>1</sup>, MUNTEANU N.<sup>1</sup>, STOLERU V.<sup>1</sup>,  
IUREA Dorina<sup>2</sup>, CĂLIN Maria<sup>3</sup>

e-mail: avasiloaiei\_dan\_ioan@yahoo.com

**Abstract.** *The paper aims to determine the influence of three different natural substances with glycoside structure (Pavstim, Ecostim and Moldstim) on tomatoes grown in polytunnel into an organic system. The efficacy of the treatments was assessed by observations and biometric determinations on plant height, number of inflorescences, number of flowers and fruits and average fruit weight for each variant. The results revealed the following variants: Pavstim 0.001% (with a total production of 54.14 t/ha), Pavstim 0.0015% (with a yield of 59.82 t/ha) and Moldstim 0.0015% (with a yield of 58.35 t/ha), reported to the control variant (44.65 t/ha). The yield differences were significant positive for the first version, respectively distinctly significant for the second and third version.*

**Key words:** organic vegetable growing, natural bioregulators substances, tomatoes.

**Rezumat.** *Lucrarea își propune să determine influența a trei substanțe naturale bioregulatorie cu structură glicozidsteroidală (Pavstim, Ecostim și Moldstim), asupra plantelor de tomatelor cultivate în solar, în sistem ecologic. Eficacitatea tratamentelor a fost evaluată prin observații și determinări biometrice referitoare la înălțimea plantei, numărul de inflorescențe, numărului de flori și fructe legate și greutatea medie a fructelor pentru fiecare variantă. Rezultatele au pus în evidență variantele: Pavstim 0,001% (cu o producție totală de 54,14 t/ha), Pavstim 0,0015% (cu o producție de 59,82 t/ha) și Moldstim 0,0015% (cu o producție de 58,35 t/ha), raportate la varianta martor (44,65 t/ha). Diferențele de producție față de martor au fost semnificative pentru prima variantă, respectiv distinct semnificative pentru a doua și a treia variantă.*

**Cuvinte cheie:** legumicultură organică, substanțe bioregulatorie naturale, tomate

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup> Institute of Biological Sciences, Iași, Romania

<sup>3</sup> Vegetable Research and Development Station Bacău, Romania

## INTRODUCTION

The Solanaceous vegetable growing in polytunnels requires performing a series of correlations between different technological links, in order to achieve their optimization. Most of the times, the difference between the success and the failure of a crop is given by the skill of the engineer/technologist arised from his own experience (Munteanu et al, 2009).

Regarding ecological vegetable growing, where the use of most of the substances from a conventional horticulture system is prohibited, the proper application of naturally substances, at the optimal dose and at the key phenophases, is a *sine qua non* element. In this context, the application of various natural a bioactive substance knows a remarkable progress, highlighted by the growing number of such new products come onto the market and also by the competition for the approval of those who have proven their effectiveness.

Among these are some natural bioactive substances extracted from plant parts of well-known species (*Hipophae rhamnoides L.*, *Capsicum annuum L.*, *Lycopersicon esculentum L.*, *Digitalis purpurea L.*, so.). Basically, these substances have glycoside steroid structure, which gives them biostimulating qualities.

Several products have been successfully used in vegetable growing practice such as Pavstim, Ecostim and Moldstim so. (Stan et al., 1996).

In fact, the contribution of this three glycoside structure substances upon which investigations have been performed in tomato culture is highlighted by the scientific literature: Pavstim stimulates the storage of biomass, photosynthetic pigment concentrations, fruit weight and productivity (Chintia et al., 1998). Moldstim used in 0.001% concentration increases the yeald by 15-20%, extend the fructification season and reduce nitrate content of fruit (Khushtov and Daurov, 2007). Ecostim applied to tomato seeds by soaking solution induced a faster development of seedlings, reducing the critical sensitivity of soil fungal pathogens attack (Chintea et al., 1998).

These results determined us to test the efficacy of these substances to a culture of tomatoes in polytunells, in ecological agriculture circumstances.

## MATERIALS AND METHODS

The researches were conducted during 2011 in "V. Adamachi" vegetable experimental field of UASVM Iasi. The tomato culture in polytunells (Primadona F1 variety), 2<sup>nd</sup> cycle (29 May – 30 October), was established by seedling produced in alveolar trays (without subculturing procedure).

The experience contained seven variants (including the control version), shown in table 1.

The treatments were performed with a Vermorel sprayer device, by spraying fine and uniform solution throughout the whole plant, weekly. The first treatment was carried out two weeks after planting.

The experience has been organized in a randomized block device with three repetitions, each repetition parcel containing five plants.

We pointed the effectiveness of treatments through observations and biometric determinations on plant height, number of inflorescences, number of flowers and fruits, average fruit weight and also on overall production for each variant.

Table 1

The experimental variants

| Variant no.        | Bioactive substance applied | Origin                         | Concentration % | How it was applied |
|--------------------|-----------------------------|--------------------------------|-----------------|--------------------|
| V <sub>1</sub> (C) | -                           | -                              | -               | -                  |
| V <sub>2</sub>     | Pavstim                     | <i>Digitalis purpurea</i>      | 0.001           | Weekly treatment   |
| V <sub>3</sub>     | Ecostim                     | <i>Lycopersicon esculentum</i> | 0.001           | Weekly treatment   |
| V <sub>4</sub>     | Moldstim                    | <i>Capsicum annuum</i>         | 0.001           | Weekly treatment   |
| V <sub>5</sub>     | Pavstim                     | <i>Digitalis purpurea</i>      | 0.0015          | Weekly treatment   |
| V <sub>6</sub>     | Ecostim                     | <i>Lycopersicon esculentum</i> | 0.0015          | Weekly treatment   |
| V <sub>7</sub>     | Moldstim                    | <i>Capsicum annuum</i>         | 0.0015          | Weekly treatment   |

## RESULTS AND DISCUSSIONS

The results highlights that the treatments performed had a significant influence on plant growth and development.

The measurements regarding the plant growth and development index took place two days before the first harvest.

The plants height emphasizes within 0.001% variants the Moldstim one, with an average height of 119.87 cm, with 17.34 cm more than the control version. Ecostim and Pavstim variants have substantially the same average height with control version. The situation changes at 0.0015% variants: Ecostim variant has its height close to control version, Moldstim and Pavstim variants having an additional of 9.27 cm, respectively 10.87 cm.

Regarding the number of flowers / plant, it has varied from 4.67 to control version, till 6.27 at Moldstim 0.001% version.

The number of flowers per plant highlighted the variants treated with 0.0015%, mainly the Pavstim variant, with an average of 16.18 flowers / plant.

The number of fruits/plant revealed the Pavstim 0.0015% and Moldstim 0.0015% variants with 12.78 fruits / plant, respectively 12.26 fruits / plant.

The average fruit weight outlined the same variants, Pavstim 0.0015% and Moldstim 0.0015%, with an average weight of 97.87 g, respectively 98.06 g.

The main growth and development index are presented in table 2.

Table 2

**The impact of bioactive substances treatments on the growth and development of the plants**

| Variant |                     | Growth and development index |                            |                |               |                          |
|---------|---------------------|------------------------------|----------------------------|----------------|---------------|--------------------------|
| No.     | Specification       | Height (cm)                  | No. of inflorescence/plant | No. of flowers | No. of fruits | Average fruit weight (g) |
| 1.      | Control (untreated) | 102.53                       | 4.67                       | 12.21          | 9.20          | 83.13                    |
| 2.      | Pavstim 0.001       | 105.4                        | 5.6                        | 14.00          | 10.86         | 94.30                    |
| 3.      | Ecostim 0.001       | 109.8                        | 4.87                       | 13.13          | 9.66          | 90.73                    |
| 4.      | Moldstim 0.001      | 119.87                       | 6.27                       | 14.13          | 11.01         | 85.22                    |
| 5.      | Pavstim 0.0015      | 113.4                        | 6.15                       | 16.18          | 12.78         | 97.87                    |
| 6.      | Ecostim 0.0015      | 103                          | 5.27                       | 15.46          | 11.20         | 95.15                    |
| 7.      | Moldstim 0.0015     | 111.8                        | 6.13                       | 15.40          | 12.26         | 98.06                    |

The dynamics of production reveals, within all variants, significant yields at the second and third harvests. The total yields fluctuated from 44.65 t/ha at control version to 59.82 t/ha at Pavstim 0.0015% version (table 3).

Table 3

**The yield dynamics (t/ha)**

| Variant |                     | The harvest appreciation date |       |       |       |       |
|---------|---------------------|-------------------------------|-------|-------|-------|-------|
| No.     | Specification       | 7 IX                          | 23 IX | 9 X   | 20 X  | Total |
| 1.      | Control (untreated) | 7.89                          | 11.67 | 15.87 | 9.22  | 44.65 |
| 2.      | Pavstim 0.001       | 9.81                          | 13.74 | 18.13 | 12.46 | 54.14 |
| 3.      | Ecostim 0.001       | 10.06                         | 11.32 | 16.57 | 13.56 | 51.51 |
| 4.      | Moldstim 0.001      | 9.63                          | 14.09 | 17.11 | 11.81 | 52.64 |
| 5.      | Pavstim 0.0015      | 11.37                         | 15.56 | 18.94 | 13.95 | 59.82 |
| 6.      | Ecostim 0.0015      | 10.25                         | 11.68 | 16.68 | 13.59 | 52.20 |
| 7.      | Moldstim 0.0015     | 11.54                         | 15.63 | 18.71 | 12.47 | 58.35 |

We should mention that in terms of quality, in an overwhelming percentage, the total production of each variant ranged in extra class, with values belonging to the interval 71.25% (control version) – 77.75% (Moldstim 0.0015 version). About one quarter of the production was part of first quality class, second quality class being underrepresented (the maximum recorded value was 4.25% at both the Control and Ecostim 0.0015 version) (table 4).

Table 4

## Fruit quality (according to STAS no. 1421-81)

| Variant |                     | Extra class (%) | First class (%) | Second class (%) |
|---------|---------------------|-----------------|-----------------|------------------|
| No.     | Specification       |                 |                 |                  |
| 1.      | Control (untreated) | 71.25           | 24.50           | 4.25             |
| 2.      | Pavstim 0.001       | 74.50           | 22.75           | 2.75             |
| 3.      | Ecostim 0.001       | 73.50           | 24.25           | 2.25             |
| 4.      | Moldstim 0.001      | 76.25           | 20.50           | 3.25             |
| 5.      | Pavstim 0.0015      | 74.75           | 21.75           | 3.5              |
| 6.      | Ecostim 0.0015      | 73.25           | 22.50           | 4.25             |
| 7.      | Moldstim 0.0015     | 77.75           | 19.50           | 2.75             |

The experimental data were processed by proper statistical and mathematical methods (ANOVA).

Thus, regarding this matter, stands out the  $V_5$  and  $V_7$  variants, with distinctly significant positive productions and  $V_2$  variant with significant positive production. Also, the relative production highlights the same variants, with an increase of 33.97%. 30.68%, respectively 21.25% (table 5).

Table 5

## The variant's yield analysis

| Variant |                     | Total production (t/ha) | Difference over the control (t/ha) | Relative production (%) | Significance |
|---------|---------------------|-------------------------|------------------------------------|-------------------------|--------------|
| No.     | Specification       |                         |                                    |                         |              |
| 1.      | Control (untreated) | 44.65                   | 0.0                                |                         |              |
| 2.      | Pavstim 0.001       | 54.14                   | 9.5                                | 121.25                  | *            |
| 3.      | Ecostim 0.001       | 51.51                   | 6.9                                | 115.36                  |              |
| 4.      | Moldstim 0.001      | 52.64                   | 8.0                                | 117.89                  |              |
| 5.      | Pavstim 0.0015      | 59.82                   | 15.17                              | 133.97                  | **           |
| 6.      | Ecostim 0.0015      | 52.2                    | 7.55                               | 116.90                  |              |
| 7.      | Moldstim 0.0015     | 58.35                   | 13.70                              | 130.68                  | **           |

LSD 5% = 8.09 t/ha

LSD 1% = 11.35 t/ha

LSD 0.1% = 16.03 t/ha

The F-test shows that differences in production are due to the studied experimental factor, mainly.

## CONCLUSIONS

1. Regarding the dynamics of plants growth in height, the most prominent is Pavstim 0.001% variant. However, it should be noted that all treated variants have greater height levels than the control version.

2. All the treated variants achieved a higher number of inflorescences, flowers and fruit, as well as a superior average fruit weight than the control version, particularly the variants treated with a concentration of 0.0015%.

3. The efficiency of applying biostimulating substances is shown by the overall production obtained at Pavstim 0.0015 and Moldstim 0.0015 variants, causing distinctly significant positive differences.

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# RESEARCH CONCERNING THE GERMINATION CAPACITY FOR *BRASSICA OLERACEA* VAR. *ACEPHALA* SEEDS UNDER THE INFLUENCE OF CERTAIN TECHNOLOGICAL FACTORS

## CERCETĂRI PRIVIND CAPACITATEA GERMINATIVĂ A SEMINTELOR DE *BRASSICA OLERACEA* VAR. *ACEPHALA* SUB INFLUENȚA UNOR FACTORI TEHNOLOGICI

BALCĂU Simina<sup>1</sup>, APAHIDEAN Maria<sup>1</sup>, ZAHARIA A.<sup>1</sup>,  
BĂRBUȚĂ Iulia<sup>1</sup>, MUNTEAN Delia<sup>2</sup>  
e-mail: simina\_balcau@yahoo.com

**Abstract.** Research was carried out to establish the germination capacity of the seeds from the storage samples of the vegetable specie *Brassica oleracea* var. *acephala*, with decorative and practical features. The experiences aimed to determine the effect of several factors on germination including: the origin of seeds, their age and various chemical treatments applied to them. In the conducted research, it was found that the source of seeds leads to significant differences in the germination of kale seeds. Different results were obtained for seed germination due to the chemical treatments applied.

**Key words:** germination, gibberellic acid, technological factors, humidity.

**Rezumat.** Cercetările întreprinse au urmărit stabilirea capacității germinative a semințelor din probele de control a speciei legumicole *Brassica oleracea* var. *acephala* cu însușiri decorative și utilitare. Experiențele au vizat stabilirea efectului asupra germinației a mai multor factori printre care: proveniența semințelor, vârsta acestora și diferite tratamente chimice aplicate acestora. În urma cercetărilor realizate s-a constatat că proveniența semințelor este cauza unor diferențe semnificative în ceea ce privește capacitatea germinativă a semințelor de varză de frunze. Rezultate diferite obținute la germinarea semințelor s-au înregistrat și datorită tratamentelor chimice aplicate semințelor.

**Cuvinte cheie:** germinație, acid giberelic, factori tehnologici, umiditate.

### INTRODUCTION

*Brassica oleracea* var. *acephala* is a vegetable species very often used as a food product, and as of lately as a decorative element in landscaping or flower arrangements, which is the motivation for this study. The kale is multiplied only through seeds, that is why new information about seed germination capacity under the influence of different technological factors can help towards obtaining higher quantity of sowing material. The duration of germination and the merge of the seedlings

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

<sup>2</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

are also influenced by the age of the seed. The older the seeds the lengthier the germination period and emergence of seedling (Indrea et. al., 2009). For the species from the cabbage group, as well as for most vegetable species, seed germination depends on temperature and soil humidity (Burzo et. al., 2000). Most often in vegetable growing biostimulators are used to direct plant growing and development by adjusting the metabolic balance, and the way and moment of application is different according to the product used and the vegetable species to which they are applied (Mihalca, 2009). Amongst these substances are gibberellic acid, used to stimulate the energy and seed germination faculty, especially in unfavorable conditions of temperature, humidity and light (Oprea, 2012).

## MATERIAL AND METHOD

The biologic material used for organizing the experience that aims to establish the influence of different technological factors on seed germination of *Brassica oleracea* var. *acephala*, is represented by seeds procured from Italy, Hungary and Romania. The seeds produced in Italy will be noted as source I and the ones produced in Hungary will be considered as source II. For organizing the experience several items were used: filter paper, seed germination stimulants and germinators. The experience had two factors and each variant had 3 repetitions. The temperature was the same for each variant and ranged between 18 and 23°C over 24 hours. The determinations that were made concerned the morphological characteristics of the seeds and seed germination for each variant. The results were statistically processed and presented in tables, and interpreted in the paper.

To accomplish the proposed objectives kale seeds were used in the experience. Morphological characteristics of the kale seeds used are shown in table 1.

Table 1

**Morphological characteristics of *Brassica oleracea* var. *acephala* seeds used in the experience**

| Species  | Source    |       |              |            |       |              |
|--|-----------|-------|--------------|------------|-------|--------------|
|  | I Italy   |       |              | II Hungary |       |              |
|  | 100 pcs/g | MMB g | Plant health | 100 pcs/g  | MMB g | Plant health |
| <b><i>Brassica oleracea</i> var. <i>acephala</i></b> | 0,31      | 3,1   | good         | 0,29       | 2,9   | good         |

The seeds were divided in groups of 100 pieces and weighed with a technical scale. It was noticed that the seeds from Italy have a mass of 3,1 g for 1000 pcs while the seeds from Hungary have a mass of 2,9 for 1000 pcs the latter being smaller, their mass representing 93,5% of the seeds from Italy.

## RESULTS AND DISCUSSIONS

To establish seed germination a bifactorial experience was organized with 4 variants, each variant having 3 repetitions. The technological factors that were taken into consideration were the source of the seed and humidity. The humidity was ensured with the help of filter paper that was soaked in water, one of which was maintained permanently in water in germinators and one that was folded and

kept in plastic trays. In the case of the latter humidity changed from an optimum level of 80-90% to 20-30% within 24 hours. The seeds were considered germinated when the roots appeared and were at least 1 cm in length. The first observations were made after 48 hours. Further observations were repeated every 24 hours. The results were statistically processed and are shown in table 2.

Table 2

**Results concerning seed germination for *Brassica oleracea* var. *acephala* under the influence of source and humidity**

| Variant |  | Percent of germinated seeds after n hours |      |      |     | Total germinated seeds |
|---------|--|---|------|------|-----|------------------------|
| Nr.     | Conținut content   | 48  | 72   | 96   | 120 | (%)                    |
| 1.      | <i>B. o. var. acephala</i><br>Source I – changing humidity (control) | 4,6                                       | 25,7 | 20,3 | 8,2 | 58,8                   |
| 2.      | <i>B. o. var. acephala</i><br>Source I – constant humidity           | 12,3                                      | 41,5 | 32,6 | 3,3 | 89,7                   |
| 3.      | <i>B. o. var. acephala</i><br>Source I – changing humidity           | 3,1                                       | 15,3 | 10,6 | 6,2 | 35,2                   |
| 4.      | <i>B. o. var. acephala</i><br>Source II – constant humidity          | 5,8                                       | 18,3 | 20,5 | 2,8 | 47,4                   |

It was found that seed germination took place over five days from the start date. Most of the seeds have germinated in day 3 and 4. In the end the percent of germination was different for each variant. The least germinated seeds (32,5 %), variant 3, are the ones from Hungary and had a changing humidity. Another low percentage was observed on variant 4 (source II Hungary) with a percent of 47.4%, a value that is also considered unsatisfactory, in spite of the optimum level of humidity. The best results were obtained by variant 2 (source I Italy) with a constant optimum level of humidity. To establish if the differences between the variants were significant the results were statistically processed using analysis of variance (table 3).

Table 3

**Summary of experimental results concernig seed germination for *Brassica oleracea* var. *acephala* under the combined influence of source and humidity**

| Variant |  | Germinated seeds |                | ±d    | Difference significance |
|---------|--|------------------|----------------|-------|-------------------------|
| Nr.     | Content  | Absolute number  | % from control |       |                         |
| 1.      | <i>B. o. var. acephala</i><br>Source I changing humidity (control) | 58,8             | 100,0          | -     | -                       |
| 2.      | <i>B. o. var. acephala</i><br>Source I constant humidity           | 89,7             | 152,6          | 30,9  | ***                     |
| 3.      | <i>B. o. var. acephala</i><br>Source II changing humidity          | 35,2             | 59,9           | -23,6 | 000                     |
| 4.      | <i>B. o. var. acephala</i><br>Source II constant humidity          | 47,4             | 80,6           | -11,4 | 0                       |

LSD<sub>5%</sub> - 9,8

LSD<sub>1%</sub> - 12,3

LSD<sub>0,1%</sub> - 18,5

Analyzing the results from the table it can be observed that there are more germinated seeds from variant 2 compared to the control, with a difference of 30,9, statistically very significantly positive, while the differences from variant 3 and 4 are very significant and significantly negative compared to the control. The results presented in the table have been obtained under the combined influence of 2 factors. To establish the unilateral influence of each factors on *B. o. var. acephala* seed germination the results have been statistically processed and portrayed in tables 4 and 5.

Table 4

**Synthesis of experimental results concerning seed germination for *B. o. var. acephala* under the unilateral influence of humidity**

| Variant |  | Germinated seeds |              | ±d   | Significance of difference |
|---------|--|------------------|--------------|------|----------------------------|
| Nr.     | Content  | Absolut number   | Relative (%) |      |                            |
| 1.      | <i>B. o. var. acephala</i><br>- changing humidity<br>(control) | 47,0             | 100,0        | -    | -                          |
| 2.      | <i>B. o. var. acephala</i><br>- constant humidity              | 68,6             | 146,0        | 21,6 | ***                        |

LSD<sub>5%</sub> - 8,3  
LSD<sub>1%</sub> - 11,2  
LSD<sub>0,1%</sub> - 17,5

The obtained results highlight the fact that the seeds that had an optimum humidity level, germinated in a percent of 146 compared to the control, with a number of 21,6 seeds that have germinated over the control amount. This difference is very significantly positive.

Table 5

**Synthesis of experimental results concerning seed germination for *B. o. var. acephala* under the unilateral influence of source**

| Variant |  | Germinated seeds |              | ±d        | Significance of difference |
|---------|--|------------------|--------------|-----------|----------------------------|
| Nr.     | Content  | Absolut number   | Relative (%) |           |                            |
| 1.      | <i>B. o. var. acephala</i><br>- source I (control) | 74,3             | 100,0        | -         | -                          |
| 2.      | <i>B. o. var. acephala</i><br>- source II          | 41,3             | 55,6         | -<br>33,0 | 000                        |

LSD<sub>5%</sub> - 7,9  
LSD<sub>1%</sub> - 11,4  
LSD<sub>0,1%</sub> - 15,8

Analyzing the data from table 5 it can be noticed that the seeds from source I Hungary have germinated in a percent of 55,6 compared to the control, which means 33 less germinated seeds. This difference is statistically ensured and is very significantly negative. This can be concluded from the fact that the seeds

from source II Hungary are significantly smaller than the ones from source I Italy, with 2,9 g for 1000 pcs compared to 3,1 g for the seeds from Italy.

Further on, the effect of giberelic acid (AG<sub>3</sub>) on *B. o. var. acephala* on seed germination was investigated. For the organization of the experience kale seeds were used, produced in 2009 and 2011. The seed samples were put in germinators at a temperature of 18-22° C and the humidity was achieved through solution of AG<sub>3</sub> in different concentrations. The results of the germination are presented in table 6.

Table 6

**Experimental results on *B. o. var. acephala* seed germination under the influence of AG<sub>3</sub> concentration and seeds age.**

| Variant |  | Germinated seeds out of 100 |              | ±d   | Significance of difference |
|---------|--|-----------------------------|--------------|------|----------------------------|
| Nr.     | Content  | Absolut number              | Relative (%) |      |                            |
| 1.      | 2009 seeds, untreated with AG <sub>3</sub> (control) | 41,2                        | 100,0        | -    | -                          |
| 2.      | 2009 seeds, treated with AG <sub>3</sub> (100 ppm)   | 43,8                        | 106,0        | 2,6  | -                          |
| 3.      | 2009 seeds, treated with AG <sub>3</sub> (500 ppm)   | 72,5                        | 175,9        | 31,3 |                            |
| 4.      | 2011 seeds, untreated with AG <sub>3</sub>           | 85,2                        | 206,8        | 44,0 | ***                        |
| 5.      | 2011 seeds, treated with AG <sub>3</sub> (100 ppm)   | 86,3                        | 209,5        | 45,1 | ***                        |
| 6.      | 2011 seeds, treated with AG <sub>3</sub> (500 ppm)   | 91,5                        | 222,1        | 50,3 | ***                        |

LSD<sub>5%</sub> - 14,3

LSD<sub>1%</sub> - 19,5

LSD<sub>0,1%</sub> - 21,3

Analyzing the data from table 6 it can be observed that the germinated seeds differ from one another based on number and percent in accordance to the variant. The weakest germination is observed on variant 1 and 2 (2009 seeds without AG<sub>3</sub> or with AG<sub>3</sub> 100 ppm. The same seeds but an AG<sub>3</sub> concentration of 500 ppm have germinated in a much higher percent compared to the control (72,5%). The situation is different for 2011 seeds. Their germination for these samples is over 85% regardless the concentration of AG<sub>3</sub>.

The statistic process of these results that are meant to establish the significance of the differences between the variants and the control indicates that the germination for the seeds from variants 3 and 6 is significantly higher than the control, a difference that is statistically very significantly positive.

The determination of the unilateral effect of the studied factors on kale seed germination is presented in table 7.

Table 7

**Synthesis of experimental results concerning seed germination for *B.o. var. acephala* under the unilateral influence of seed age**

| Nr. | Variant<br>Content   | Germinated seeds |                 | ±d   | Significance<br>of difference |
|-----|----------------------|------------------|-----------------|------|-------------------------------|
|     |                      | Absolut<br>(nr.) | Relative<br>(%) |      |                               |
| 1.  | 2009 seeds (control) | 52,5             | 100,0           | -    | -                             |
| 2.  | 2011 seeds           | 87,7             | 167,1           | 35,2 | ***                           |

LSD<sub>5%</sub> - 10,5  
LSD<sub>1%</sub> - 14,2  
LSD<sub>0,1%</sub> - 18,5

It can be observed that the results obtained are closer in value in the case of variants 1 and 2 (63,2 and 65,2), while for variant 3 seed germination is higher than the control with a positive difference of 18,8 seeds. This difference is statistically ensured and is distinctly significantly positive.

### CONCLUSIONS

Based on the obtained results the following conclusions can be drawn:

1. Kale seeds germinate in a percent of 85 under optimum conditions of environment and technology used.
2. Kale seed germination is positively influenced by the size of the seeds.
3. Out of all the technological factors studied in the experience the level of humidity influences kale seeds germination more.
4. Kale seeds that are stored a number of years can be stimulated towards a good germination by using a solution of AG<sub>3</sub> in a concentration of 500 ppm.

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# ASSESSMENT OF VEGETABLE RESOURCES CONSERVATION WORK AT VEGETABLE RESEARCH STATION BACAU ROMANIA

## EVALUAREA ACTIVITĂȚII DE CONSERVARE A RESURSELOR GENETICE LEGUMICOLE LA STATIUNEA DE CERCETARE DEZVOLTARE PENTRU LEGUMICULTURA BACAU

**BREZEANU Creola<sup>1</sup>, BREZEANU P.M.<sup>1</sup>, AMBARUS Silvica<sup>1</sup>, CALIN Maria<sup>1</sup>**  
e-mail: creola.brezeanu@yahoo.com

**Abstract** *The aim of this study is to present our research activity for: identification, collection, evaluation and conservation of germplasm resources useful in breeding works; creation and patenting of new cultivars, with high production capacity and quality, suitable for organic and conventional system and diversification of vegetables assortment by introducing of species, varieties and local populations. More than forty-nine plant varieties developed in Vegetable Research and Development Station Bacau have been patented by State Office for Inventions and Trademarks (OSIM). Part of our cultivars can be successfully cultivated in ecological culture system. The paper presents information about our accessions and some new genotypes created by our researchers.*

**Key words:** biodiversity, preservation, breeding

**Abstract** *Scopul acestei lucrări este de a prezenta activitatea de cercetare bazată pe identificarea, colectarea, evaluarea și conservarea resurselor de germoplasmă utile în ameliorare, în vederea creării și patentării de noi cultivare și hibrizi cu capacitate productivă ridicată, cu indici de calitate superiori care să se preteze la condițiile agriculturii convenționale și biologice, (diversificării sortimentului de legume cultivate prin introducerea de specii noi, mai puțin cunoscute și răspândite și a unor populații locale cu potențial cantitativ și calitativ. Mai mult de 49 de varietăți de plante au fost create la Stațiunea de Cercetare Dezvoltare pentru Legumicultură Bacău și patentate de către Oficiul de Stat pentru Invenții și Mărci (OSIM). O parte dintre aceste cultivare sunt cultivate cu succes în sistemul de agricultură biologică. Lucrarea prezintă informații cu privire la accesii colectate și la genotipurile obținute prin ameliorare de către cercetătorii stațiunii.*

**Cuvinte cheie:** biodiversitate, prezervare, ameliorare

### INTRODUCTION

Conservation work starts from the most urgent environmental problem all over recognized in our society: continuing loss of biodiversity (Wood, 2000). In Romania alarming losses were recorded in all plant species including vegetable. The researchers from VRDS Bacau are involved in international projects regarding management of genetic resources. Our priority is to ensure efficient

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<sup>1</sup> Vegetable Research and Development Station Bacau, Romania

management of vegetable genetic resources in order to preserve and use in breeding of proper biological material, valuable from the view point of resistance to biotic and abiotic stress, with high qualitative and quantitative potential. The mini gene bank from VRDS Bacau started its activities in 1995.

## MATERIAL AND METHOD

We organized special expedition at small farms in Moldova, in order to collect seeds of old varieties, local population and cultivars under erosion risk. We also collected accessions for abroad. The purpose of the collection of plant genetic resources is to capture the maximum amount of useful genetic variation in the smallest number of samples (Marshall and Brown, 1975). Our interest is on genotypes with high level of resistance to pathogen attack and drought.

Seeds were cleaned to remove debris, inert material, damaged and infected seeds or seeds of other species in order to improve the quality of samples. Storage facilities are represented by fields for "in vivo" conservation and seed chambers for vegetable and other herbaceous crops. All accessions are stored at +4° C, after previous desiccations process. Cold chambers at -18 ° C for long term preservation are not available.

## RESULTS AND DISCUSSIONS

*Number of accessions:* Table 1 provides an overview of the most important accessions stored in our mini gene bank.

Table 1

The most important accessions stored in our mini gene bank

| Botanical family | Species                              | Number of accessions | Number of traits |
|------------------|--------------------------------------|----------------------|------------------|
| Solanaceae       | <i>Capsicum annuum</i> L.            | 350                  | 12               |
|                  | <i>Lycopersicon esculentum</i> Mill. | 391                  | 12               |
|                  | <i>Solanum melongena</i> L.          | 40                   | 6                |
| Cucurbitaceae    | <i>Cucumis melo</i> L.               | 10                   | 15               |
|                  | <i>Cucumis sativus</i> L.            | 15                   | 13               |
|                  | <i>Citrulus lanatus</i> L.           | 6                    | 10               |
| Leguminosae      | <i>Phaseolus vulgaris</i> L.         | 212                  | 9                |
|                  | <i>Phaseolus coccineus</i> L.        | 26                   | 9                |
|                  | <i>Phaseolus aureus</i> Roxb.        | 22                   | 9                |
| Cruciferae       | <i>Brassica campestris</i> L.        | 2                    | 6                |
|                  | <i>Brassica rapa</i> L.              | 2                    | 6                |
|                  | <i>Brassica oleracea</i> L.          | 34                   | 6                |
|                  | <i>Raphanus sativus</i> L.           | 44                   | 8                |
| Umbeliferae      | <i>Daucus carota</i> L.              | 9                    | 8                |
|                  | <i>Pastinaca sativa</i> L.           | 9                    | 6                |
|                  | <i>Petroselinum hortense</i> L.      | 9                    | 6                |
| Alliaceae        | <i>Allium cepa</i> L.                | 164                  | 8                |
|                  | <i>Allium porrum</i> L.              | 24                   | 8                |
|                  | <i>Allium sativum</i> L.             | 5                    | 8                |
|                  | <i>Allium ampeloprasum</i> L.        | 2                    | 8                |
|                  | <i>Allium schoenoprasum</i> L.       | 4                    | 8                |



Currently our mini gene bank holds more than 2500 accessions of horticultural crops (vegetables and plants with multiple purposes: aromatics, spices, medicinal, ornamentals), mainly those belonging to the *Solanaceae*, *Cucurbitaceae*, *Leguminosae*, *Cruciferae*, *Umbeliferae*. Large part of accessions is collected from: Romania, Spain, China, and Republic of Moldova. Portugal, India.

*Regeneration and characterization:* Seeds lose viability even under good storage conditions and it is necessary to regenerate accessions from time to time; the frequency of regeneration depends on the initial viability, the rate of loss of viability and the regeneration standard decided to regenerate the accession (Roberts 1984). The aim of regeneration is to increase the quantity of seed of any accession where the number of seeds available has been depleted, or to restore maximum viability to a seed lot. Regeneration of germplasm is one of the most crucial processes in gene bank management. It is costly in terms of resources and time, and it involves the risk to genetic integrity.

A big part of accessions has been characterized following the descriptors. Germplasm characterization is the recording of distinctly identifiable characteristics, which are heritable. This needs to be distinguished from preliminary evaluation, which is the recording of a limited number of agronomic traits considered to be important in crop improvement. (Upadhyaya, 2008) Germplasm characterization is carried out in precision fields by spaced planting under adequate agronomic conditions and plant protection. Our purpose in characterization process of accessions is:

- to describe accessions, establish their diagnostic characteristics;
- to classify groups of accessions;
- to identify accessions with desired agronomic traits and select entries for more precise evaluation;
- to estimate the extent of variation in the collection.

*Evaluation and utilization:* most part of accessions stored in our institution is used in different breeding programs carried out, mainly in *Solanaceae*, *Leguminosae* and *Compositae*. Drought, pathogen resistance and breeding for quality are the most important areas of our research interest.

Organoleptic and nutritional traits of (tomatoes, pepper, eggplant and melon) are performed by determination of titratable acidity, carotenes and lycopene, sugars, proteins, ascorbic acids content.

More than forty-nine plant varieties created in VRDS Bacau have been patented by state office for inventions and trademarks (OSIM). Most cultivars are suitable for ecological culture system, omitting synthetic herbicides and mineral nitrogen fertilizers, along with more diverse crop rotations, reduces detrimental impacts on biodiversity (Bengtsson et al., 2005). Positive effects of organic farming are found especially in biodiversity and also in health problems associated with nutrition. (Biao X, 2003).

The most important vegetable cultivars developed at Vegetable Research and Development Station, Bacau are: endive: Bacău 3, Magura; sweet pepper: Ceres, Dariana Bac, Dariochea; long pepper: Siret, Ionel; round pepper: Lider, Creola; hot pepper: Iute Delicios; faba bean: Productiv 31; Onion: Orizont;

cucumber: Mapamond, Cornibac F1; cauliflower: Timpurie de Bacău and Dumbrava; savory: De Moldova, Daria; climbing bean: Auria Bacăului, Verba, Verdana; garden bean: Cristina, Mileniu, Marinică, Perlata; lettuce: Silvia, Marilena, Serata; lovage: Rarău; gill: De Brad 3; carrot: Ceahlău; melon: Brilliant; sweet corn: Dulce de Bacau; moon radish: Roșioară; summer radish: Bianca, Țepușă de Bacău; spinach: Premier; Red beet: Rubiniu; Early cabbage: Flavius F<sub>1</sub>, Rolis F<sub>1</sub>; summer cabage: Rovana; tomato: Moldoveanca; celery: Bistrita. The main breeding objectives proposed were: productivity, quality, earliness, genetic resistance to the pathogen agents attack, ecological plasticity etc.

## CONCLUSIONS

In the context of climate change in Romania, it is increasingly obvious that it is extremely important that national genetic resources have to be protected through the implementation of national programs for conservation feasible. Despite of current economical situation, Vegetable Research Station Bacau continue the researches on breeding, in order to develop new genotypes suitable to climatic condition and to market request.

Improving the economic importance of species must take into account the heritage of genetic resources held at this time for the success of the experiments to be integrated specific climatic conditions of our country.

In addition it is essential for species threatened by climate change to have real chances of survival through the implementation of national programs for “ex situ” conservation.

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# THE STUDY OF INITIAL BREEDING MATERIAL WITH HIGH GENETIC VARIABILITY, IN ORDER TO OBTAIN NEW TOMATO CULTIVARS, SUITABLE FOR ORGANIC FARMING

## STUDIUL MATERIALULUI ÎNȚIAL DE AMELIORARE CU VARIABILITATE GENETICĂ RIDICATĂ, PENTRU OBTINEREA UNOR CULTIVARE NOI DE TOMATE PENTRU AGRICULTURĂ BIOLOGICĂ

**BREZEANU P.M.<sup>1</sup>, BREZEAN<sup>1</sup> Creola, AMBARUS Silvica, MUNTEANU N.<sup>2</sup>**  
e-mail: brezeanumarian@yahoo.com

**Abstract.** *The main purpose of research was to develop a new semi early cultivar of tomato for industrialization and fresh consumption, with a broad genetic base: increased productivity (2.0-3.0 kg / plant), with improved quality of fruit (high content of in soluble solids, uniformity of fruit's shape and color), shape index between 0.98-1.02, average weight 120 g/ fruit, with tolerance and / or resistance to pathogen attack, adapted to specific environmental conditions, with increased chances of achieving, screening and selection biotype. Thus, we proceeded to identify and collect useful sources of germplasm for tomato breeding programme.*

**Key words:** genotype, resistance, quality, ecological system culture

**Rezumat.** *Scopul principal al cercetării a fost de a obține un soi nou, semitimpuriu de tomate destinat industrializării și consumului în stare proaspătă, cu o bază genetică largă, productivitate sporită (2,0-3,0 kg/planta), cu calitate îmbunătățită a fructului (conținut crescut în substanță uscată solubilă, uniformitate a formei și a culorii fructului la maturitate), indice de formă cuprins între 0,98-1,02, greutate medie a fructului 120 g, cu toleranțe și/sau rezistențe la atacul agenților patogeni, adaptat la condiții specifice de mediu, cu șanse mărite de realizare, depistare și selecție a biotipului. În acest sens s-a procedat la identificarea și colectarea surselor de germoplasmă de tomate, utile lucrărilor de ameliorare.*

**Cuvinte cheie:** genotip, rezistența, calitate, sistem ecologic de cultură

### INTRODUCTION

Cultivar is a defining quality standard that consumers are accustomed. Standard cultivars generally have qualities that are valued at a time. Newer cultivars with similar qualities are tested and studied in culture compared to the standard being the most effective information on the achievements and potential in creating new vegetable open pollinated or hybrid cultivars. Knowledge of morphological and physiological characteristics of the parents is a prerequisite

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<sup>1</sup> Vegetable Research and Development Station Bacău, Romania

<sup>2</sup> University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

condition for breeding and especially for obtaining new cultivars with high level of performance. Cubero (1982) reported no significant genetic correlations between yield and fruit weight. Estimates of variance components and heritability have been determined in tomato for fruit weight, fruit size, and fruit set in studies (Cuartero, 1982).

The aim of our study was to save precious resources by collecting local native populations, old, primitive varieties, which are under risk in order to reduce genetic erosion and save entire material obtained by breeding. The study presents six promising tomato genotypes (superior plant characteristics, such as fruit firmness, color, and resistance to crack, pathogens, and storage)

## MATERIAL AND METHOD

A selection criterion was individual positive selection followed by selection of lines and mass selection.

As a calculation method, we used sequence variations, considering values of  $s\%$ :

- $s < 10$  – the traits is less variable,
- $10 < s < 20$  - the traits present middle variability,
- $20$  - the traits is very variable.

Biological material consists of six lines that have met stabilized features over the time. As control was used „Moldoveanca”, a variety developed in breeding programme at Vegetable Research and Development Station, Bacau.

Our studies on the main features were promoted from field base (collection - which holds over thirty cultivars), in work field, six genitors, with indeterminate growth. Promoting the genitors from the field of basic in work field was based on genetic stability of the main features.

There were performed biometric measurements and observations, using the evaluation criteria UPOV standards in case of all promoted to work field genitors.

The main characteristics investigated for all genitors (SP) were: plant height (cm); the number of shoots per plant; the number of leaves (below the first inflorescence and per plant); type and structure of inflorescence; presence of pedicle, pedicle length; average weight of fruit; the total weight of fruit per plant; fruit firmness, crack and storage resistance; number of seeds in fruit; predominant shape of the fruit; fruit height, fruit diameter; external color of immature and mature fruit; the aspect of fruit surface; number of seminal lodges; skin and flesh color of fruit.

## RESULTS AND DISCUSSIONS

Synthesis of all phenological observation and biometrical measurements permitted us a concise characterization of studied material. Four from six lines presents bifurcate type of inflorescence. The structure of inflorescence was lax in five cases at L12, L75, L73, L83, L53 and compact at L68. The pedicle was absent only at L12, in rest was present and long (tab. 1).

The total number of shoots per plant varies from 5 at L12 to 12 at L83. The number of leaves below the first inflorescence was 3 at L12, 5 at L68, 6 at L75 and 8 at L73, L83 and L53. We registered a large variation in case of total number of leaves per plant from 18 at L12 to 85 at L83. Plant height varies in small limits from 60 cm at L12, L75 and L68 to 75 cm at L73 and 80 cm at L73 (fig. 1).

Table 1

The main characteristics of tomatoes plants at genitors

| No. | Type of inflorescence | Inflorescence structure | Pedicle | Length pedicle |
|-----|-----------------------|-------------------------|---------|----------------|
| L12 | compose               | lax                     | absent  | -              |
| L75 | compose               | lax                     | present | long           |
| L68 | bifurcate             | compact                 | present | long           |
| L73 | bifurcate             | lax                     | present | long           |
| L83 | bifurcate             | lax                     | present | long           |
| L53 | bifurcate             | lax                     | present | long           |

Comparing each of the studied lines were noticed two: L12 with the lowest plant height – 18 cm, the smallest number of shoots - 5 and leaves (per plant – 18 and below the first inflorescence - 3) and L83 with the biggest number of shoots - 12 and leaves (per plant – 85 and below the first inflorescence - 8).

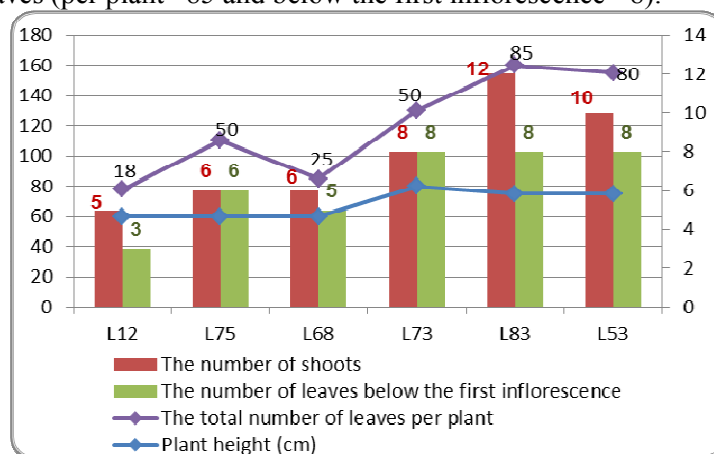


Fig. 1 - Variation of plant height, number of leaves (below the first inflorescence and per plant), number of shoots

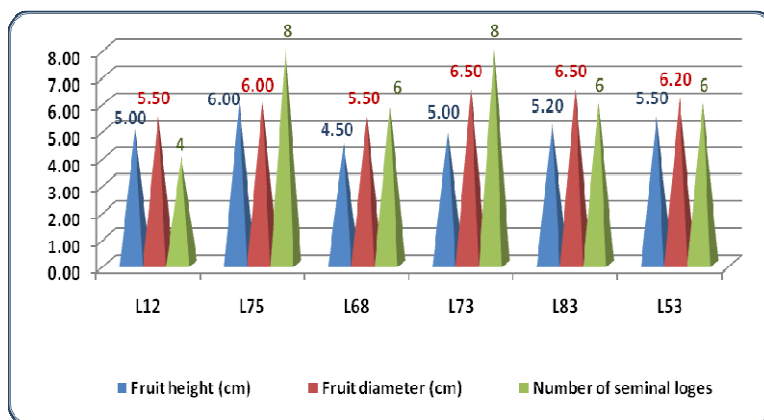
The shape of fruits was round, easy flattened and flattened. All lines presented at maturity fruits colored in light to dark red. The highest content of lycopene was in fruits of L 75 (the fruit flesh color was dark red) (tab. 2).

Table 2

The main characteristics of tomatoes fruits at genitors

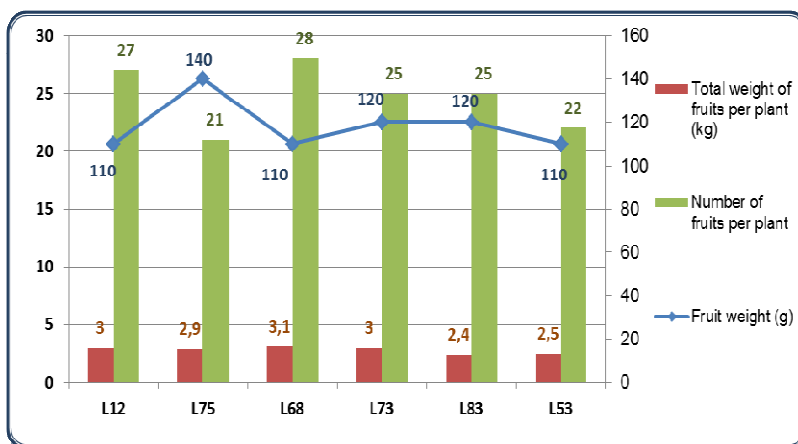
| No. | Dominant fruit shape | Immature fruit color | Mature fruit color | Fruit surfaces | Skin color | Flesh color |
|-----|----------------------|----------------------|--------------------|----------------|------------|-------------|
| L12 | round                | light green          | red                | smooth         | red        | red         |
| L75 | round                | light green          | dark red           | least cost     | red        | dark red    |
| L68 | easy flattened       | light green          | red                | smooth         | red        | red         |
| L73 | easy flattened       | with lid             | dark red           | least cost     | dark red   | red         |
| L83 | flattened            | light green          | light red          | cost           | bright red | red         |
| L53 | round                | with lid             | dark red           | cost           | dark red   | red         |

The fruit height and fruit diameter registered a small variation in limit of one cm: between 4,5 and 5,5 cm at fruit height and from 5,5 cm to 6,5 in case of fruit diameter. The number of seminal loges varies from 4 at L12 to 8 at L75 and L73 (fig. 2).



**Fig. 2** - Variation of fruit height, fruit diameter and number of seminal loges

L 68 noted by the largest amount of fruit harvested from a plant, 3,1 kg and also by a highest number of fruits per plant, 28 (fig. 3). L83 registered the lowest yield of fruits per plant. The heaviest fruits were the fruits of L75, 140g.



**Fig. 3** - Variation of fruit weight, weight of fruits per plant and number of fruits per plant

For successful production of tomatoes, yield and fruit size (measured as weight) must be considered (Wessel-Beaver, 1992).

Genotypes L12 and L75, were distinguished by firmness of fruits and resistance to crack and storage (tab. 3). L83 presented a low level of fruit firmness and medium resistance to crack and storage.

Table 3

## The main characteristics of tomatoes fruits at SP genitors

| No. | Fruit weight (g) | Total weight of fruits per plant (kg) | Number of fruits per plant | Fruits firmness | Crack resistance | Store resistance | Number of seeds in fruit |
|-----|------------------|---------------------------------------|----------------------------|-----------------|------------------|------------------|--------------------------|
| L12 | 110              | 3,0                                   | 27                         | good            | very good        | very good        | 277                      |
| L75 | 140              | 2,9                                   | 21                         | very good       | very good        | very good        | 165                      |
| L68 | 110              | 3,1                                   | 28                         | good            | good             | good             | 190                      |
| L73 | 120              | 3,0                                   | 25                         | medium          | good             | medium           | 380                      |
| L83 | 120              | 2,4                                   | 25                         | low             | medium           | medium           | 220                      |
| L53 | 110              | 2,5                                   | 22                         | good            | medium           | medium           | 180                      |

Regarding resistance to pest and disease four lines presents very good resistance: L12, L75, L68 and L73, and two lines were resistant to pest attack and disease (tab. 4).

All lines obtained a proper yield, quantitative superior to control variant, “Moldoveanca”, as follows: 90 t/ha at L12 and L73, 87 t/ha at L75, 75 t/ha at L53 and 72 t/ha at L83. The best yield was registered to L68, 93 t/ha (33 t/ha more than witness variant). The witness variant registered the lowest level of yield, 60 t/ha (Fig 4).

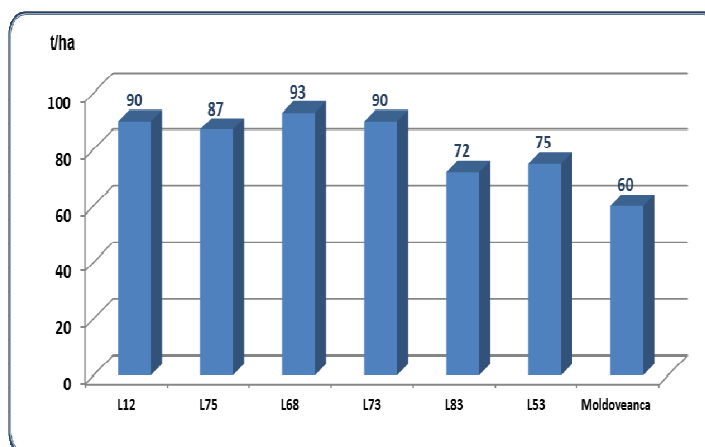
Table 4

## Synthesis of results regarding yield

| Variant                | Total yield |      | Difference on (control) | Signification of differences | Resistance to disease | Pest resistance |
|------------------------|-------------|------|-------------------------|------------------------------|-----------------------|-----------------|
|                        | %           | t/ha |                         |                              |                       |                 |
| L12                    | 150         | 90   | +30                     | ***                          | very good             | very good       |
| L75                    | 145         | 87   | +27                     | ***                          | very good             | very good       |
| L68                    | 155         | 93   | +33                     | ***                          | very good             | very good       |
| L73                    | 150         | 90   | +30                     | ***                          | very good             | very good       |
| L83                    | 120         | 72   | +12                     | ***                          | good                  | good            |
| L53                    | 125         | 75   | +15                     | ***                          | good                  | good            |
| Moldo-veanca (control) | 100         | 60   | -                       |                              | medium                | medium          |

DI 5% = 2,6 t/ha  
 DI 1% = 3,7 t/ha  
 DI 0,1% = 4,8 t/ha

Although the obtained yield is in accordance with the primary objective of tomato growers, to maximize the harvest of fruit per cultivation area, consumers put a great pressure on growers to improve both tomato yield and quality (Žnidarčič et al, 2003).



**Fig. 4** - Comparison of yield of six cultivars and witness variant

## CONCLUSIONS

The study aimed to relieve the effect of cross between a large number of bred genitors, in order to establish their combinative ability and a correlation between the factors that contribute to obtain valuable cultivars. Combinative ability of parents is one of the most important attributes that determine the value of new created cultivars.

The data presented indicates that total production increase and decrease compared with the maternal genitor, but depending on paternal used genitors.

All six lines achieved total production of over 70 t / ha in organic system culture (more with 12-33 t/ha comparing with control „Moldoveanca”).

Four of the lines have a very good resistance to attack of pests and pathogens, which entitles us to conclude that they are suitable for organic culture.

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# COMPARATIVE STUDY OF SOME FRENCH BEAN CULTIVARS CONCERNING THE SEEDS YIELD QUALITY AND QUANTITY

## STUDIUL COMPARATIV AL UNOR SOIURI DE FASOLE DE GRĂDINĂ CU PRIVIRE LA CALITATEA ȘI CANTITATEA PRODUCȚIEI DE SEMINȚE

CENUȘĂ Ana-Emilia<sup>1</sup>, POPESCU V.<sup>1</sup>  
e-mail: ema\_april@yahoo.com

**Abstract.** *The paper presents the results concerning a study on the optimum plant density obtained in a comparative culture in some dwarf garden bean cultivars used for seed production. The research works were carried out during the year 2011. The variants were placed in an experimental field according to the method of randomised blocks disposed in four replications. The observation and determination concerning the plants phenology and morphologic characters (the number of pods per plant, length of pod, MMB) permit the studied varieties characterisation and the sowing scheme recommendation. The experimental data obtained with regard to seed yields were statistic calculated according to the method of variant analyze. The maximum seed yields were obtained in the variant with three rows on bed at 37 cm apart, but we recommend the variant with two rows on bed at 70 cm apart, considering the necessity to ease the mechanical hoeing and harvesting.*

**Key words:** french bean, cultivar, morphological character, sowing scheme, seed production.

**Rezumat.** *Lucrarea prezintă rezultatele cu privire la studiul densității optime a plantelor obținute în cultură comparativă a unor soiuri de fasole de grădină pitică pentru producere de sămânță. Cercetările au fost efectuate în anul 2011. Variantele au fost amplasate în câmpul experimental conform metodei în blocuri randomizate, cu patru repetiții. Observațiile privind fenologia și caracterele morfologice ale plantelor (număr de păstăi pe plantă, lungimea păstăii, MMB) permit caracterizarea soiurilor studiate și recomandarea schemelor de semănat. Valorificarea datelor de producție seminceră s-a făcut după metoda analizei varianței. Producțiile maxime de semințe s-au realizat la varianta cu trei rânduri pe brazdă la 37 cm distanță, dar noi recomandăm varianta cu două rânduri pe brazdă la 70 cm distanță, deoarece asigură efectuarea mecanizată a lucrărilor de întreținere.*

**Cuvinte cheie:** fasole de grădină, soi, caractere morfologice, scheme de semănat, producția de semințe.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Bucharest, Romania

## INTRODUCTION

Permanent assurance of the seed amounts required for extension in the culture of the valuable varieties which are able to maintain their initial characteristics implies a constant application of some methods aiming this goal (Crăciun, 1987). Such techniques of intervention are developed on scientifically bases according to the biological specific of the species and variety in a well defined system under the name of “selected seed and planting material” (Dumitrescu et al., 1977).

So knowing the factors which have influence on the quality and seed yield obtained per ha (genetic background of the cultivar, maturity stage of the pods at the yield, seed size/weight of 1,000 seeds, environment and plant nutrition, phytosanitary status) become a main objective (Munteanu, 1994; Szilagy, 2002; Țigăieru, 2009).

## MATERIAL AND METHOD

Several autochthonous and foreign varieties of French bean recommended for their value by numerous authors (Dumitrescu et al., 1998; Ionescu and Ionescu, 1998; Glăman et al., 2002) were studied in comparative trials. Biological material under trial was represented by three dwarf Romanian varieties having green pods (Fantastica, Delicioasă de Pasărea, Ișalnița 43), three dwarf Romanian varieties having yellow pods (Margareta, Ioana, Mileniu), a foreign dwarf variety characterized by green pods (Prelude), a foreign dwarf variety with yellow pods (Gondola).

Aiming to establish the influence of the cultivar as well of the sowing scheme on the quality and quantity of seed production, an experience of bifactorial type (2x8) was set up consisting in randomized blocks of four replications, where the A factor was represented by the eight varieties while B factor consisted in two sowing densities having 500,000 plants/ha and 330,000 plants/ha respectively. By the combination of these factors and gradations resulted 16 experimental variants.

Agrotechnical works were specific for the seed production in dwarf French bean (Popescu and Atanasiu, 2001; Drăghici, 2006; Glăvan, 2000). Biological purifications were made and mass selection according to negative traits was applied aiming to remove plants which deviated from the main established traits being ill or untypical for the authorized varieties (Ciofu and Drăghici, 2002).

During the vegetative period some phenological observations and biometrical determinations were carried out (Hălmăgean, 2000). The results of production per ha were statistically computed according to the method of variance analysis of the blocks (Ceapoiu, 1968).

## RESULTS AND DISCUSSIONS

The results proved that from physical point of view (pod size, weight of the seeds) seeds are strongly influenced by the genotype and by the nutritive space of the plants (Ciofu et al., 2003).

Experimental data regarding some morphological characters of the plants, green pods and seeds are shown in the tab. 1.

Table 1

**Main morphological characteristics and yield of some  
French bean varieties under investigation**

| Variety               | Morphological traits of |                        |           |          |                    |                         |
|-----------------------|-------------------------|------------------------|-----------|----------|--------------------|-------------------------|
|                       | Plant                   | Green pods             |           |          | Dried seeds        |                         |
|                       | Stem height-cm          | Number of pods / plant | Length-cm | Width-cm | Number of seed/pod | Weight of 1,000 seeds-g |
| Fantastica            | 53.2                    | 15                     | 11.3      | 0.7      | 4                  | 207.0                   |
| Delicioasă de Pasărea | 48.3                    | 15                     | 14.4      | 0.9      | 4                  | 251.9                   |
| Işalniţa 43           | 55.7                    | 16                     | 12.4      | 0.7      | 3                  | 244.6                   |
| Prelude               | 40.6                    | 13                     | 8.3       | 0.7      | 3                  | 237.2                   |
| Margareta             | 43.0                    | 25                     | 9.6       | 0.6      | 4                  | 141.8                   |
| Ioana                 | 44.9                    | 18                     | 11.9      | 0.7      | 4                  | 178.3                   |
| Mileniu               | 52.3                    | 9                      | 12.1      | 1.0      | 3                  | 432.0                   |
| Gondola               | 44.7                    | 11                     | 12.3      | 0.6      | 3                  | 145.0                   |

The vegetative period was expressed by the number of days from the sprouting to physiological maturity by phenological stages (tab. 2).

Table 2

**Main phenological stages of the French bean varieties under investigation**

| Variety               | Number of days from the sprouting to |             |                                    |                                     |
|-----------------------|--------------------------------------|-------------|------------------------------------|-------------------------------------|
|                       | Flowering                            | Pod setting | Technological maturity of the pods | Physiological maturity of the seeds |
| Fantastica            | 38                                   | 44          | 62                                 | 76                                  |
| Delicioasă de Pasărea | 35                                   | 39          | 67                                 | 82                                  |
| Işalniţa 43           | 32                                   | 35          | 58                                 | 78                                  |
| Prelude               | 31                                   | 34          | 64                                 | 73                                  |
| Margareta             | 29                                   | 36          | 58                                 | 72                                  |
| Ioana                 | 31                                   | 40          | 68                                 | 89                                  |
| Mileniu               | 45                                   | 48          | 58                                 | 92                                  |
| Gondola               | 38                                   | 41          | 70                                 | 83                                  |

Seed yield obtained in the comparative culture for the orientation by yielding of every replication plot and referred per ha was statistically processed according to variance analysis using computing method for bifactorial trials organized in randomized blocks and were compared with the average production of the experimental variants (table 3).

Table 3

**Synthesis of the results from the comparative culture for the orientation of some French bean seed varieties under investigation**

| Variety               | Plant density 500,000 plants/ha |            |                 |              | Plant density 330,000 plants/ha |            |                 |              |
|-----------------------|---------------------------------|------------|-----------------|--------------|---------------------------------|------------|-----------------|--------------|
|                       | Seed yield                      |            |                 |              | Seed yield                      |            |                 |              |
|                       | t/ha                            | relative % | Difference t/ha | Significance | t/ha                            | relative % | Difference t/ha | Significance |
| Margareta             | 2.53                            | 131.1      | 0.60            | XXX          | 1.74                            | 131.8      | 0.42            | XXX          |
| Delicioasă de Pasărea | 2.47                            | 128.0      | 0.54            | XXX          | 1.60                            | 121.2      | 0.28            | XXX          |
| Ioana                 | 2.13                            | 110.4      | 0.20            | XXX          | 1.56                            | 118.2      | 0.24            | XXX          |
| Prelude               | 2.11                            | 109.3      | 0.18            | XXX          | 0.98                            | 74.2       | -0.34           | 000          |
| Fantastica            | 1.98                            | 102.6      | 0.05            | XXX          | 1.24                            | 93.9       | -0.08           | 000          |
| Işalniţa 43           | 1.94                            | 100.5      | 0.01            | -            | 1.58                            | 119.7      | 0.26            | XXX          |
| Media                 | 1.93                            | 100        | -               | -            | 1.32                            | 100        | -               | -            |
| Mileniu               | 1.42                            | 73.6       | -0.51           | 000          | 1.24                            | 93.9       | -0.08           | 000          |
| Gondola               | 0.83                            | 43.0       | -1.10           | 000          | 0.60                            | 45.5       | -0.72           | 000          |

LSD 5%=0,02

LSD 1%=0,03

LSD 0,1%=0,04

The differences registered between the variants and the averages of the trial are statistically assured being very significant.

The highest seed yield was registered in Margareta variety (2.14 t/ha) followed by Delicioasă de Pasărea variety (2.04 t/ha) while the lowest yield was obtained in Gondola variety (0.72 t/ha) for the both sowing densities.

Considering the influence of the plant density on the seed productivity some very significant yield gains were obtained in the variant having 500,000 plants/ha by comparison with a density of 330,000 plants/ha (0.61 t/ha). Margareta variety registered a yield of 2.53 t/ha for a density of 500,000 plants/ha and it gave 1.74 t/ha for a density of 330,000 plants/ha. On the other hand, Gondola variety registered a yield of 0.83 t/ha for a growing density of 500,000 plants/ha and of 0.60 t/ha for a density of 330,000 plants/ha.

### CONCLUSIONS

1. Both quantity of seed and its quality are evidently influenced by the two factors under investigation: growing density and variety.

2. The number of pods per plant and weight of 1,000 dried seeds are first of all variety characteristics having a decisive influence on the yield, but they can be influenced by technological links.

3. Maximum seed yields were obtained in the variant having a growing density of 500,000 plants/ha (three rows on the furrow at 37 cm distance) by comparison with the variant having 330,000 plants/ ha (two rows on the furrow at 70 cm distance).

4. The distance between the rows of plants of 37 cm does not assure an optimum space for the mechanical hoe application and many dwarf French bean plants were affected and even destroyed, because their roots were damaged. So, for this reason the yield was very much diminished. This is why we recommend the variant having two rows on the bed, at 70 cm distance, which allows application of the mechanical works in optimum conditions.

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# ASSESSMENT OF AGROBIOLOGICAL POTENTIAL OF THE *ORIGANUM VULGARE* L. SPECIE UNDER THE INFLUENCE OF THE DIFFERENT DENSITIES OF CULTIVATION

## EVALUAREA POTENTIALULUI AGROBIOLOG AL SPECIEI *ORIGANUM VULGARE* L. SUB INFLUENȚA DIFERITELOR DENSITATI DE CULTIVARE

*HOBINCU Marlina*<sup>1</sup>, *MUNTEANU N.*<sup>1</sup>  
e-mail: mhobincu@gmail.com

**Abstract.** *The study aims to highlight the influence of density of cultivation on the agrobiological potential of the oregano crop. The main characteristics considered in this experiment were: plant height, bush diameter, number of shoots/bush, number of inflorescences/shoot, number of leaves/shoot, the weight of the aerial fresh part, the weight of the aerial dry part, the weight of underground fresh part, the weight of underground dry part. Analyses show that the density of cultivation of 70 cm between rows and 35 cm between plants in turn, is most appropriate to achieve good results in terms of culture of oregano. Influence of the densities cultivation was determined by calculating the coefficient of variability.*

**Key words:** *Origanum vulgare*, agrobiological characteristics, density of cultivation

**Rezumat.** *Studiul urmărește să evidențieze influența densității de cultivare asupra potențialului agrobiologic al culturii de oregano. Principalele caracteristici avute în vedere în cadrul acestei experiențe au fost: înălțimea plantelor, diametrul tufelor, numărul de lăstari/tufă, numărul de inflorescențe/lăstar, numărul de frunze/lăstar, greutatea părții aeriene proaspete, greutatea părții aeriene uscate, greutatea părții subterane proaspete, greutatea părții subterane uscate. Analizele demonstrează că densitatea de cultivare, de 70 de cm între rânduri și 35 cm între plante pe rând, este cea mai indicată pentru a obține rezultate bune în privința culturii de oregano. Influența densității de cultivare a fost apreciată prin calculul coeficientului de variabilitate.*

**Cuvinte cheie:** *Origanum vulgare*, caracteristici agrobiologice, densitate de cultivare

### INTRODUCTION

In Romanian agricultural landscape, *Origanum vulgare* is a species known as the wild and the cultivated flora. The most important species of oregano are presented at the high altitude mountains of the Mediterranean countries, which are common in Greece, Asia Minor, Italy and even in the Balkans (Sarlis, 1994).

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

## MATERIAL AND METHOD

Biological material, in this experiment, consist of plants and plant fragments and fresh and dry harvest. Research was performed in the experimental field of Department of Vegetable growing from Faculty of Horticulture, held at the BIAROM Farm, in 2010-2011.

Oregano crop was established in May 2010, by seedlings of 50 days old. The seedlings were produced in the greenhouse conditions.

During the vegetation, the observations and the measurements were performed on the main biometric morphophysiological characteristics under the influence of different density of cultivation: 140 cm between rows and 70 cm between plants in the row, 70 cm between rows and 35 cm between plants in the row and the third density of 60 cm between rows and 30 cm between plants in the row. Each variant had three rehearsals.

Morphological study of plants of oregano was followed by comparative growth stages on phenophases (phenophase shoots issue, phenophase issue floriferous stems, flower buds and the formation phenophase complete flowering).

Influence of cultivation density was determined by calculating the standard deviation, amplitude variation and coefficient of variation. Data were processed by specific statistical methods.

Coefficient of variation was calculated in accordance with the literature, applying the known formulas (Saulescu and Saulescu, 1967) and respecting the following formula:  $V\% = \text{standard deviation} \times 100 / \text{arithmetic average}$ .

- low (close to lower limit) of the indicator indicates a homogeneous series (mean, median, modal value representative),
- high values (close to upper limit) of the indicator shows a heterogeneous series ((mean, median, modal value are unrepresentative).

$V\% \leq 10$  - character variability is considered small;

$V\% = 10$  to  $20$  - character variability is considered medium;

$V\% \geq 20$  - character analysis variability is high.

## RESULTS AND DISCUSSIONS

Visible growth of oregano plant, both in the phenophases period and from one year to another is a first observation that emerges from the results for the three densities analyzed cultivation.

Phenophase shoots issue is that you can see character analyzed the average variability (tab. 1). Its values are above the threshold of 10%, hovering around 14%.

Although in the formation phenophase flower stems, in 2010, this character indicates a low variability in the same phenophase, only a year later, the situation changes and the value easily exceeds ten percent. Values are somewhat stable in formation stage floral bud.

If the dynamics of the oregano plant height could talk about a important leap both in terms of development and growth phenophases and during this study, the same can't be said about the results of oregano bushes average diameters (tab.2).



Table 1

**Dynamics of oregano plant height (2010-2011)**

| Phenophase  | Issue shoots |       | Floriferous stems |       | Flower buds |      | Full flowering |       |
|---|--------------|-------|-------------------|-------|-------------|------|----------------|-------|
|   | 2010         | 2011  | 2010              | 2011  | 2010        | 2011 | 2010           | 2011  |
| The distance between rows x distance between plants in the row (cm) |              |       |                   |       |             |      |                |       |
| 140 X 70  | 16.2         | 22.1  | 37.9              | 42.2  | 54.6        | 60   | 62.6           | 67.2  |
| 70 X 35   | 19.8         | 21.2  | 42.3              | 53    | 57.9        | 67.4 | 77.6           | 80.2  |
| 60 X 30   | 15.3         | 16.7  | 39.6              | 48.2  | 56.8        | 61.5 | 72.3           | 76.7  |
| average standard deviation  | 1.37         | 1.67  | 1.28              | 3.12  | 0.97        | 2.26 | 4.39           | 3.88  |
| amplitude variation   | 4.50         | 5.40  | 4.40              | 10.80 | 3.30        | 7.40 | 15.00          | 13.00 |
| V %   | 13.93        | 14.47 | 5.56              | 11.32 | 2.98        | 6.21 | 10.74          | 9.01  |

Size of these plants is well-defined and no major differences that distinguish preference to factor concerned.

Table 2

**Dynamics of shrub diameter of oregano (2010-2011)**

| Phenophase  | Issue shoots |      | Floriferous stems |      | Flower buds |       | Full flowering |       |
|---|--------------|------|-------------------|------|-------------|-------|----------------|-------|
|   | 2010         | 2011 | 2010              | 2011 | 2010        | 2011  | 2010           | 2011  |
| The distance between rows x distance between plants in the row (cm) |              |      |                   |      |             |       |                |       |
| 140 X 70  | 60.6         | 65.3 | 63.2              | 67.6 | 65          | 75.3  | 77.5           | 80.4  |
| 70 X 35   | 62.1         | 68.8 | 73.8              | 74   | 81          | 90.9  | 88.6           | 97.6  |
| 60 X 30   | 62.0         | 64.1 | 63.4              | 73.7 | 68          | 77.5  | 83.3           | 98.7  |
| average standard deviation  | 0.48         | 1.41 | 3.50              | 2.09 | 4.91        | 4.87  | 3.21           | 5.93  |
| amplitude variation   | 1.50         | 4.70 | 10.60             | 6.40 | 16.00       | 15.60 | 11.10          | 18.30 |
| V %   | 1.36         | 3.70 | 9.08              | 5.03 | 11.92       | 10.39 | 6.68           | 11.13 |

At the beginning and end of the vegetation can be the difference of the coefficient of variation from one year to another, within the same phenophase, it has the ascending values. An average degree of homogeneity was determined in the third stage (V% 11.92 - 10.39 V%).

Known that plants need nutrition and development space relatively large, about the number of shoots per plant (table 3) can be identified increases in those two years, but the values differentiated variants particularly those with a distance between rows of 70 cm and 35 cm between plants in the row.

One can appreciate that density affects the number of shoots per plant and hence yield of oregano.

Table 3

**Results on the dynamics of the number of shoots/bush on plants of oregano  
(2010-2011)**

| Phenophase  | Issue shoots |       | Floriferous stems |       | Flower buds |       | Full flowering |       |
|---|--------------|-------|-------------------|-------|-------------|-------|----------------|-------|
|   | 2010         | 2011  | 2010              | 2011  | 2010        | 2011  | 2010           | 2011  |
| The distance between rows x distance between plants in the row (cm) |              |       |                   |       |             |       |                |       |
| 140 X 70  | 78           | 93    | 82                | 100   | 86          | 180   | 95             | 107   |
| 70 X 35   | 83           | 90    | 87                | 102   | 93          | 111   | 93             | 116   |
| 60 X 30   | 80           | 83    | 84                | 84    | 96          | 88    | 96             | 98    |
| average standard deviation  | 1,45         | 2,96  | 1,45              | 5,70  | 2,96        | 27,64 | 0,88           | 5,20  |
| amplitude variation   | 5,00         | 10,00 | 5,00              | 18,00 | 10,00       | 92,00 | 3,00           | 18,00 |
| V %   | 3,13         | 5,79  | 2,98              | 10,35 | 5,60        | 37,90 | 1,61           | 8,41  |

Average coefficient of variability met in this parameter has a low significance level, coelctivitatea tending to be heterogeneous, especially in flower bud formation stage where the figure recorded is above the average, V% - 37.90, although it is estimated that the coefficient over 35-40%, average is not representative.

The inflorescences means also very much to the success of a culture of oregano, as these crops because of the quality print in oils. In table 4, can be viewed as the number of inflorescences per plant dynamics show clear evidence, from year to year.

Table 4

**Dynamics of the number of inflorescences/shoot at the oregano plants 2010-2011)**

| Phenophase  | Issue shoots |       | Floriferous stems |       | Flower buds |       | Full flowering |       |
|---|--------------|-------|-------------------|-------|-------------|-------|----------------|-------|
|   | 2010         | 2011  | 2010              | 2011  | 2010        | 2011  | 2010           | 2011  |
| The distance between rows x distance between plants in the row (cm) |              |       |                   |       |             |       |                |       |
| 140 X 70  | 4            | 6     | 7                 | 8     | 9           | 11    | 12             | 13    |
| 70 X 35   | 7            | 8     | 8                 | 12    | 11          | 15    | 14             | 16    |
| 60 X 30   | 6            | 5     | 6                 | 9     | 9           | 12    | 12             | 17    |
| average standard deviation  | 0.88         | 0.88  | 0.58              | 1.20  | 0.67        | 1.20  | 0.67           | 1.20  |
| amplitude variation   | 3.00         | 3.00  | 2.00              | 4.00  | 2.00        | 4.00  | 2.00           | 4.00  |
| V %   | 26.96        | 24.12 | 14.29             | 21.53 | 11.95       | 16.43 | 9.12           | 13.58 |

In this case, the coefficient of variation values are averages chart, with one exception that we find in 2010, the full flowering, V% -9.12. High values of this coefficient we find in the first period of vegetation phenophases over both years, which is between V% - 26.96% and -24.12 V, see very high variability in the number of inflorescences.

Another feature to which we focused is the number of leaves on a shoot (table 5). How they are distributed on the shoot, leaves the important functions they perform (photosynthesis, respiration and perspiration) justify the choice. As can be seen in the table above dynamic number of leaves have a tendency to increase, while presenting the best results for the two density cultivation.

Table 5

**Dynamics of the number of leaves/shoot at the oregano plants (2010-2011)**

| Phenophase  | Issue shoots |      | Floriferous stems |       | Flower buds |      | Full flowering |      |
|---|--------------|------|-------------------|-------|-------------|------|----------------|------|
|   | 2010         | 2011 | 2010              | 2011  | 2010        | 2011 | 2010           | 2011 |
| The distance between rows x distance between plants in the row (cm) |              |      |                   |       |             |      |                |      |
| 140 X 70  | 28           | 37   | 50                | 59    | 73          | 84   | 88             | 90   |
| 70 X 35   | 36           | 43   | 62                | 70    | 73          | 86   | 96             | 99   |
| 60 X 30   | 33           | 41   | 58                | 67    | 71          | 82   | 89             | 96   |
| average standard deviation  | 2.33         | 1.76 | 3.53              | 3.28  | 0.67        | 1.15 | 2.52           | 2.65 |
| amplitude variation   | 8.00         | 6.00 | 12.00             | 11.00 | 2.00        | 4.00 | 8.00           | 9.00 |
| V %   | 12.50        | 7.57 | 10.78             | 8.70  | 1.60        | 2.38 | 4.79           | 4.82 |

Analyzing the coefficient of variation for this character, it is clear that the media has a high degree of representativeness, the community is homogeneous and uniform, especially in the last two phenophases of vegetation period, where the values are largely constant.

Although the influence of cultivation densities, analyzed in this study reveal no significant differences, however, best results were obtained and this time, the two of densities cultivation.

Table 6

**Dynamic values of the aerial weight, fresh and dried oregano plant (2010-2011)**

| Phenophase  | Aerial part of fresh mass (Kg) |       | Aerial part of dry mass (Kg) |       |
|---|--------------------------------|-------|------------------------------|-------|
|   | 2010                           | 2011  | 2010                         | 2011  |
| The distance between rows x distance between plants in the row (cm) |                                |       |                              |       |
| 140 X 70  | 2.04                           | 2.08  | 1.33                         | 1.19  |
| 70 X 35   | 2.57                           | 2.6   | 1.68                         | 1.47  |
| 60 X 30   | 2.42                           | 2.47  | 1.58                         | 1.4   |
| average standard deviation  | 0.16                           | 0.16  | 0.10                         | 0.08  |
| amplitude variation   | 0.53                           | 0.52  | 0.35                         | 0.28  |
| V %   | 11.66                          | 11.35 | 11.78                        | 10.77 |

Biomass is a character regarded as one of the most important indicators in assessing productivity. Comparing reference values of the coefficient of variability of results, there is a variability average secondary between and V -

11.35% and V -11.66%, the aerial part fresh mass between V and V% -11.78% and V - 10.77 the aerial part dry mass.

Another aspect studied was the weight of the underground, dried and fresh oregano plant. Studied through the cultivation density in this case, the recommended density 70 cm/35 cm seems.

Table 7

**Dynamic values of the weight of the underground fresh and dried oregano plant (2010-2011)**

| Phenophase  | Underground part of fresh mass (Kg) |       | Underground part of dry mass (Kg) |       |
|---|-------------------------------------|-------|-----------------------------------|-------|
|   | 2010                                | 2011  | 2010                              | 2011  |
| The distance between rows x distance between plants in the row (cm) |                                     |       |                                   |       |
| 140 X 70  | 2.06                                | 2.10  | 1.35                              | 1.20  |
| 70 X 35   | 2.60                                | 2.63  | 1.70                              | 1.49  |
| 60 X 30   | 2.45                                | 2.50  | 1.60                              | 1.42  |
| average standard deviation  | 0.16                                | 0.16  | 0.10                              | 0.09  |
| amplitude variation   | 0.54                                | 0.53  | 0.35                              | 0.29  |
| V %   | 11.76                               | 11.46 | 11.63                             | 11.05 |

Also, the follow calculations, variability indicator is considered average, falling within a fairly small interval (V% - 11.46% and V% -11.76 ,underground part of fresh biomass and V% -11.05 and V %-11.63 , underground part of dry biomass).

## CONCLUSIONS

1. The morphological characterization of oregano plants reveal that plants had a morphology that fits in botanical description of this species, experimental conditions presented satisfying environmental requirements of plants, allowing their proper development.

2. Phenological observations have revealed characteristics of oregano plants, is carried out under optimum phenophases ontogenetic evolution of the species, allowing us to conclude that *O. vulgare* found optimal growth and development.

3. The effective density cultivation for this crop, in this study, is 70 cm between rows and 35 cm between plants in the row, and use appropriate technology based on continuous optimization of technological factors is of primary importance.

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# QUANTIFYING THE INFLUENCE A SOME DIFFERENT ORGANIC SUBSTRATES ON SEED GERMINATION OF *ORIGANUM VULGARE* L.

## CUANTIFICAREA INFLUENȚEI UNOR SUBSTRATURI ORGANICE ASUPRA GERMINĂRII SEMINTELOR DE *ORIGANUM VULGARE* L.

HOBINCU MARLENA<sup>1</sup>, MUNTEANU N.<sup>1</sup>

e-mail: mhobincu@gmail.com

**Abstract.** Germination is one of the most important technological phase, in order to get a quality harvest. Knowing that the oregano seeds presents a low germination, this study aims to find the best organic substrate whose formula is best suited to a level as high germination. Thus, there was used seven soil mixing formulas which have in their composition, peat, manure, perlite and sand in different proportions. Quantifying the influence of organic substrates on seed germination of oregano was estimated through the rate of germination (emergence, dynamic of germination rate, dynamic of germination velocity and germination velocity coefficient).

**Key words:** *Origanum vulgare*, germination, organic substrates

**Rezumat.** Germinația este una dintre cele mai importante secvențe tehnologice în vederea obținerii unei recolte de calitate. Știut fiind că semințele de oregano prezintă un grad scăzut de germinare, acest studiu își propune să găsească cel mai bun substrat organic, al cărei formulă să fie optim pentru un nivel de germinare cât mai ridicat. Astfel, s-au utilizat șapte formule de amestec, având în componența lor turbă, mranită, perlit și nisip în diverse proporții. Cuantificarea influenței substraturilor organice asupra germinării semințelor de oregano s-a estimat prin prin rata de germinare (răsărire, dinamica ratei de germinare, dinamica vitezei de germinare și a coeficientului vitezei de germinare).

**Cuvinte cheie:** *Origanum vulgare*, germinație, substraturi organice

### INTRODUCTION

Many studies made, such as Putievsky (1983) and Thanos et al. (1995), the germination of different species of *Origanum*, have confirmed their low germination capacity. This was previously observed by Theophrastus (371-287 B.C.), in his *Historia Plantarum*, who observed that the maximum percentage and germination speed were obtained under certain conditions, seeds germination in 62% of the five days.

Importance of sowing beds on the seed germination is well known (Stan and Stan, 1999) and have recently confirmed (Stan et al., 2008). In this respect, our studies are targeted on the influence of different organic soil mixing formulas on the oregano seed germination.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

## MATERIAL AND METHOD

Experience was organized as a bifactorial type (3Ax7B) and was conducted in the Vegetable growing laboratory of University of Agricultural Sciences and Veterinary Medicine from Iasi, using oregano seeds from different origins (Romania, Italy and Greece).

Factor A was the cultivar, while factor B was the substrate of cultivation. Factor A consists in three unnamed cultivars from Romania, Italy and Greece. Factor B consists in seven combinations of mixtures between peat, manure, perlite and sand. Mixtures were placed in sterile Petri dishes. In each Petri dish were sown with 100 seeds.

After sowing, the dishes were placed in germinator (SANYO MLR Germinator 351 H), ensuring oregano seed germination conditions (temperature, humidity, light). Thus, the temperature fluctuated between 22°C and 24°C, humidity ranging between 70-80%, and the light was about 10.000 luxes, especially in the second part of the experience. Germination for all three cultivars was compared with standard germination (germination carried out at Inspectorate for Seed Quality from Iasi). Germination and emergence substrates are detailed in table 1:

Table 1

**Substrates used in the study of the influence on the process of germination of the oregano seeds**

| Crt. no. | Variant        | Specification  | Composition of nutrient substrates (% volumetric) |
|----------|----------------|--|---|
| 1.       | V <sub>1</sub> | T <sub>100</sub> - peat  | 100% peat   |
| 2.       | V <sub>2</sub> | T <sub>75</sub> +M <sub>25</sub>                                   | 75% peat +25% manure                              |
| 3.       | V <sub>3</sub> | T <sub>75</sub> +N <sub>25</sub>                                   | 75% peat +25% sand                                |
| 4.       | V <sub>4</sub> | T <sub>75</sub> +P <sub>25</sub>                                   | 75% peat +25% pearl stone                         |
| 5.       | V <sub>5</sub> | T <sub>70</sub> +M <sub>20</sub> +N <sub>10</sub>                  | 70% peat +20% manure +10% sand                    |
| 6.       | V <sub>6</sub> | T <sub>70</sub> +M <sub>20</sub> +P <sub>10</sub>                  | 70% peat +20% manure +10% pearl stone             |
| 7.       | V <sub>7</sub> | T <sub>50</sub> +M <sub>20</sub> +N <sub>10</sub> +P <sub>10</sub> | 50%peat +20%manure +10%sand+10%pearl stone        |

Influence of nutritional substrates mixtures of crop was assessed by the rate of germination (emergence, growth rate of germination, growth velocity of germination and germination velocity coefficient). Velocity of germination (germination velocity or speed of germination) represents the percentage of germinated plants per unit time (day) and is given by:

$$V_G = \frac{G_i}{n}, \text{ where:}$$

G<sub>i</sub> = germination in the time unit;

n = number of days the germination was achieved G<sub>i</sub>.

Coefficient of germination velocity is velocity final germination compared with germination of seeds and is calculated (after Kotowski, 1926, cited by Stan, 2010):

$$CV_G = \frac{G_i}{G_f \cdot n} \times 100, \text{ where:}$$

CV<sub>G</sub> = coefficient of germination velocity;

G<sub>i</sub> = germination in the time unit;

G<sub>f</sub> = final germination;

n = number of days the germination was achieved G<sub>i</sub>.

In the assessment methods used in the study recognized and germination, in the present study we treated emergence of germination, emergence and appreciated that when the seed/soil strains surfaced about 0.5 to 0.7 mm.

## RESULTS AND DISCUSSIONS

Dynamics emergence of oregano plants was strongly influenced by the composition of substrates (table 2). Start the process of emergence seeds was 3-4 days after sowing. Looking through the growing substrates of crop was observed that the most important influence in Italy seeds was  $V_4$  ( $T_{75}+P_{25}$ ) where the germination percentage was 89%.

In second place, in the three categories of seed, was the nutritive substrate of peat 100%. The mixture  $V_5$ , the composition which is peat, manure and sand has led a number of seed germination considerably higher, whose Italian origin is (87%).

Table 2

**Dynamics of germination/emergence of oregano seeds  
(population of Romania, Greece and Italy)**

| Variant                       |         | Dates / Germination rate (%) |       |       |       |       |       |       |       |
|-------------------------------|---------|------------------------------|-------|-------|-------|-------|-------|-------|-------|
| Specification                 | Country | 20.03                        | 21.03 | 22.03 | 23.03 | 24.03 | 25.03 | 26.03 | Total |
| $T_{100}$                     | Romania | 2                            | 17    | 14    | 14    | 6     | 2     | 5     | 60    |
|                               | Greece  | 4                            | 14    | 20    | 18    | 1     | 1     | 12    | 70    |
|                               | Italy   | 6                            | 35    | 29    | 10    | 2     | 2     | 3     | 87    |
| $T_{75}+M_{25}$               | Romania | 2                            | 17    | 23    | 12    | 2     | 3     | 23    | 82    |
|                               | Greece  | 2                            | 15    | 20    | 22    | 5     | 3     | 0     | 67    |
|                               | Italy   | 6                            | 23    | 25    | 13    | 9     | 1     | 4     | 81    |
| $T_{75}+N_{25}$               | Romania | 1                            | 13    | 13    | 13    | 4     | 5     | 4     | 53    |
|                               | Greece  | 6                            | 12    | 13    | 7     | 1     | 1     | 7     | 47    |
|                               | Italy   | 5                            | 32    | 24    | 2     | 9     | 5     | 5     | 82    |
| $T_{75}+P_{25}$               | Romania | 0                            | 8     | 19    | 6     | 5     | 2     | 9     | 49    |
|                               | Greece  | 1                            | 13    | 18    | 16    | 2     | 2     | 0     | 52    |
|                               | Italy   | 2                            | 25    | 25    | 5     | 17    | 7     | 8     | 89    |
| $T_{70}+M_{20}+N_{10}$        | Romania | 6                            | 6     | 9     | 4     | 0     | 1     | 15    | 41    |
|                               | Greece  | 2                            | 12    | 21    | 25    | 3     | 1     | 8     | 72    |
|                               | Italy   | 3                            | 26    | 47    | 2     | 4     | 1     | 4     | 87    |
| $T_{70}+M_{20}+P_{10}$        | Romania | 5                            | 8     | 15    | 16    | 2     | 0     | 4     | 50    |
|                               | Greece  | 2                            | 7     | 30    | 17    | 5     | 1     | 6     | 68    |
|                               | Italy   | 1                            | 25    | 20    | 7     | 6     | 3     | 7     | 69    |
| $T_{50}+M_{20}+N_{10}+P_{10}$ | Romania | 3                            | 3     | 21    | 8     | 4     | 8     | 13    | 60    |
|                               | Greece  | 0                            | 12    | 31    | 9     | 4     | 0     | 2     | 58    |
|                               | Italy   | 2                            | 26    | 22    | 8     | 4     | 4     | 3     | 69    |

For the Romanian origin seeds the same substrate is in last place, with a germination percentage of only 40% as opposed to those of Greek origin where he obtained a value of 72%.

Table 3

**Emergence velocity dynamics of oregano plants**  
(population of Romania, Greece and Italy)

| Variant  |         | Emergence plants | v/cv* | Dates/velocity/velocity ratio |       |       |       |       |       |       |       |
|--|---------|------------------|-------|-------------------------------|-------|-------|-------|-------|-------|-------|-------|
| Specif.  | Country |                  |       | 20.03                         | 21.03 | 22.03 | 23.03 | 24.03 | 25.03 | 26.03 | av.   |
| T <sub>100</sub>   | Ro.     | 60               | v     | 0,66                          | 4,75  | 6,6   | 7,83  | 7,57  | 6,87  | 6,66  | 5,84  |
|  |         |                  | cv    | 1,11                          | 7,91  | 11    | 13,05 | 12,61 | 11,45 | 11,11 | 9,81  |
|  | Gr.     | 70               | v     | 1,33                          | 4,5   | 7,6   | 9,33  | 8,14  | 7,25  | 7,77  | 6,56  |
|  |         |                  | cv    | 1,9                           | 6,42  | 10,85 | 13,33 | 11,63 | 10,35 | 11,11 | 9,37  |
|  | It.     | 87               | v     | 2                             | 10,25 | 14    | 13,33 | 11,71 | 10,25 | 9,33  | 10,12 |
|  |         |                  | cv    | 2,29                          | 11,78 | 16,09 | 15,32 | 13,46 | 12,06 | 11,11 | 11,73 |
| T <sub>75+M<sub>25</sub></sub>                               | Ro.     | 82               | v     | 0,66                          | 4,75  | 8,4   | 9     | 8     | 7,37  | 9,11  | 6,75  |
|  |         |                  | cv    | 0,81                          | 5,79  | 10,24 | 10,97 | 9,75  | 8,99  | 11,11 | 8,23  |
|  | Gr.     | 67               | v     | 0,66                          | 4,25  | 7,4   | 9,83  | 9,14  | 8,37  | 7,44  | 6,72  |
|  |         |                  | cv    | 0,99                          | 6,34  | 11,04 | 14,67 | 13,64 | 12,5  | 11,11 | 10,04 |
|  | It.     | 81               | v     | 2                             | 7,25  | 10,8  | 11,16 | 10,85 | 9,5   | 8,55  | 8,58  |
|  |         |                  | cv    | 2,46                          | 8,95  | 13,33 | 13,78 | 13,48 | 11,88 | 11,11 | 10,71 |
| T <sub>75+N<sub>25</sub></sub>                               | Ro.     | 53               | v     | 0,33                          | 3,5   | 5,4   | 6,66  | 6,28  | 6,12  | 5,88  | 4,88  |
|  |         |                  | cv    | 0,62                          | 6,6   | 10,18 | 12,57 | 11,85 | 11,55 | 11,11 | 9,21  |
|  | Gr.     | 47               | v     | 2                             | 4,5   | 6,2   | 6,33  | 5,57  | 5     | 5,22  | 4,97  |
|  |         |                  | cv    | 4,25                          | 9,57  | 13,19 | 13,47 | 11,85 | 10,63 | 11,11 | 10,58 |
|  | It.     | 82               | v     | 1,66                          | 9,25  | 12,2  | 10,5  | 10,28 | 9     | 8,5   | 8,77  |
|  |         |                  | cv    | 2,03                          | 11,28 | 14,87 | 12,88 | 12,54 | 11,73 | 11,11 | 10,92 |
| T <sub>75+P<sub>25</sub></sub>                               | Ro.     | 49               | v     | 0                             | 2     | 5,4   | 5,5   | 5,42  | 5     | 5,44  | 4,1   |
|  |         |                  | cv    | 0                             | 4,08  | 11,02 | 11,22 | 11,07 | 10,24 | 11,11 | 8,39  |
|  | Gr.     | 52               | v     | 0,33                          | 3,5   | 6,4   | 8     | 7,14  | 6,5   | 5,77  | 5,37  |
|  |         |                  | cv    | 0,64                          | 6,73  | 12,3  | 15,38 | 13,73 | 12,5  | 11,11 | 10,34 |
|  | It.     | 89               | v     | 0,66                          | 6,75  | 10,4  | 9,5   | 10,57 | 10,12 | 9,88  | 8,26  |
|  |         |                  | cv    | 0,74                          | 7,58  | 11,68 | 10,67 | 11,87 | 11,37 | 11,11 | 9,28  |
| T <sub>70+M<sub>20</sub>+N<sub>1</sub></sub>                 | Ro.     | 41               | v     | 2                             | 3     | 4,2   | 4,16  | 3,57  | 3,25  | 4,55  | 3,53  |
|  |         |                  | cv    | 4,87                          | 7,31  | 10,24 | 10,16 | 8,7   | 7,92  | 11,11 | 8,61  |
|  | Gr.     | 72               | v     | 0,66                          | 3,5   | 7     | 10    | 9     | 8     | 8     | 6,59  |
|  |         |                  | cv    | 0,92                          | 4,86  | 9,72  | 13,88 | 12,5  | 11,11 | 11,11 | 9,15  |
|  | It.     | 87               | v     | 1                             | 7,25  | 15,2  | 13    | 13,66 | 10,37 | 9,66  | 10,02 |
|  |         |                  | cv    | 1,14                          | 8,33  | 17,47 | 14,94 | 13,46 | 11,92 | 11,11 | 11,19 |
| T <sub>70+M<sub>20</sub>+P<sub>10</sub></sub>                | Ro.     | 50               | v     | 1,66                          | 3,25  | 5,6   | 7,33  | 6,57  | 5,75  | 5,55  | 5,1   |
|  |         |                  | cv    | 3,33                          | 6,5   | 11,2  | 14,66 | 13,14 | 11,5  | 11,11 | 10,2  |
|  | Gr.     | 68               | v     | 0,66                          | 2,25  | 7,8   | 9,33  | 8,71  | 7,75  | 7,55  | 6,29  |
|  |         |                  | cv    | 0,98                          | 3,3   | 11,47 | 13,72 | 12,81 | 11,39 | 11,11 | 9,25  |
|  | It.     | 68               | v     | 0,33                          | 6,25  | 9     | 8,66  | 8,28  | 7,62  | 7,55  | 6,81  |
|  |         |                  | cv    | 0,49                          | 9,19  | 13,23 | 12,74 | 12,18 | 11,21 | 11,11 | 10,02 |
| T <sub>50+M<sub>20</sub>+N<sub>10</sub>+P<sub>10</sub></sub> | Ro.     | 60               | v     | 1                             | 1,5   | 5,4   | 5,83  | 5,57  | 5,87  | 6,66  | 4,54  |
|  |         |                  | cv    | 1,66                          | 2,5   | 9     | 9,72  | 9,28  | 9,79  | 11,11 | 7,58  |
|  | Gr.     | 58               | v     | 0                             | 3     | 8,6   | 8,66  | 8     | 7     | 6,44  | 5,95  |
|  |         |                  | cv    | 0                             | 5,17  | 14,82 | 14,94 | 13,79 | 12,06 | 11,11 | 10,27 |
|  | It.     | 67               | v     | 0,66                          | 7     | 10    | 9,66  | 8,85  | 8,25  | 7,44  | 7,4   |
|  |         |                  | cv    | 0,99                          | 10,44 | 14,92 | 14,42 | 13,21 | 12,31 | 11,11 | 11,05 |



Comparing these results with those obtained from Inspectorate of Seed Quality Iasi, the inspectorate results were significantly lower in all three cases analyzed. Thus, germination of seeds from Italy determined was 63%, Greek seed representative result was only 46% and the Romanian seeds was 10 percent higher. During emergence of monitoring emergence that the highest rate of germination occurs within days of the beginning emergence. An important step of germination rate can be easily seen in variants  $V_1$  ( $T_{100}$ -turbă) and  $V_2$  ( $T_{75}+M_{25}$ ) from the fourth day. Dynamics of emergence or "acceleration" of the oregano plant emergence, the regions of Greece and Italy have numbers higher than in this country, which was in close correlation with the particular requirements for temperature, but with some specific biological features.

Emergence velocity and coefficient of velocity were favorably influenced by the presence in all recipes and nutritional mixtures of peat, in different quantities, which were substrates for germination/emergence the oregano seeds.

A suggestive comparison of velocity coefficient values belonging the seeds of Greek and Romanian origin, is that the seeds of Greek origin values are between 9 and 10, unlike those whose origin is Romanian, where the range of values is more generous.

The aspect we show that the seeds of Greek origin have a more uniform emergence on the one hand, and a more obvious germination, on the other part. In this experience, it seems that a positive for both varieties presented so far, has the composition of the nutrient, which is peat and manure ( $T_{75}+M_{25}$ ). Very good results were recorded, especially if seeds of Italian origin from the second day, with a peak in the third day. Dynamics of average values of velocity of emergence falling from 6.81 plants/day, the  $V_6$  ( $T_{70}+M_{20}+P_{10}$ ) to 10.12 plants/day, nutrient substrate composed of peat 100%. Clearly, this time, the speed of seed germination of oregano, Italian provenance amplitude shows a much higher average values seen in parallel with the other two varieties analyzed during this study. Velocity coefficient of dynamic emergence presents a similar evolution velocity, both from variant to the variant, but also in terms of seed origin. It may be noted that emergence best results were achieved by the entire experience, in order, the following:  $V_1$  (CV=11.73 %),  $V_5$  (CV= 11.19%) și  $V_7$  (CV=11.05%). The average value for all period of emergence of velocity coefficient of these variables ranged from 7.58% at version  $V_7$  ( $T_{50}+M_{20}+N_{10}+P_{10}$ ) for seeds of Romanian origin and 11.73% at  $V_1$  ( $T_{100}$ ) for seeds from Italy.

## CONCLUSIONS

1. From this study show that adding peat and manure the nutrient substrate causes an increase in both the average rate of germination and emergence. They are highest values in the early days, and are greatly diminished over time reaching a few percent in the last days of emergence, which shows a good uniformity of emergence.

2. Emergence velocity and velocity ratio were favorably influenced by the presence in all recipes and nutritional mixtures of peat, in different quantities, which were substrates for germination/emergence of the oregano seeds.

3. It notice that the velocity coefficient values are generally the same dynamic path, as velocity. This is possible in the final emergence has values close to all variants.

4. In this experience, it seems that a positive effect varieties in Greece and Romania present a nutritiv mixture, in the composition of which is peat and mraniță (T<sub>75</sub>+M<sub>25</sub>) and the crop substrate composed of peat 100% only seed from Italy.

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# PRELIMINARY STUDIES FOR THE INTRODUCTION OF THE JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS* L.) IN THE ROMANIAN VEGETABLE PRODUCTION

## STUDII PRELIMINARE PENTRU INTRODUCEREA ÎN CULTURĂ A TOPINAMBURULUI LEGUMICOL (*HELIANTHUS TUBEROSUS* L.)

MUNTEANU N.<sup>1</sup>, DASCALU T.<sup>1</sup>, GUTIERREZ Patricia<sup>2</sup>,  
e-mail: dascalu\_teofil@yahoo.com

**Abstract.** *The paper is based on a documentary as well as on a field study regarding the importance of the culture, the biological and ecological characteristics of the Jerusalem artichoke species (*Helianthus tuberosus* L.). Moreover, some extremely important information regarding the importance of the Jerusalem artichoke in man's diet, the risk factors of the culture has been detailed, which provides a more thorough documentation of a growth technology for this species in Romania's climate.*

**Key words:** Jerusalem artichoke, botanical characteristics, importance of the culture, ecological characteristics, risk factors.

**Rezumat.** *Lucrarea constă într-un studiu documentar și în teren privind importanța culturii, particularitățile biologice și cele ecologice ale speciei legumicole topinambur (*Helianthus tuberosus* L.). În plus, au fost evidențiate și unele informații deosebit de utile privind importanța topinamburului în alimentația omului, factorii de risc ai culturii, care asigură o mai completă fundamentare a unei tehnologii de cultivare a acestei specii în condițiile din România.*

**Cuvinte cheie:** topinambur, particularități botanice, importanța culturii, caracteristici ecologice, factori de risc.

### INTRODUCTION

Jerusalem artichoke is grown for its tubers which can be eaten boiled (in most cases, in soups or other flavored dishes), baked or even raw, for their special taste and nutrient value. When eaten raw, the tubercles can be thinly sliced for different types of simple or assorted salads especially for enriching the salad with a crunchy texture and a slightly taste of walnut. They can also be canned, used as simple pickles or assorted.

In Europe, Jerusalem artichoke tubers are used especially for preparing the so-called „Palestina soup” (Grigson, 1978). In France, the tubercles have been avoided for a long period of time because of their culinary value, but lately more and more exquisite restaurants have added a variety of Jerusalem artichoke dishes on their menu lists (Henning, 2000).

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

<sup>2</sup> Tuscia University ,Viterbo, Italy

The chemical composition of Jerusalem artichoke tubers for 100 g of fresh product is : 80% water, 15% carbohydrates, 1-2% proteins, 0,1-0,6g lipids, 0,6-4 g fibers. The content of the tuber in macro elements, calculated for 100 grams of fresh product is: 560 mg potassium, 0,25 mg nitrogen, 25 mg calcium, 3,4 mg iron, 16 mg magnesium, 3 mg sodium, 78 mg phosphor, 0,1 mg zinc, 0,6 µg vitamin A, 0,3 mg vitamin B1, 0,005 mg vitamin B2, 0,5 mg niacin, 0,09 mg vitamin B6, 13 µg acid folic.

Jerusalem artichoke is a species with certain distinctive biochemical properties. Inulin is stored in the tubers as reserve substance while other species have starch as reserve, the main source of carbohydrates. Inulin, together with a high content of mineral salts render the Jerusalem artichoke a great importance in human diets, especially, for the diets of the persons suffering of obesity and diabetes.

The energetic value of the fresh Jerusalem artichoke tubercles is 650 kcal, and cooked (boiled tubercles) 410 kcal.

The agronomic importance of the specie comes from the fact that Jerusalem artichoke can be very well grown in fields with low fertility as a crop that requires minimal works. Actually, great productivity can be achieved, in quantity as well as in quality, by using modern technologies in growing Jerusalem artichoke. Jerusalem artichoke can be grown on a variety of systems and types of cultures: in the field, in intensive or sustainable systems (such as ecological culture) or for household use, on his own field or in vegetable associations.

The economical and social importance derives from the fact that Jerusalem artichoke ensures considerable revenues within a consolidated market, with demands for this product in a balanced report with the expenses.

The profit of the culture is low if there is no incisive scientifically documented promotion on the market in order to increase the demand for this product. In the case of the ecological culture, the demand for Jerusalem artichoke tubers is higher due to better information of the customers, so the profit of the culture is higher. The tubers can be easily stored for 6 to 12 months, if the appropriate technical solutions are used, maintaining a relative higher humidity in the storage spaces and a low temperature (between 0-2°C) in order to determine a decrease in the breathing process of the stored tubers.

The advantages and disadvantages of the Jerusalem artichoke culture must be completed by some risk factors which can compromise the crop and the measures that must be taken to avoid this.

In their chronological order of manifestation, the main risks are (synthesis according to Stanley, 2008):

- using planting material that has been infected with a virus determines a delay of the culture, reduced growth, decrease in production from the point of view of its quantity as well as of its quality,
- setting up a culture within an improper rotation (the species being extremely sensitive to *Sclerotinia sclerotiorum* Lib) which leads to a compromised culture.

Our paper wants to present the main aspects regarding crop importance, biological and ecological features in connection with some cultivar practices.

## MATERIAL AND METHOD

The present study was realized based on the analysis of the information existent in the specialized literature.

The biological and ecological characteristics, the risk factors and the importance of the Jerusalem artichoke have been analyzed and the work method was the comparative analyze method of the information in the specialized literature in Romania and the one abroad.

## RESULTS AND DISSCUSSIONS

*Helianthus tuberosus* L. species belongs to the *Helianthus* type, which is part of the *Asteraceae* family, *Asterales* order, genomic formula  $2n=102$ .

There are 10 species of the *Helianthus* type, but because of the high number of natural hybrids, some authors classified more than 70 species within the same gender (Heiser, 1995).

**Biological characteristics.** Jerusalem artichoke, when in a culture is behaving like an annual species, although at its origin places and in its spontaneous form it is a perennial species.

The plants are herbaceous, but in the second period of their life cycle in a year it begins to wooden around the area of the main roots and the inferior ramifications.

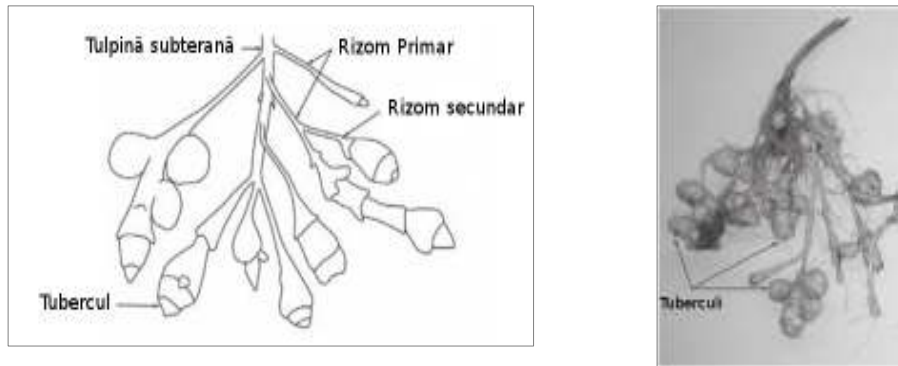
The root presents itself in a fibrous system, strongly developed in the above soil layer as well as deep in the ground. The weight of the dry matter of the root is bigger in the case of the cultivated clones (15 g), in comparison with the spontaneous populations (12,7g / plant) (Swanton, 1986).

Jerusalem artichoke has two types of stems: underground and aerial. Underground stems are represented by rhizomes (incorrectly called stools by some authors) and tubers. Rhizomes are slightly thickened underground stems with a length of up to 1,5 m (fig. 1), generally of white color, which can also form tubers by turning their ends into tubercles. When the formation of rhizomes is inhibited, just as it is the case of the cultures on compacted soils, the tuber production greatly decreases.

The tubers can have a variety of shapes, from rounded-elongated to an irregular shape with a lot of secondary tubercles, cluster type (Alex and Switzer, 1976), of smaller or bigger dimensions according to the clone and climate conditions. Three types have been found: big, more than 50g / tuber; medium, 20-50g / tuber, small, less than 20g / tuber (Pas'ko, 1973).

The tubers can also present nodes where secondary tubercles appear (an undesirable fact for commercial cultures).

The external color of the tubercles can be: white, red and violet or violet or dark-brown; the internal color, the most frequent, is white or white with a shade of pink (Pas'ko, 1973).

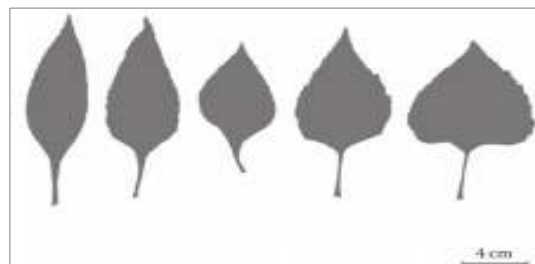


**Fig. 1** - General morphology of the underground stem, of the Jerusalem artichoke rhizomes and tubercles. (after Kays, 2006)

On the surface of the tubercles, there can be noticed the “eyes” situated in a pocket, being formed of several buds. The eyes are disposed along a spiral line around the tuber, being scarce at the base and denser towards the ends, just like the potato tubers (Stan and Munteanu, 2001). In proper conditions, from an eye aerial stems are formed. The number of stems that emerge from a single eye varies from one to three main stems, depending on the cultivar.

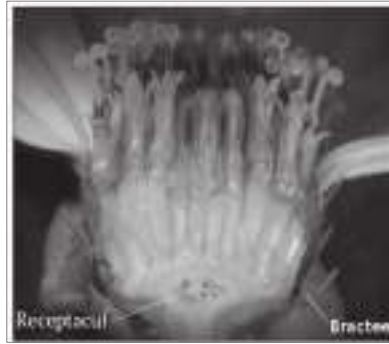
The aerial stem is straight, with a well developed mechanic tissue and can reach a height of 3 m or even more. In the culture there are also known „dwarf” clones, with a height of about 1 meter (Zubr and Pedersen, 1993). The number of the ramifications varies very much according to their position on the main stem. In the case of the clones with very tall stems, for example 4 m, the number of the tubers is lower.

The leaf is simple, lanceolate or lance-ovate of 10-20 cm in length and 5-10 cm wide, acuminate, serrated, pubescent on the inferior side, with acuminate at the end (fig. 2).



**Fig. 2.**The form of the leaf differs in function of the clone (according to Tsvetoukhine, 1960)

The flowers are grouped in a calathidium type of inflorescence (fig. 3), specific to the species of *Asteraceae* family. The inflorescence has on the exterior a row of lingule sterile flowers, golden yellow, counting 10-20 flowers and on the rest of the surface of the calathidium a lot of tubular hermaphrodite fertile flowers with successive blooming.



**Fig. 3** - Section at the inflorescence level to highlight the receptacle, the bracts and the flowers in different stages of development (according to Betty Schroeder, 2008)

Considering the number of inflorescences on the plant, Jerusalem artichoke cultivars are classified in three categories:

- with a reduced number, 1-15 inflorescences
- with a medium number, 16-49 inflorescences
- with a high number, 50-155 inflorescences (Pas'ko, 1973).

The fruit is an achene, in normal conditions the fruits are formed in a reduced number (Russel, 1979). Their external color can be black, gray, brown with dark spots (Kanvalinkova, 2003). Spontaneous populations produce more fruits (3-50 for every inflorescence), in comparison with the ones in cultivation (0,08-0,66 for every inflorescence) (Westley, 1993).

**Ecologic characteristics.** As a perennial species, the Jerusalem artichoke, is cold resistant and it can usually stand temperatures of  $-10^{\circ}\text{C}$  and  $-15^{\circ}\text{C}$ , even if the ground is not covered by snow. It is a rustic specie because it can be found in areas like Alaska (Duke, 1983), but at the same time it can appear in extremely dry areas with high temperatures (center Spain, south Italy, North Africa). The part of the plant that grows outside the ground is sensitive to freezing temperatures and can be destroyed at temperatures below  $0^{\circ}\text{C}$ . The optimal temperature for its growth and development is  $26,6^{\circ}\text{C}$  (Xiao Yong Ma et al., 2011).

Regarding the light, Jerusalem artichoke needs full daylight during its vegetation period up to its maturity and for forming tubers short daylight conditions are sufficient. (Huxley et al., 1992). Thus it can be stated that Jerusalem artichoke is sensitive to light during its vegetation period.

Jerusalem artichoke can tolerate medium precipitations during the year which go between 310 and 2820 mm. it can endure drought conditions although in its original location the precipitations are medium. This phenomenon was demonstrated through experiments done in south of Italy: thus the Jerusalem artichoke grown in a system without any irrigations, with precipitations of 125 mm from June to September, still in this conditions the yield of tubers was 10 to/ha (Macella et al., 1996).

The topinambur can be grown in most of the soils, but it has better results in soils with a light texture and a slightly alkaline pH. Although the production is

better in clay soil, rich in nutrient elements, because of the difficult harvesting it is not recommended to cultivate the Jerusalem artichoke in these soils.

Jerusalem artichoke is generally considered to be tolerant to soil salinity (Long et al., 2008, Newton et al., 1991), as its culture is possible in partially saline soils or in irrigated system with saline sea water (Chittendon, 1951).

## CONCLUSIONS

1. After this study realized, based on information comprised in specialized literature, sufficient data has been structured for documenting the nutrient value, agro technical and economical importance of Jerusalem artichoke.

2. It also could be raised significant environmental and biological features necessary for introducing the vegetable species of *Helianthus tuberosus* L. into the crop production from Romania.

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# THE ASSESSMENT ON THE FERTILITY POTENTIAL OF VEGETABLES CULTIVATED SOIL FROM TÂRGU FRUMOS MICROREGION

## EVALUAREA POTENȚIALULUI DE FERTILITATE A TERENURILOR CU VOCAȚIE LEGUMICOLĂ DIN MICROZONA TÂRGU FRUMOS

MUNTEANU N.<sup>1</sup>, ȚIBULCĂ L.<sup>1</sup>, BIREESCU L.<sup>2</sup>, STOLERU V.<sup>1</sup>

e-mail: nmunte@uaiasi.ro

**Abstract.** *The research was carried out in the vegetable ecosystem of Târgu Frumos microregion in 2010 - 2011. The assessment of the fertility potential of vegetable cultivated soil from Târgu Frumos microregion was evaluated using synthetic biological indicators of soil fertility as expressed through: potential indicator of enzyme activity (IPAE%), potential indicator of vital activity (IPAV%) and synthetic biological indicator (ISB%) (Ștefanic et al 1994). In this study were analyzed two vegetable microfarms from Târgu Frumos such as A.F. Maxim and A.F. Vavilov. The results reveal a high fertility potential of analyzed soil from Târgu Frumos, which provides a high suitability regarding sustainability of the vegetable crops and also stabilize the production process.*

**Key words:** fertility potential, potential indicator of enzyme activity (IPAE%), potential indicator of vital activity (IPAV%) and synthetic biological indicator (ISB%).

**Rezumat.** *Cercetările au fost desfășurate în cadrul ecosistemului legumicol a microzonei Târgu Frumos în 2010 - 2011. Evaluarea potențialului de fertilitate a terenurilor cu vocație legumicolă din microzona Târgu Frumos s-a realizat cu ajutorul indicatorilor biologici sintetici de fertilitate și calitate ai solului exprimați prin: indicatorul potențialului activității enzimatică (IPAE%), indicatorul potențialului activității vitale (IPAV%) și indicatorul sintetic biologic (ISB%) (Ștefanic și colab 1994). În cadrul acestui studiu au fost analizate două microferme legumicole din microzona Târgu Frumos, A.F. Maxim respectiv A.F. Vavilov. Rezultatele demonstrează un potențial ridicat de fertilitate a terenurilor din microzona Târgu Frumos, ceea ce asigură o mare preabilitate privind sustenabilitatea culturilor legumicole vizând totodată posibilitatea stabilizării procesului de producție.*

**Cuvinte cheie:** potențial de fertilitate, indicatorul potențialului activității enzimatică (IPAE%), indicatorul potențialului activității vitale (IPAV%), indicatorul sintetic biologic (ISB%).

## INTRODUCTION

Târgu Frumos microregion is one of the most important areas of vegetables crops from Moldova. From tens or even hundreds of years, the populations of

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup> Institute of Biological Sciences Iasi, Romania

Târgu Frumos have practice the vegetable growing and the products are delivered to markets and retail stores in major cities (Munteanu, 2009).

Soil fertility is the main attribute of the fundamental life processes and pedogenesis of the soil, through which provides the structure and functionality of natural biocenoses (Stefanic et al. 2006, Bireescu Geanina 2001). Highlighting the action and intensity of inputs on quality indicators of fertility and soil resources, is performed using synthetic indicators like PIVA, PIEA and SBI proposed by Stefanic et al (1994).

In these circumstances the question appear to ensuring increased values of fertility and biological properties and mitigation under the stress of the risk factors determined by conventional vegetable growing system in which risk factors have a significant role in limiting and stressful.

In our country, soil respiration testing was possible in 1988 when Stefanic made an original respirometer, able to replace the oxygen consumed automatically in the process of soil respiration and capture CO<sub>2</sub> released (Stefanic, 1999). Soil respiration is a parameter that assesses the soil microflora activity and is representing a measure of the intensity with which it engages soil processes involving soil microflora (Stefanic, 1999).

## MATERIAL AND METHOD

The research was carried out in 2010 - 2011 in the vegetable ecosystem of Târgu Frumos microarea for two microfarms, AF Maxim and A.F. Vavilov, in order to evaluate the fertility potential of land in this area.

The study of the main characteristics of soil fertility was possible with a series of indicators of soil fertility such as:

- Potential indicator of vital activity (PIVA), expressed as semisum of respiratory activity of soil and cellulose decomposition activity ( $PIVA\% = (A + At) / 2$ );
- Potential indicator of enzyme activity (PIEA), expressed as semisum of sucrase and urease activity in soil ( $PIEA\% = (Z + U) / 2$ );
- Synthetic biological indicator - (SBI%), expressed as semisum of the above mentioned two indicators ( $BI = (PIVA\% + PIEA\%) / 2$ ).

Calculation methodology for PIVA% and PIEA% is based on the equal importance of each determination, considering each determination that the expression of one aspect of soil life event.

Samples for laboratory testing were collected using a sampling drill from the depth 0-20 cm by taking systematic agrochemicals samples applied in Romania at two different times, 18.03.2010 and 20.07.2010 from among the plants and the interval between rows of plants.

The test results for indicators were statistically processed by multiple test method of Duncan (Snedecor, 1965).

## RESULTS AND DISCUSSIONS

### Potential Indicator of Vital Activity - PIVA%

In Table 1 can be seen the results on synthetic indicators of fertility of soil resources: PIVA; PIEA and SBI.

At A.F. Maxim (18/03/2010) stationary, the values of potential indicator of vital activity (breathing and cellulose) from vegetables crops in solar plants vary slightly among the 16.88% to 20.08%.

At A.F. Vavilov (18.03.2010) stationary, the values of vital potential from the plants cultivated in greenhouse are average among comparable to those of A.F. Maxim 13.45 to 15.64% respectively.

At A.F. Maxim (20/07/2010), the values that indicates the synthetic indicator of potential cellulolytic vital breath from the samples that was taken from the greenhouse varying slightly from 20.07 to 22.27%.

At the samples from the interval between rows of plants the values halved.

At A.F.Vavilov (20/07/2010) stationary, the values are comparable to the A.F. Maxim, 20.93 to 22, 30%.

At the samples from the interval between rows of plants the values halved.

#### **Potential indicator of enzyme activity - PIEA%**

Stationary Târgu Frumos – A.F.Maxim (18/03/2010), the depth of 0-20 cm, the values for the enzymathic potential varying slightly from 7.11 to 8.45%.

Stationary Târgu Frumos – A.F. Vavilov (18/03/2010), the 0 -20 cm depth, the values for the enzymathic potential are submedii of greenhouse crops are comparable to those of A.F. Maxim, but somewhat smaller 6.25 to 6.71%.

Stationary Târgu Frumos – A.F. Maxim (20/07/2010), the depth of 0-20 cm, the values of synthetic indicator of potential enzyme for the sample taken between the rows in greenhouse, varying slightly from 10.26 to 11.41 %

At the samples from the interval between rows of plants the values halved. Stationary Târgu Frumos – A.F. Vavilov (20/07/2010), the 0 -20 cm depth, the values are comparable to those of A.F. Maximum, 11.26 -11.91% respectively.

At the samples from the interval between rows of plants the values halved.

#### **Synthetic Biological Indicator - SBI%**

Stationary Târgu Frumos - A.F. Maxim (18.03.2010), the 0 -20 cm depth, the values of biological potential from the vegetables cultivated in greenhouses are varying slightly from 11.99 to 14.26%.

Stationary Târgu Frumos - A.F. Vavilov (18.03.2010), the 0 -20 cm depth, the values of potential biological are smaller comparable to those of A.F. Maxim 9.85 to 11.02% respective.

Stationary Târgu Frumos - A.F. Max (20.07.2010), the 0 -20 cm depth, the values of biological potential of greenhouse crop between the rows of the plants, varying slightly from 15.16 to 16.59%.

At the samples from the interval between rows of plants the values halved.

Table 1

The Potential of Vital and enzymatic study of vegetable from  
Târgu Frumos 2010

| Stationary                                      | Culture                       | Specification          | POTENȚIAL BIOTIC                     |                         |        | POTENȚIAL ENZIMATIC             |                 |                               |                       |        |       |
|---|-------------------------------|------------------------|--------------------------------------|-------------------------|--------|---------------------------------|-----------------|-------------------------------|-----------------------|--------|-------|
|   |                               |                        | soil breathing (mg CO <sub>2</sub> ) | Celulozolis (%celulose) | PIVA % | Catalasis (cmc O <sub>2</sub> ) | Sucrose (mg gl) | Ureasis (mg NH <sub>4</sub> ) | Total Fosfatis (mg P) | PIEA % | SBI % |
| Tg.Frumos<br>A.F.Maxim<br>0-20cm<br>18.03. 10   | Onion                         | Small tunnel lake ,row | 21,15                                | 22,45                   | 18,27  | 225                             | 524             | 5                             | 3,1                   | 8,16   | 13,22 |
|   | Letuce                        | Small tunnel lake ,row | 23,41                                | 24,56                   | 20,08  | 234                             | 568             | 4                             | 3,8                   | 8,45   | 14,26 |
|   | Orach+Letuce                  | Tunnel hill, row       | 20,74                                | 21,63                   | 17,73  | 207                             | 453             | 6                             | 2,7                   | 7,49   | 12,61 |
| Tg.Frumos<br>A.F.Vavilov<br>0-20cm<br>18.03. 10 | Vegetables                    | Tunnel lake, row       | 17,71                                | 16,43                   | 13,45  | 187                             | 364             | 4                             | 2,6                   | 6,25   | 9,85  |
|   | Cucumbers                     | Tunnel lake, row       | 18,32                                | 19,06                   | 15,64  | 193                             | 376             | 3                             | 3,2                   | 6,40   | 11,02 |
|   | Peppers                       | Tunnel edge, row       | 17,27                                | 18,54                   | 15,03  | 201                             | 384             | 5                             | 2,5                   | 6,71   | 10,87 |
| Tg.Frumos<br>AFMaxim<br>0-20cm<br>20.07.10      | Tomato<br><i>Granadero F1</i> | Row                    | 25,86                                | 26,31                   | 21,77  | 317                             | 756             | 6                             | 4,2                   | 11,41  | 16,59 |
|   |                               | Interval               | 14,71                                | 15,44                   | 12,62  | 152                             | 273             | 3                             | 2,1                   | 4,87   | 8,75  |
|   | Tomato<br><i>Caliope F1</i>   | Row                    | 24,31                                | 25,21                   | 21,01  | 336                             | 684             | 5                             | 3,7                   | 10,84  | 15,93 |
|   |                               | Interval               | 13,17                                | 14,36                   | 11,57  | 125                             | 315             | 2                             | 1,8                   | 5,63   | 8,60  |
|   | Peppers<br><i>Maradona</i>    | Row                    | 23,42                                | 24,53                   | 20,07  | 351                             | 568             | 4                             | 4,6                   | 10,26  | 15,16 |
|   |                               | Interval               | 11,31                                | 12,65                   | 10,09  | 172                             | 276             | 2                             | 2,3                   | 5,03   | 7,56  |
| Tg.Frumos<br>A.F.Vavilov<br>0-20cm<br>20.07.10  | Tomato <i>Belle F1</i>        | Row                    | 26,14                                | 27,18                   | 22,30  | 312                             | 754             | 7                             | 4,4                   | 11,55  | 16,93 |
|   |                               | Interval               | 14,05                                | 13,24                   | 12,32  | 165                             | 471             | 4                             | 3,0                   | 6,84   | 9,58  |
|   | Cucumbers<br><i>Merengue</i>  | Row                    | 24,81                                | 25,33                   | 20,93  | 322                             | 788             | 9                             | 3,7                   | 11,91  | 16,42 |
|   |                               | Interval               | 17,03                                | 18,27                   | 14,81  | 161                             | 442             | 4                             | 1,8                   | 6,29   | 10,55 |

Stationary Târgu Frumos - A.F. Vavilov (20.07.2010), the 0 -20 cm depth, the values are the average of the biological potential of solar crop plants among comparable to those of AF Maxim respectiv 16.42 to 16.93%.

At the samples from the interval between rows of plants the values halved.

The environmental conditions in greenhouses and in the open field in 2010, is considered an atypical climatic year for the investigated area, with a rainy summer and not dry out as multi-media shows, correlated with the type of operation (conventional) and position among the plants (drip irrigation) or range of rows (irrigated and sunk by anthropogenic impact and technology) influence, cautions, limited, or promote the resumption and multiplication of soil biological activity in the qualitative transformation of organic waste.

Biological and synthetic fertility indicator as pedobiologic SBI, show high values in the two stationary studied plants row regardless of culture.

The values decreased significantly in the interval between rows of plants and can reach over 50% due to soil compaction in terms of fertile soils because of the high clay content, consistency summer regime and poor aeration.

Synthetic indicators of fertility values and quality PIVA% and PIEA% are influenced by environmental conditions and the microclimate of a technological nature.

Biological activity is much lower range especially because poor physical and mechanical properties (medium-fine texture soil, low aeration porosity and consistency summer hard soil) and anthropogenic impact by subsidence phenomenon and especially the impact of stressful and limiting the risk factors of conventional technology system on life in soil.

## CONCLUSIONS

1. In conventional technology system in the two analyzed stationary the vital indicators of fertility and quality synthetic, PIVA% SBI% and PIEA% are influenced by environmental conditions and the microclimate of a technological nature, emphasizing the positive mitigate the effects of stressful and limiting risk factors;

2. The biological activity is much lower range especially because poor physical and mechanical properties (medium-fine texture soil, low aeration porosity and consistency summer hard soil) and anthropogenic impact by subsidence phenomenon and especially from the impact of stressful and limiting the risk factors of conventional technology system on life in the soil;

3. The biotic potential physiological activity illustrate the totality of soil microbiota (microflora, edaphic mezofauna) which is involved in biochemical transformation of organic matter, humus and mineral soil material.

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# CONSUMER PERCEPTIONS OF ORGANIC VEGETABLES, IN IASI

## PERCEPTIA CONSUMATORILOR FATA DE LEGUMELE ECOLOGICE, IN IASI

*STOLERU V.<sup>1</sup>, MUNTEANU N.<sup>1</sup>, MAFTEI Adriana<sup>1</sup>*

e-mail: vstoleru@uaiasi.ro

**Abstract.** *This study presents the results of a survey regarding consumer perceptions of organic vegetable in Iasi. The survey was conducted to determine consumer attitudes towards organic vegetables and the reasons for consumption or non-consumption of organic vegetables. A total of 100 consumers from different occupational groups living in Iasi participated in the research. The results of the survey revealed that more than half of the survey participants had knowledge about organic vegetables. The majority of the participants were positive towards organic vegetable and purchased them as well. The results of crosstabulation analysis showed that the consumption of organic foods varied with respect to income, profesion and education. The results of the survey also indicated that the participants confidence in organic vegetable was at moderate level. About 21% of the participants was found to rely on organic vegetable Organic vegetable were purchased because they were perceived as healthy, environmentally friendly and more nutritious than conventional foods.*

**Key words:** survey, attitude, consumers, organic vegetable

**Rezumat.** *Studiul prezintă rezultatele unui sondaj cu privire la percepția consumatorilor față de legumele ecologice, din Iasi. Sondajul a fost efectuat pentru a determina atitudinile consumatorilor față de astfel de produse, precum și motivele pentru consumul sau non-consumul de legume ecologice. La acest studiu au participat 100 de consumatori din diferite categorii profesionale, care locuiesc în Iași. Rezultatele studiului au scos în evidență că mai mult de jumătate dintre participanții la sondaj au cunoștințe despre produsele ecologice. Majoritatea participanților au păreri pozitive față de legumele produse ecologic. Rezultatele analizelor încrucișate au arătat că consumul de alimente ecologice, variază în funcție de: venit, profesie și educație. Rezultatele sondajului au indicat, de asemenea, că încrederea participanților în consumul de legume ecologice a fost la nivel moderat. Aproximativ 21% dintre participanți au indicat faptul că pot fi găsite în mod constant pe piață legume ecologice. Legumele ecologice sunt achiziționate deoarece sunt percepute ca fiind sănătoase, sunt obținute într-un mediu ecologic și mai hrănitoare decât cele convenționale.*

**Cuvinte cheie:** sondaj, atitudine, consumatori, legume ecologice

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

## INTRODUCTION

Attitudes can be defined as a behavioral predisposition known before, a result of inner feelings, showing favorable or unfavorable way about a given topic or service.

In the world, a consumer attitude on pesticide treatment histories of fresh produce has been measured since 1990. In a case study, consumers initially selected the conventionally produce as cabbage and sweetcorn. After being presented with treatment histories, the majority of the consumers chose no input or reduced input treatments. Over 70% of consumers were concerned or very concerned with pesticide residues and were willing to pay over 10% premium for pesticide residue-free produce (Collins et al., 1992).

In response to food scares related to high levels of pesticide residues sometimes found on vegetables and fruits, consumers in Thailand increasingly demand 'safe' foods, in a number of initiatives and labels indicating 'pesticide safe' vegetables. However, the pesticide-residue problem has proved enduring. This opens a market opportunity for organic foods, which are produced entirely without using synthetic chemicals (Roitner-Schobesberger et al., 2010).

In Romania, the first studies on the behavior of respondents to the production of vegetables have been conducted in 2004 (Stan et al., 2005). Recent studies highlighted the fact that both respondents attitude and preference varies with education level of respondents (Stoleru, 2008, Teliban et al., 2009).

Food consumption is associated with various environmental impacts, and consumers' food choices therefore represent important environmental decisions, consumers' beliefs about ecological food consumption and their willingness to adopt such behaviors (Tobler et al. 2011).

Consumers belong to different backgrounds, age, sex, education and occupation and may have different attitudes and perceptions even selection of the store for buying of food items. In case of food products, factors such as demographic variables, quality, price, food security and information of products and labels have been found to play a great role towards purchasing decisions (Pratibha Goyal et al., 2011).

Moreover, the more income households earn, and the more consumers perceive a potential negative impact on health from pesticides usage, the more likely they would be willing to pay a premium for fresh organic produce (Haghiri et al., 2011). In general, consumers were very well informed about organic vegetables practices, though their subjective knowledge was on moderate to rather low level. Subjective knowledge is shown to be an important factor in explaining organic vegetables consumption. It is significantly, relatively strongly and directly associated with organic vegetables consumption. Objective knowledge, in contrast, is only indirectly associated with organic vegetables consumption, through increased subjective knowledge and more favourable general attitudes towards organic vegetables. Attitudes towards organic vegetables have a direct positive and relatively strong relationship with organic vegetables consumption (Pieniak et al., 2010).



## MATERIAL AND METHOD

In any social analysis, it is important that those involved in the analysis based on questionnaire survey to represent a sample that accurately express how social structure, professional and other population.

On the other hand, some analyzes of this type, designed to highlight the socio-professional groups who are interested in the context of the problem analyzed. Therefore, within the context analysis, socio-professional group of respondents is of major importance.

Consumer perception towards organic vegetables was based on their questionnaire, which included the following aspects: socio-professional attitude to vegetables in general and attitudes towards organic vegetables in particular.

**Presentation of analysis questionnaire.** The questionnaire contains a list of questions forms, which were addressed in writing to obtain relative information as our goal. Developing the questionnaire is a difficult problem, especially for market research, because there is no standard formulation of leading to a relevant model. In fact, a questionnaire is considered to be good when providing expected answers, real and usable. Drafting the questionnaire, took into account primarily the investigation. Questions and possible problems when developing their objectives are strictly followed. In this study, we used an own questionnaire, designed by rules of the literature (Oppenheim, 1997, Buiga, 2003, Stoleru, 2008).

Socio-professional are represented by six questions, and refers to: sex, age, number of family members, education level, net income and occupation of respondents. Attitudinal aspects from survey, concerns the consumption of vegetables, as conventional and environmental goals and summarizes 21 questions.

**Interviewing.** First, all persons who participated in the collection of information, have been trained about the subject from survey and how to do the interviewing. To achieve its purpose, 110 people were surveyed from different social and professional categories (persons with 8 years of schooling, vocational school, high school, college and university) from which we obtained 110 questionnaires. The interviewing was carried out on the area of Iasi, in the following locations: domestic markets (Nicolina, Alexandru cel Bun, C.U.G), super-market (Careffoure) and other locations with smaller area. Following validation, have only 100 questionnaires remained for analysis because they had complete responses to all questions, responses assigned within acceptable limits "serious answers" etc.

**The pilot phase of the questionnaire (check survey).** Whatever the experience of drawing up the questionnaire, are very rare situations when it is presented in perfect form, that does not require improvements. Therefore, questionnaire testing is needed, that is verifying the understanding, interpretation and acceptability of questions. The pilot phase is the phase where check questionnaire in terms of form and fund, a standard number of respondents. Phase check pilot survey was carried out on 15 persons (the sample being comprised of individuals with different training levels).

**Data processing** - taken place using SPSS 20 (Statistical Package for the Social Sciences) is a comprehensive and flexible statistical analysis. With such a program can process data from the simplest to most complex. For a correct analysis, it is imperative that data be placed on the variables; they are set as accurately and as data is entered correctly. The success of establishing the correctness of input variables and depend on the final results of the questionnaire analysis. Each row of the data editor is a case or an observation of the survey, each respondent is a case survey. In measuring consumer attitudes, it was intended to analyze the frequency response and the correlations

between different variables, depending on the socio-professional group of respondents.

## RESULTS AND DISSCUTION

The data presented in table 1 can be seen that 46% of respondents currently buying vegetables in the free market. This highlights the fact that there is certainty vegetable product quality, structure and some marketing for vegetable products. Of respondents, 31% frequently buy vegetables from supermarkets.

The analyzing respondents age and location of where they buy vegetables can be seen that active people, between 25 and 65 years old, mainly buys vegetables from the domestic market.

Older people (over 65), having more free time, buying vegetables from the free market frequently (80%) and confirmed the correlation between respondents' profession and the market response, the location where they shopping (fig. 1).

Table 1

| Respondents age correlation with the location for buying vegetables |                |             |             |              |               |       |
|---|----------------|-------------|-------------|--------------|---------------|-------|
| Location of buying vegetables                                       | under 18 years | 18-25 years | 25-40 years | 41- 65 years | over 65 years | Total |
| Domestic market   | 3              | 7           | 15          | 16           | 5             | 46    |
| Neighborhood store  | 1              | 2           | 6           | 2            | 0             | 11    |
| Other market  | 0              | 2           | 1           | 2            | 0             | 5     |
| Supermarket   | 1              | 7           | 14          | 8            | 1             | 31    |
| Directly from the producer  | 0              | 2           | 3           | 2            | 0             | 7     |
| Total   | 5              | 20          | 39          | 30           | 6             | 100   |

Regarding the correlation between the profession and their perception of respondents to buy vegetables on the free market, we can see that it is determined mostly by free time had available. Of the respondents, those employed in healthcare, education and agriculture buying vegetables from domestic market lower percentage compared to pensioners, students or those working in services.

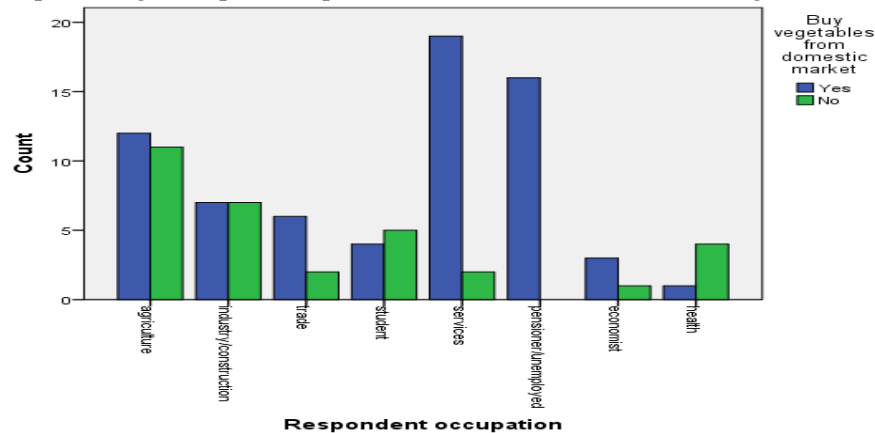


Fig. 1 - Correlation between the profession and answer the respondent on the market as a location where they shopping

So the data is confirmed by results presented in table 2 which shows certainly that the probability is very low correlation of these data ( $p < 0.001$ ).

Table 2

**Person Chi-square test for correlation between profession and domestic market shopping**

| Specification                | Value               | df | Asymp. Sig. (2-sided) |
|------------------------------|---------------------|----|-----------------------|
| Pearson Chi-Square           | 24,996 <sup>a</sup> | 7  | ,001                  |
| Likelihood Ratio             | 30,050              | 7  | ,000                  |
| Linear-by-Linear Association | 5,229               | 1  | ,022                  |
| N of Valid Cases             | 100                 |    |                       |

a. 7 cells (43,8%) have expected count less than 5. The minimum expected count is 1,28.

If respondents' level of education correlates underlying motivation of the respondents purchase vegetables can be observed that those with higher education are most interested in long-term issues such as price, appearance and taste of vegetables purchased. The data presented in tab. 3, can see an increase in dependence between education level of respondents and the number of criteria underlying purchase vegetables. This highlights the growing interest in quality vegetables among people with advanced studies at the expense of those with basic education.

Table 3

**Correlation of respondents' level of motivation studies underlying acquisition vegetables**

| Motivation purchase vegetables | Gymnasium | Vocational school | High school | College  | University | Total |
|--------------------------------|-----------|-------------------|-------------|----------|------------|-------|
| Vegetable price                | 4         | 5                 | 11          | 2        | <b>14</b>  | 36    |
| Availability                   | 1         | 1                 | 0           | <b>0</b> | 3          | 5     |
| Cultivation system             | 1         | 2                 | 4           | 1        | 5          | 13    |
| Appearance                     | 1         | 4                 | 6           | 1        | <b>15</b>  | 27    |
| Taste                          | 1         | 3                 | 5           | 1        | <b>7</b>   | 17    |
| Other criterion                | 0         | 1                 | 0           | 1        | 0          | 2     |
| Total                          | 8         | 16                | 26          | 6        | 44         | 100   |

The price, appearance and taste of vegetables are the most important criteria that determine acquisition vegetables.

Of all respondents can be seen that for 13% of them, how to obtain vegetables is an important criterion, which determines a positive environmental and consumer health. Ask if they know the difference between organic vegetables and those grown with synthetic chemicals, 82% of all respondents said "yes", know this difference and provided answers based on taste, health benefits and superior nutritional properties of ecological vegetables.

Levels of pesticides (chemicals) of vegetables, is a big enough concern for most respondents (88% of them are worried about the level of pesticides), but

most concerned, seem to be that with incomes between 400 and 600 RON (20%) and those with incomes between 800 and 1,000 RON (17%). If sex respondent is correlated with care on the level of pesticides, there is an equal (51% of the women concerned and 49% are men). Correlating age with the same care respondents, shows that respondents most concerned are between 25 and 40, followed by those 41 and 65 aged (fig. 2).

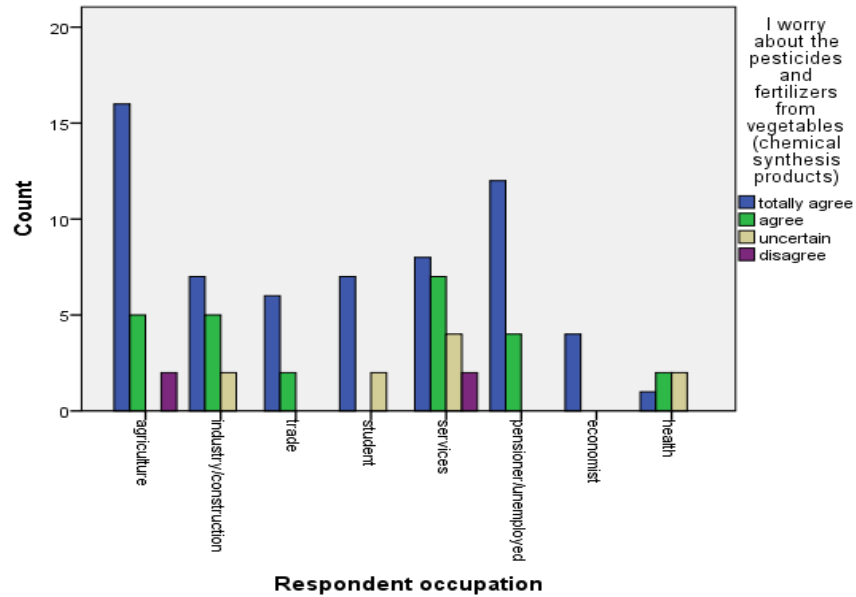
Data presented in table 4 and figure 2 highlight that the data obtained in 78.1% of respondents coincided with probability one can say that the data of 88.5% of respondents profession and caring for the pesticide to correlate .

Table 4

**Person Chi-square test for correlation between respondents towards pesticides from vegetables by income**

| Specification                | Value               | df | Asymp. Sig. (2-sided) |
|------------------------------|---------------------|----|-----------------------|
| Pearson Chi-Square           | 28,977 <sup>a</sup> | 21 | ,115                  |
| Likelihood Ratio             | 36,426              | 21 | ,020                  |
| Linear-by-Linear Association | ,336                | 1  | ,562                  |
| N of Valid Cases             | 100                 |    |                       |

a. 25 cells (78,1%) have expected count less than 5. The minimum expected count is ,16.



**Fig. 2 - Attitude of respondents towards pesticides from vegetables by income**

From the total of respondents (table 5), 90% believe that the level of pesticides imported vegetables is higher than organic vegetables (fig. 3), mostly present perception ( $p < 0.077$ ) at persons with average salary between 400 and 1.500 RON (fig. 4).

Table 5

I think the level of pesticides and fertilizers from imported vegetables is higher than organic vegetables (chemical synthesis products)

| Specification       | Frequency | Percent | Valid Percent | Cumulative Percent |
|---------------------|-----------|---------|---------------|--------------------|
| Valid totally agree | 72        | 72,0    | 72,0          | 72,0               |
| agree               | 18        | 18,0    | 18,0          | 90,0               |
| uncertain           | 10        | 10,0    | 10,0          | 100,0              |
| Total               | 100       | 100,0   | 100,0         |                    |

Table 6

Person Chi-square test for attitude than level of pesticides and fertilizers from imported vegetables is higher than organic vegetables

| Specification                | Value               | df | Asymp. Sig. (2-sided) |
|------------------------------|---------------------|----|-----------------------|
| Pearson Chi-Square           | 14,187 <sup>a</sup> | 8  | ,077                  |
| Likelihood Ratio             | 14,958              | 8  | ,060                  |
| Linear-by-Linear Association | 1,465               | 1  | ,226                  |
| N of Valid Cases             | 100                 |    |                       |

a. 10 cells (66,7%) have expected count less than 5. The minimum expected count is ,14.

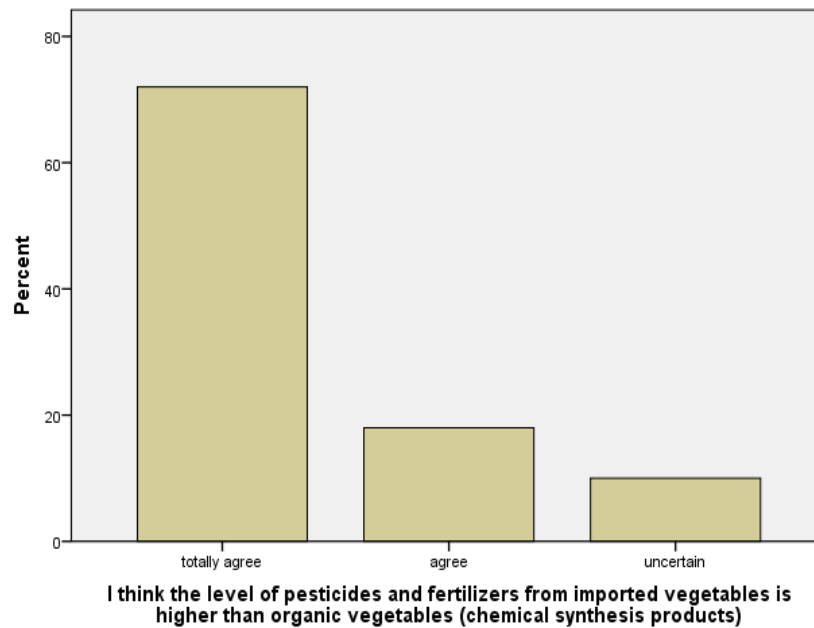
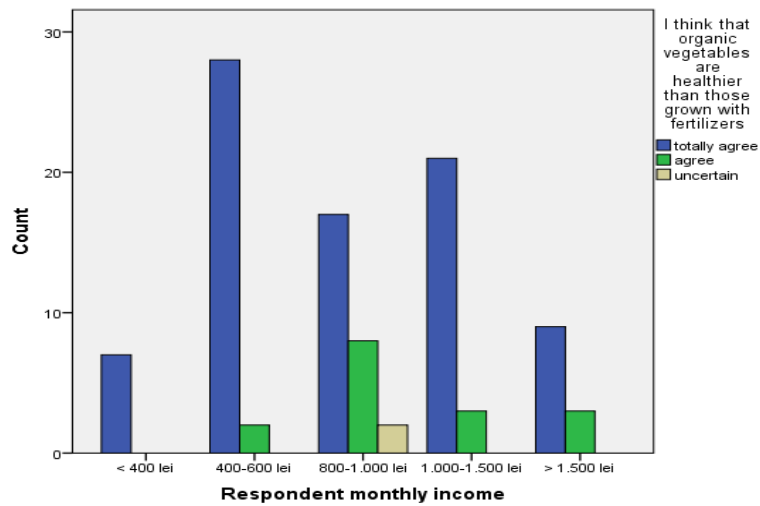


Fig. 3 - The attitude of respondents to the pesticide from organic vegetables



**Fig. 4** - The correlation between income respondents and the consideration that organic vegetables are healthier

If the correlation between respondents and profession perception that organic vegetables are healthier than those imported, it appears that people working in services, agriculture and pensioners are most aware of this (fig. 5). From the data presented in figure 5 we can be observed that the large majority of respondents consider that organic vegetables are healthier than conventional ones.

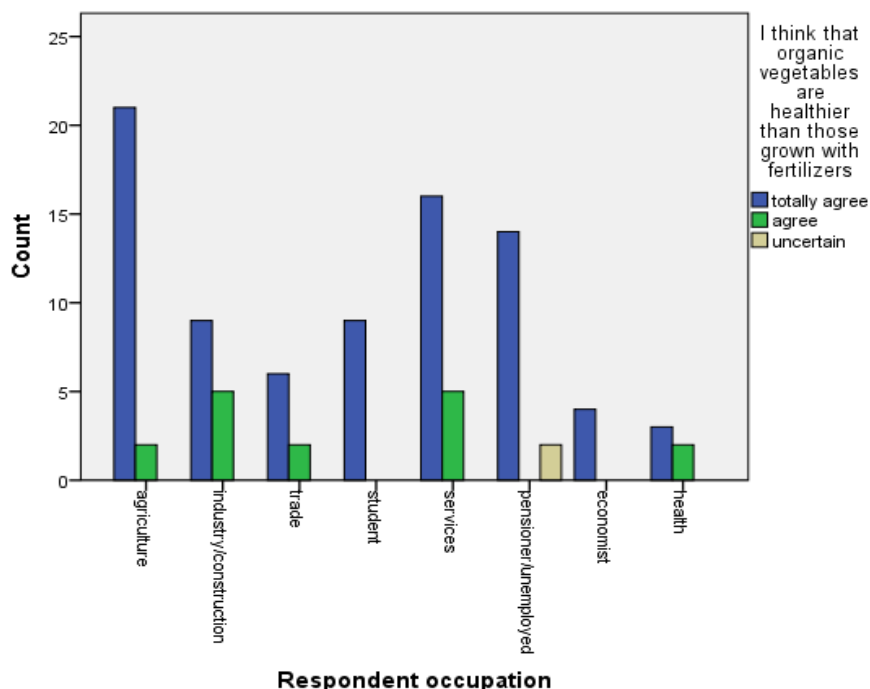
According to occupation of respondents find that the services, agriculture and pensioners realize this in greater proportion. We can say with certainty that the data presented in figure 5 correlates with probability of 95.5% (tab. 7).

*Table 7*

**Person Chi-square test for correlation between respondents towards organic vegetables and their profession**

|                              | Value               | df | Asymp. Sig. (2-sided) |
|------------------------------|---------------------|----|-----------------------|
| Pearson Chi-Square           | 24,094 <sup>a</sup> | 14 | ,045                  |
| Likelihood Ratio             | 24,160              | 14 | ,044                  |
| Linear-by-Linear Association | ,404                | 1  | ,525                  |
| N of Valid Cases             | 100                 |    |                       |

a. 18 cells (75,0%) have expected count less than 5. The minimum expected count is ,08.



**Fig. 5** - Perception of respondents towards organic vegetables according to their profession

The perception that organic vegetables are available in sufficient quantity the consumer market, we can see that 79% of respondents are conscious that vegetables that are in use are not organic. This is positively correlated with that in Romania there are few certified organic vegetable farm (tab. 8).

From the analysis, uncertainty of 66.70% leads us to believe that a majority can not distinguish the organic vegetables grown conventionally. Due to lack of information and promotion of organic vegetable growing.

*Table 8*

**Organic vegetables are available in sufficient quantity to consumer market**

| Result          | Frequency | Percentage  | Cumulative percentage |
|-----------------|-----------|-------------|-----------------------|
| total agreement | 12        | 12,0        | 12,0                  |
| agreement       | 9         | 9,0         | 21,0                  |
| uncertain       | <b>46</b> | <b>46,0</b> | <b>67,0</b>           |
| disagreement    | 19        | 19,0        | 86,0                  |
| disagree        | 14        | 14,0        | 100,0                 |
| Total           | 100       | 100,0       |                       |

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# RESEARCH CONCERNING THE MAIN CHARACTERISTICS OF SOME LOCAL BEAN POPULATION FROM BUZĂU VEGETABLE AREA

## STUDIUL PRINCIPALELOR CARACTERE ALE UNOR POPULAȚII LOCALE DE FASOLE DIN BAZINUL LEGUMICOL BUZĂU

*TEODORESCU Eliza*<sup>1</sup>, *MUNTEANU N.*<sup>1</sup>, *VÎNĂTORU C.*<sup>2</sup>,  
*STOLERU V.*<sup>1</sup>, *ZAMFIR Bianca*<sup>2</sup>,  
e-mail: neicu\_eliza@yahoo.com

**Abstract.** *The study is part of a conservation and utilization program of germplasm sources at common bean (*Phaseolus vulgaris* L. convar. nanus) from traditional vegetable areas in Romania. The research were organized in 2010 – 2011 at V.R.D.S. Buzău, using a collection of fourteen local populations from Buzău vegetable area having the purpose to mark out the main morphological and physiological characteristics, and also agro productive. The results obtained were compared to a control variety – Ioana. Thus, was dignified a great variability of the studied cultivars, and also the productive superiority of two local populations (B<sub>z</sub> 11 and B<sub>z</sub> 40) that surpasses with over 10% the control variant.*

**Key words:** breeding, germplasm collection, biodiversity, *Phaseolus vulgaris* L. convar. nanus.

**Rezumat.** *Studiul face parte dintr-un program de conservare și valorificare a resurselor de germoplasmă la fasolea comună de grădină (*Phaseolus vulgaris* L. convar. nanus) din unele bazine legumicole tradiționale din România. Cercetările au fost organizate în perioada 2010 – 2011, la Stațiunea de Cercetare și Dezvoltare Legumicolă (S.C.D.L.) Buzău, în cadrul unei colecții de paisprezece populații locale din bazinul legumicol Buzău, cu scopul de a pune în evidență principalele caracteristici morfologice, fiziologice agroproductive. Rezultatele obținute au fost comparate cu un soi martor – Ioana. A fost pusă în evidență largă variabilitatea a cultivarelor studiate, precum și superioritatea productivă a două populații locale (B<sub>z</sub> 11 și B<sub>z</sub> 40) care depășesc cu peste 10% martorul.*

**Cuvinte cheie:** ameliorare, colecție de germoplasă, biodiversitate, *Phaseolus vulgaris* L. convar. nanus.

### INTRODUCTION

Local bean population are in a great number in the traditional vegetable area, being kept among time by the cultivators in their own vegetable gardens.

In 1932, I. M. Rădulescu obtains 3000 elites from 800 initial types of bean collected from Moldova area (Rădulescu, 1940).

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup> Vegetable Research and Development Station Buzău, Romania

Buzău vegetable area known in our country for the great number of vegetable local population, including bean. In these circumstances the Breeding Laboratory from Vegetable Research and Development Station (V.R.D.S.) Buzău has as a main objective the valuation of this biological treasure through creating new varieties more productive and qualitative superior than the existent ones.

The first step in order to create a breeding program is collecting the biological material which has the genes that can offer the wanted characteristics to the new cultivar (Munteanu, 2008).

The number of the garden bean varieties (*Phaseolus vulgaris* L. convar. *nanus*) inscribed in the „Official catalogue of the culture plants varieties from România in 2012” is 12, in case of the yellow pod variety, and 9, in case of the green pod varieties. These varieties are kept either in stations or research institutes (10 varieties with yellow pod and 6 varieties with green pod), or commercial societies (2 varieties with yellow pod and 3 varieties with green pod).

The inland genetic patrimony is menaced by the nowadays decrease tendency of the varieties homologation and maintenance made by the national research institutes in favor of the national or foreign commercial societies. Therefore, our activity concerning collecting and using local populations as an initial breeding material, concurs in order to save the inland genetic patrimony and as well concurs to biodiversity conservation at these species. Similar actions were made in institutes and research station for horticulture and as well in profile universities (Munteanu, 1985).

Since 2010, at V.R.D.S. Buzău developed activities of enriching the germplasm bean collection through collecting the local populations from Buzău vegetable area and as well from the station’s influence area.

The initial descriptions of the collected biological material were frequently brief. The discussions with the cultivators had not given details about the culture compartment of the purchased biotypes. Thereby, the proveniences were numbered and there was added the symbol of the origin locality. In just a few cases we found the names used in the past (Munteanu, 1994; Rădulescu, 1940).

## MATERIAL AND METHOD

The study started with the procurement of the biological material. First, there was made an initial stocktaking of the biological material by making the initial observation papers. There were in view the following aspects:

- provenience (locality and name – if there was possible),
- initial description received from the supplier of the biological material,
- main characteristics of the seed.

There were determined the main characteristics of the seeds: weight (g), length (cm), width (cm), thickness (cm), shape and color.

Nowadays, the bean collection detains over 50 de provenances, among which 14 manifested phenotypical stability and valuable characteristics, being comparatively studied with *Ioana* cultivar.

The experimental variants were the following:

- **V<sub>1Mt</sub>** – *Ioana* variety, control variant;
- **V<sub>2</sub>** – local population B<sub>2</sub> 2;

- V<sub>3</sub> – local population B<sub>z</sub> 6;
- V<sub>4</sub> – local population B<sub>z</sub> 11;
- V<sub>5</sub> – local population B<sub>z</sub> 12;
- V<sub>6</sub> – local population B<sub>z</sub> 13;
- V<sub>7</sub> – local population B<sub>z</sub> 14;
- V<sub>8</sub> – local population B<sub>z</sub> 21;
- V<sub>9</sub> – local population B<sub>z</sub> 36;
- V<sub>10</sub> – local population B<sub>z</sub> 40;
- V<sub>11</sub> – local population B<sub>z</sub> 41;
- V<sub>12</sub> – local population B<sub>z</sub> 42;
- V<sub>13</sub> – local population B<sub>z</sub> 46;
- V<sub>14</sub> – local population B<sub>z</sub> 48;
- V<sub>15</sub> – local population B<sub>z</sub> 49.

The biological material was cultivated in open field, according to the technology recommended by the specialty literature (Ruști, 2008). During the experiment were made many mensurations according to the U.P.O.V. guide, in order to determine distinction, uniformity and stability of the bean plants (TG 12/9). In order to establish the intensity or the gradation of different characteristics was consulted the *Color scales for identification characters of common bean* (Genchev, 2005).

The experiment was organized according to the randomized blocks method with 3 repetitions and there were made mensurations in order to determine the variability of the main yield characteristics.

## RESULTS AND DISCUSSIONS

The color of the seed represents the morphological character that detains the greatest variability. In what it concerns the color (fig. 1), according to the classification made by Rădulescu in 1940, there were ascertained the existences of three categories:

- three experimental variants (V<sub>1M6</sub>, V<sub>2</sub> and V<sub>10</sub>) have white seeds;
- three experimental variants (V<sub>4</sub>, V<sub>5</sub> and V<sub>6</sub>) au black seeds;
- nine experimental variants have spotty seeds.

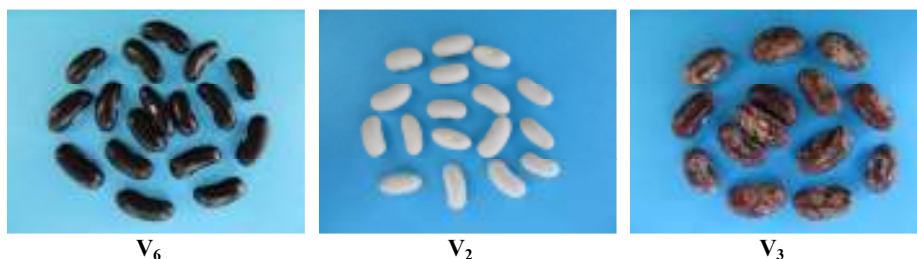


Fig. 1 - Seeds of different colors belonging to the local populations studied

In what it concerns the spotty seeds (fig. 2), taking into consideration the distribution of the colors on the tegument, we can distinguish the following categories – according to the description made by Olaru, (1982):

- zebrinus beige + violet V<sub>3</sub>, V<sub>7</sub> and V<sub>9</sub> variants;
- punctatus at V<sub>11</sub> (beige + mauve) and V<sub>12</sub> (beige + brown) variants;

- maculatus at V<sub>13</sub> (white + red), V<sub>14</sub> (white + mauve) and V<sub>15</sub> (white+ brown) variants;
- variegatus at V<sub>8</sub> (brown + black) variant.

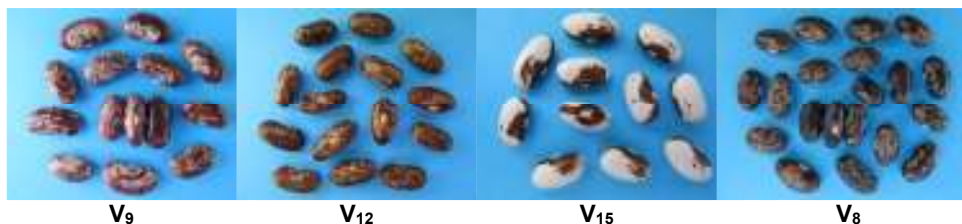


Fig. 2 - Different types of spotty seeds taken into study

During the experiment the weight of one thousand seeds (MMB) varied between 730 g (V<sub>13</sub>) and 250 g (V<sub>5</sub>), the seeds being middling and big (table 1).

The shape of the seeds taken into study varies between: oval, kidney and cylindrical.

Table 1

Main characteristics of the seeds

| Variants         | Shape       | Color                   | Seeds dimensions (cm) |       |           | M.M.B (g) |
|------------------|-------------|-------------------------|-----------------------|-------|-----------|-----------|
|                  |             |                         | Length                | Width | Thickness |           |
| V <sub>1Mt</sub> | kidney      | white                   | 1,3                   | 0,6   | 0,5       | 270       |
| V <sub>2</sub>   | kidney      | white                   | 1,2                   | 0,6   | 0,5       | 310       |
| V <sub>3</sub>   | cylindrical | spotty (beige + violet) | 1,6                   | 0,8   | 0,6       | 620       |
| V <sub>4</sub>   | oval        | black                   | 1,2                   | 0,8   | 0,6       | 540       |
| V <sub>5</sub>   | cylindrical | black                   | 1,0                   | 0,6   | 0,5       | 250       |
| V <sub>6</sub>   | kidney      | black                   | 1,5                   | 0,6   | 0,4       | 370       |
| V <sub>7</sub>   | cylindrical | spotty (beige + violet) | 1,6                   | 0,8   | 0,6       | 650       |
| V <sub>8</sub>   | cylindrical | spotty (brown +black)   | 1,2                   | 0,6   | 0,5       | 320       |
| V <sub>9</sub>   | cylindrical | spotty (beige + violet) | 1,6                   | 0,8   | 0,6       | 630       |
| V <sub>10</sub>  | cylindrical | white                   | 1,4                   | 0,7   | 0,5       | 440       |
| V <sub>11</sub>  | oval        | spotty (beige +mauve)   | 1,2                   | 0,9   | 0,7       | 570       |
| V <sub>12</sub>  | cylindrical | spotty (beige + brown)  | 1,3                   | 0,7   | 0,6       | 420       |
| V <sub>13</sub>  | cylindrical | spotty (white + red)    | 1,8                   | 0,8   | 0,7       | 730       |
| V <sub>14</sub>  | cylindrical | spotty (white +mauve)   | 1,5                   | 0,8   | 0,6       | 570       |
| V <sub>15</sub>  | cylindrical | spotty (white +brown)   | 1,4                   | 0,7   | 0,5       | 330       |

The main length of the pods is 11,58 cm. The maximum value (14,38 cm) was registered at V<sub>7</sub> variant, and the least value (8,58 cm) was registered at V<sub>5</sub> variant. The variability quotient of the pods length (15,95%) shows a mean variability of this character. The main characteristics of the pods are presented in table 2.

The main width of the pods is 1,18 cm, having values within 1,6 cm lat V<sub>4</sub> variant and 0,9 cm at V<sub>1Mt</sub> variant. The width variability is mean because the variability quotient was lower than 20% (18,49%).

The variability of the pods thickness is high because the value of the variability quotient was 20,42%, with an average equal to 0,81 cm.

In what it concerns the pods color, the number of the local populations with yellow pod (V<sub>2</sub>, V<sub>3</sub>, V<sub>6</sub>, V<sub>7</sub>, V<sub>8</sub>, V<sub>9</sub> and V<sub>15</sub>) was equal to the number of the populations with green pod. At the technological maturity the control variant had yellow pods.

Table 2

The main characteristics of the pods

| Variants         | Pods length |                                   |                         | Pods width |                                   |                         | Pods thickness |                                   |                         |
|------------------|-------------|-----------------------------------|-------------------------|------------|-----------------------------------|-------------------------|----------------|-----------------------------------|-------------------------|
|                  | cm          | differences than V <sub>1Mt</sub> | % than V <sub>1Mt</sub> | cm         | differences than V <sub>1Mt</sub> | % than V <sub>1Mt</sub> | cm             | differences than V <sub>1Mt</sub> | % than V <sub>1Mt</sub> |
| V <sub>1Mt</sub> | 12,00       | 0,00                              | 100,00                  | 0,90       | 0,00                              | 100,00                  | 0,90           | 0,00                              | 100,00                  |
| V <sub>2</sub>   | 12,74       | 0,74                              | 106,17                  | 0,94       | -0,04                             | 104,44                  | 0,82           | -0,08                             | 91,11                   |
| V <sub>3</sub>   | 12,18       | 0,18                              | 101,50                  | 1,28       | -0,38                             | 142,22                  | 0,70           | -0,20                             | 77,78                   |
| V <sub>4</sub>   | 10,42       | -1,58                             | 86,83                   | 1,60       | -0,70                             | 177,78                  | 0,86           | -0,04                             | 95,56                   |
| V <sub>5</sub>   | 8,58        | -3,42                             | 71,50                   | 0,98       | -0,08                             | 108,89                  | 0,46           | -0,44                             | 51,11                   |
| V <sub>6</sub>   | 13,94       | 1,94                              | 116,17                  | 0,94       | -0,04                             | 104,44                  | 0,82           | -0,08                             | 91,11                   |
| V <sub>7</sub>   | 14,38       | 2,38                              | 119,79                  | 1,35       | -0,45                             | 150,00                  | 0,80           | -0,10                             | 88,89                   |
| V <sub>8</sub>   | 9,70        | -2,30                             | 80,83                   | 1,00       | -0,10                             | 111,11                  | 0,83           | -0,07                             | 92,59                   |
| V <sub>9</sub>   | 12,95       | 0,95                              | 107,92                  | 1,35       | -0,45                             | 150,00                  | 0,98           | 0,08                              | 108,33                  |
| V <sub>10</sub>  | 12,87       | 0,87                              | 107,22                  | 1,10       | -0,20                             | 122,22                  | 0,90           | 0,00                              | 100,00                  |
| V <sub>11</sub>  | 9,37        | -2,63                             | 78,06                   | 1,40       | -0,50                             | 155,56                  | 0,87           | -0,03                             | 96,30                   |
| V <sub>12</sub>  | 12,40       | 0,40                              | 103,33                  | 0,97       | -0,07                             | 107,41                  | 1,17           | 0,27                              | 129,63                  |
| V <sub>13</sub>  | 12,97       | 0,97                              | 108,06                  | 1,33       | -0,43                             | 148,15                  | 0,80           | -0,10                             | 88,89                   |
| V <sub>14</sub>  | 9,85        | -2,15                             | 82,08                   | 1,35       | -0,45                             | 150,00                  | 0,55           | -0,35                             | 61,11                   |
| V <sub>15</sub>  | 9,43        | -2,57                             | 78,61                   | 1,17       | -0,27                             | 129,63                  | 0,73           | -0,17                             | 81,48                   |

In what it concerns the threads presence, three experimental variants (V<sub>5</sub>, V<sub>13</sub> and V<sub>14</sub>) presented pods with threads even from the first development stages.

The mean weight of the pods was 6,16 g. V<sub>9</sub> and V<sub>10</sub> variants had pods with 8,5g weight. The control variant registered a 6,12 g weight, being very close to the average of the experiment (table 3).

Table 3

Main productivity characteristics

| Variants         | Mean weight/pod (g) | Mean number of seeds/pod | Number of pods/plant | Mean yield/plant (g) |
|------------------|---------------------|--------------------------|----------------------|----------------------|
| V <sub>1Mt</sub> | 6,12                | 6,03                     | 41,00                | 256,20               |
| V <sub>2</sub>   | 4,62                | 5,80                     | 35,00                | 161,70               |
| V <sub>3</sub>   | 5,80                | 4,20                     | 34,00                | 197,20               |
| V <sub>4</sub>   | 7,92                | 4,40                     | 42,00                | 332,64               |
| V <sub>5</sub>   | 2,80                | 6,00                     | 59,00                | 165,20               |
| V <sub>6</sub>   | 4,70                | 4,60                     | 52,67                | 247,53               |
| V <sub>7</sub>   | 8,30                | 4,75                     | 28,00                | 232,40               |
| V <sub>8</sub>   | 4,87                | 5,67                     | 17,00                | 82,73                |
| V <sub>9</sub>   | 8,50                | 3,25                     | 27,75                | 235,88               |
| V <sub>10</sub>  | 8,50                | 5,33                     | 40,67                | 345,67               |
| V <sub>11</sub>  | 5,47                | 4,33                     | 20,00                | 109,33               |
| V <sub>12</sub>  | 8,17                | 6,00                     | 28,00                | 228,67               |
| V <sub>13</sub>  | 7,87                | 4,67                     | 33,00                | 259,60               |
| V <sub>14</sub>  | 4,70                | 3,00                     | 59,00                | 277,30               |
| V <sub>15</sub>  | 4,43                | 3,67                     | 25,50                | 113,05               |

In what it concerns the number of pods/plant we can observe the fact that V<sub>5</sub> had the highest value (59), V<sub>8</sub> had the least value (17), while the control variant had 41 pods/plant.

The most productive variants were V<sub>10</sub> and V<sub>4</sub> these variants surpassed the control variant with 89,47 g and 76,44 g in what it concerns the mean yield/plant.

The mean yield/plant for V<sub>14</sub> and V<sub>13</sub> was superior to Ioana cultivar with 8,24 % and 1,33 %.

The mean yield/plant for the V<sub>8</sub> variant represented only 32,29 % from the control variant yield.

## CONCLUSIONS

1. V<sub>4</sub> and V<sub>10</sub> variants remarked through characteristics superior to the control variant.

2. The three experimental variants (V<sub>5</sub>, V<sub>13</sub> and V<sub>14</sub>) which presented pods with threads since the first development stages will be recommended for berries.

3. V<sub>3</sub>, V<sub>7</sub> and V<sub>9</sub> variants resemble even if there were procured from different locations.

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# THE ASSESSMENT OF THE SOIL QUALITY FUND USING ECO-PEDOLOGICAL DIAGNOSIS OF EFFECTIVE TROPHICITY OF SOIL RESOURCES FROM TÂRGU FRUMOS MICROREGION

## EVALUAREA FONDULUI CALITATIV AL SOLULUI PRIN UTILIZAREA DIAGNOZEI ECO-PEDOLOGICE A TROFICITĂȚII EFFECTIVE A RESURSELOR DE SOL DIN MICROZONA TÂRGU FRUMOS

ȚIBULCĂ L.<sup>1</sup>, MUNTEANU N.<sup>1</sup>, BIREESCU L.<sup>2</sup>,  
STOLERU V.<sup>1</sup>, GHIȚĂU Carmen<sup>1</sup>.  
e-mail: tibulca\_laurentiu@yahoo.com

**Abstract.** The research was carried out in the vegetable ecosystem of Târgu Frumos microregion in 2010 - 2011. The assessment of soil quality in Târgu Frumos microregion was performed using the ecopedological diagnosis matrix of soil trophicity resources (EPDETSR). To characterize the trophicity effective of soil resources for the studied stationary, AF Maxim and A.F. Vavilov in vegetable ecosystems exploited in a conventional system. The value of synthetic indicator and soil quality fund Eco-Pedological Diagnosis of Effective Trophicity of Soil Resources (EPDETRS-points), was obtained by summing the score of each of the 10 indicators of quality. The results of the analysis and evaluation of ecopedological diagnosis using these matrix of soil resources demonstrates a high fertility potential of the analyzed microregion, highlighting the effects of uncontrolled and negative human impact in a conventional culture system.

**Key words:** sustainability, soil quality fund, effective trophicity, ecopedological diagnosis

**Rezumat.** Cercetările au fost desfășurate în cadrul ecosistemului legumicol al microzonei Târgu Frumos în 2010 - 2011. Evaluarea fondului calitativ al solului din microzona Târgu Frumos s-a realizat cu ajutorul matricei diagnozei ecopedologice a troficității resurselor de sol (DEPTERS). Pentru caracterizarea troficității efective a resurselor de sol din staționarele de cercetare luate în studiu A.F. Maxim și A.F. Vavilov în ecosisteme legumicole exploatate în sistem convențional. Valoarea indicatorului ecologic general și sintetic al fondului de calități a solului Diagnoza Eco-Pedologică a Troficității Efective a Resurselor de Sol (DEPTERS-puncte), s-a obținut prin însumarea notelor acordate pentru fiecare din cei 10 indicatori analitici de calitate studiați. Rezultatele analizei și evaluării troficității efective cu ajutorul matricei diagnozei ecopedologice, a resurselor de sol demonstrează un potențial ridicat de fertilitate a terenurilor din microzona Târgu Frumos, evidențiând efectele impactului antropic necontrolat și negativ în sistemul de cultură convențional.

**Cuvinte cheie:** sustenabilitate, fond calitativ al solului, troficitate efectivă, diagnoză ecopedologică.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup> Biological Research Institute of Iași, Romania

## INTRODUCTION

The vegetable crop represent a subject wich is in attention of the producers and consumers from Târgu Frumos micro-area. The farmers are interested in producing fresh vegetables that are free of toxic substances from synthetic products and the profit can be as good (Munteanu, 2009).

The highlight of the action and intensity of inputs on quality indicators of fertility and soil resources, such pedobiological nature, is performed using synthetic indicator of general environmental and soil quality fund (Bireescu, 2001): Eco-Soil Diagnosis of Effective Trophic Soil Resources (DEPTERS-points) (Stefanic, 1994). In these circumstances the question is to ensuring the increase of values to fertility and biological properties and mitigation under the stress of the risk factors determined by conventional vegetable growing system in which risk factors have a significant role in limiting and stressful (Montanarella, 2008).

Our research is focused on the evaluation of eco-pedological diagnosis of effective trophicity of soil researches from Targu Frumos vegetable region.

## MATERIAL AND METHOD

The research was conducted in 2010 - 2011 in the ecosystem of Târgu Frumos micro-area for two microfarms that cultivate vegetables, A.F. Maxim, respective A.F. Vavilov, in order to evaluate the fertility potential of land in this area.

To characterize the trophic effective of soil resources in research from those two studied stationary in conventional vegetable ecosystems was considered that the soil quality evaluation form must include a list of the top 10 pedo-ecological factors and determinants: soil texture (Tx), edaphic volume (Ve) and soil consistency (Con); Indicator of Synthetic Biologycal Potential (SBI%); the content of total N (Nt), mobile P content (PAL) and K content (KAL); soil reaction (pH H<sub>2</sub>O), content of humus (Hum%) and degree of base saturation (V%).

The main mechanical, physical, chemical and biological analysis were placed in six size ecological classes and the notes are rated from 0 ... 10 points. Trophicity effective action is the result of soil properties and interrelations of phisical, chemical and biological qualities, considered at the same time, indicators of soil fertility and quality (Chirița, 1974; Bireescu et al., 2001,2005 si 2010).

The value of the synthetic indicator and soil quality fund: Eco-Soil Diagnosis of effective trophic Soil Resources (DEPTERS-points), is obtained by summing the score of each of the 10 indicators of quality

$$\text{DEPTERS} = \sum (Tx + PA + Con + Biol + pH + Hum + V + Nt + P + K)$$

To compare the results, has established a reliability scale of quality, 5-levels: very good, good, medium, satisfactory and poor: less than 20 points - effective trophicity poor, oligotrophic soil - rating: poor; between 21 and 40 points - effective trophicity less than mediocre, oligo-mesotrophic soil - rating: satisfactory; between 41 and 60 points - effective trophicity mediocre, mesotrophic soil - rating: medium; between 61 and 80 points - effective trophicity superior, eutrophic soil - rating: good; between 81 and 100 points - very good trophicity effective, soil megatrophic - rating: very good.



## RESULTS AND DISCUSSIONS

Results of the analysis and evaluation of effective trophic ecopedological diagnosis using the matrix from the soil resources of the two stationary vegetables studied, in an representative and traditional vegetable area in the NE region for 2010 at Maxim greenhouses are presented in table 1 and for Vavilov greenhouses are presented in table 2.

The quality of general environmental indicators value and synthetic soil quality fund called Eco-Soil Diagnosis of effective trophic Soil Resources (DEPTERS-points), is a result by summing the grades given for each of the 10 indicators analytical quality and fertility analyze, in greenhouses and open field, 0-20cm depth (table 1 and table 2).

Aggregate amount of notes for the 10 quality indicators for diagnosis ecopedological score indicates as follows (tab. 1):

- 52 points value for greenhouses soil among tomato Granadero F1 - trophicity effective medium;
- 56 points value for greenhouses soil range from tomato Granadero F1-trophicity effective medium;
- 60 points value for soil greenhouses Caliope F1 tomato row- trophicity effective medium;
- 62 points of the greenhouses value for soil culture Caliope tomatoes trophicity effective good;
- 58 points of the greenhouses value for soil cultivated with pepper Maradona plant rows sample, trophicity effective medium;
- 56 points value for the greenhouses for soil cultivated with pepper on the interval between rows trophicity effective medium;
- 62 points for soil greenhouses value cucumbers trophicity effective good;
- 58 points value for cucumber soil in greenhouses trophicity effective medium;

Aggregate amount of notes for the 10 quality indicators for diagnosis ecopedological score indicates the actual trophic soil resources as follows (tab. 2):

- 56 points of the solar value for soil Belle F1 tomato row – trophicity average effective;
- 62 points value for soil solar Belle F1 tomato trophicity effective-medium range;
- 58 points value for cucumber soil in solar Merengue trophicity turn-average effective;
- 62 points value for cucumber soil in solar Merengue on the interval - effective trophicity good;
- 64 points value for soil solar Vedrana pepper, turn-effective trophicity good;

Table 1

**Ecopedological diagnosis of actual trophic matrix of soil resources (DEPTERS)-conventional vegetable system  
Târgu Frumos 2010 – Maxim greenhouses**

| Specifi-<br>cation               | Quality indicators and fertility ecopedological and biopedological |     |                      |     |                  |     |                               |     |            |     |     |     |                |     |                       |     |                            |     |     |     |                   |
|----------------------------------|--|-----|----------------------|-----|------------------|-----|-------------------------------|-----|------------|-----|-----|-----|----------------|-----|-----------------------|-----|----------------------------|-----|-----|-----|-------------------|
|                                  | texture<br>%   |     | wet soil<br>consist. |     | soil<br>reaction |     | Base<br>saturatio<br>degreeV% |     | Humus<br>% |     | Nt% |     | mobil P<br>ppm |     | asimilab.<br>K<br>ppm |     | aerație<br>porozit.<br>PA% |     | SBI |     | depters<br>points |
|                                  | val  | pct | val                  | pct | val              | pct | val                           | pct | val        | pct | val | pct | val            | pct | val                   | pct | val                        | pct | val | pct | val               |
| Tomato<br>row<br>Granadero       | 36   | 6   | t                    | 6   | 6,4              | 6   | 78                            | 6   | 3,2        | 6   | 0,1 | 4   | 18             | 4   | 135                   | 6   | 12                         | 4   | 17  | 4   | 52                |
| Tomato<br>interval<br>Granadero  | 35   | 6   | f.t.                 | 4   | 6,8              | 8   | 85                            | 8   | 3,3        | 6   | 0,2 | 6   | 31             | 6   | 178                   | 8   | 6                          | 2   | 9   | 2   | 56                |
| Tomato<br>row Cliope             | 35   | 6   | t                    | 6   | 6,6              | 8   | 77                            | 6   | 3,2        | 6   | 0,2 | 6   | 24             | 6   | 142                   | 6   | 18                         | 6   | 16  | 4   | 60                |
| Tomato<br>interval<br>Caliopie   | 35   | 6   | f.t.                 | 4   | 6,9              | 10  | 82                            | 8   | 3,2        | 6   | 0,2 | 8   | 30             | 6   | 176                   | 8   | 11                         | 4   | 9   | 2   | 62                |
| Pepper<br>row<br>Maradona        | 36   | 6   | t                    | 6   | 6,4              | 6   | 85                            | 8   | 3,0        | 4   | 0,2 | 6   | 23             | 6   | 167                   | 6   | 16                         | 6   | 15  | 4   | 58                |
| Pepper<br>interval<br>Maradona   | 35   | 6   | f.t.                 | 4   | 6,4              | 6   | 88                            | 8   | 3,2        | 6   | 0,2 | 8   | 31             | 6   | 181                   | 6   | 10                         | 4   | 8   | 2   | 56                |
| Cucumber<br>row<br>Merengue      | 34   | 6   | t                    | 6   | 6,7              | 8   | 86                            | 8   | 3,1        | 6   | 0,2 | 8   | 18             | 4   | 148                   | 6   | 16                         | 6   | 16  | 4   | 62                |
| Cucumber<br>interval<br>Merengue | 34   | 6   | f.t.                 | 4   | 6,9              | 10  | 88                            | 8   | 3,2        | 6   | 0,2 | 8   | 27             | 6   | 168                   | 6   | 8                          | 2   | 9   | 2   | 58                |

Table 2

**Ecopedological diagnosis of actual trophic matrix of soil resources (DEPTERS)-conventional vegetable system  
Târgu Frumos 2010 – Vavilov greenhouses**

| Specifi-<br>cation           | Quality indicators and fertility ecopedological and biopedological |     |                      |     |                  |     |                               |     |            |         |     |         |                |         |                       |         |                            |         |     |     |                           |
|------------------------------|--|-----|----------------------|-----|------------------|-----|-------------------------------|-----|------------|---------|-----|---------|----------------|---------|-----------------------|---------|----------------------------|---------|-----|-----|---------------------------|
|                              | texture<br>%   |     | wet soil<br>consist. |     | soil<br>reaction |     | Base<br>saturatio<br>degreeV% |     | Humus<br>% |         | Nt% |         | mobil P<br>ppm |         | asimilab.<br>K<br>ppm |         | aerație<br>porozit.<br>PA% |         | SBI |     | dept<br>ers<br>point<br>s |
|                              | val  | pct | val                  | pct | val              | pct | val                           | pct | val        | pc<br>t | val | pc<br>t | val            | pc<br>t | val                   | pc<br>t | val                        | pc<br>t | val | pct | val                       |
| Tomato<br>row Belle          | 34   | 6   | t                    | 6   | 6,7              | 8   | 78                            | 6   | 3,0        | 4       | 0,1 | 4       | 17             | 4       | 143                   | 6       | 22                         | 8       | 17  | 4   | 56                        |
| Tomato<br>int Belle          | 34   | 6   | f.t.                 | 4   | 6,9              | 10  | 83                            | 8   | 3,3        | 6       | 0,2 | 8       | 31             | 6       | 169                   | 6       | 15                         | 4       | 10  | 4   | 62                        |
| Cucumber<br>row<br>Merengue  | 34   | 6   | t                    | 6   | 6,6              | 6   | 85                            | 8   | 3,1        | 6       | 0,2 | 6       | 18             | 4       | 152                   | 6       | 20                         | 6       | 16  | 4   | 58                        |
| Cucumber<br>int.<br>Merengue | 34   | 6   | f.t.                 | 4   | 6,8              | 8   | 88                            | 8   | 3,4        | 6       | 0,2 | 8       | 23             | 6       | 187                   | 8       | 14                         | 4       | 11  | 4   | 62                        |
| Pepper<br>row<br>Verdana     | 36   | 6   | t                    | 6   | 6,8              | 8   | 85                            | 8   | 3,2        | 6       | 0,2 | 6       | 21             | 6       | 171                   | 6       | 18                         | 6       | 16  | 6   | 64                        |
| Pepper<br>int.<br>Verdana    | 36   | 6   | f.t.                 | 4   | 6,9              | 10  | 87                            | 8   | 3,4        | 6       | 0,2 | 8       | 27             | 6       | 186                   | 8       | 12                         | 4       | 9   | 2   | 62                        |
| Tomato<br>row Belle          | 34   | 6   | t                    | 6   | 6,7              | 8   | 78                            | 6   | 3,0        | 4       | 0,1 | 4       | 17             | 4       | 143                   | 6       | 22                         | 8       | 17  | 4   | 56                        |
| Tomato<br>int Belle          | 34   | 6   | f.t.                 | 4   | 6,9              | 10  | 83                            | 8   | 3,3        | 6       | 0,2 | 8       | 31             | 6       | 169                   | 6       | 15                         | 4       | 10  | 4   | 62                        |

## CONCLUSIONS

1. The matrix analysis of the actual soil organic diagnosis as their characters, the synthetic indicator of interaction of correlation and ecological factors (climate and soil) of biotopes, highlights the effects of uncontrolled human impact and negative conventional culture system, and shows that the food fund soil is high, but it is not fully exploited and stressed, the limited nutrition and physiological processes like development and productivity of vegetables in the local context, especially in excessively dry summer;

2. Aggregate amount of notes for the 10 quality indicators scores indicate more differentiated diagnosis of trophic ecopedological effective soil resources of protected land on which the assessment is qualitative and emphasizes the effects and intensity of risk factors on fertility and quality traits;

3. The ecopedological diagnosis soil after their characters, and integrating the synthetic indicator of soil quality is the result of correlation and factor interaction environmental (climate, soil, pedobiological) of biotopes analyzing and highlighting the potential and actual soil food context and local context that can mitigate stress, or increase the effective trophic level of the fund;

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# RESEARCH ON THE BIOTIC AND ENZYMATIC POTENTIAL OF SOIL FROM TÂRGU FRUMOS MICROREGION

## CERCETARI PRIVIND POTENTIALUL BIOTIC ȘI ENZIMATIC AL SOLULUI DIN CADRUL MICROZONEI TARGU FRUMOS

**ȚIBULCĂ L.<sup>1</sup>, MUNTEANU N.<sup>1</sup>,  
BIREESCU L.<sup>2</sup>, STOLERU V.<sup>1</sup>, GHITĂU Carmen<sup>1</sup>**  
e-mail: tibalca\_laurentiu@yahoo.com

**Abstract.** *The research was carried out in the vegetable ecosystem of Târgu Frumos microregion in 2010 – 2011. The biotic potential level illustrate an physiological activity of the totality of soil microbiota (microflora, edaphic mezofauna) which is involved in biochemical transformation of organic matter, humus and mineral soil material. The assessment of potential biotic and soil enzyme from Târgu Frumos microarea was possible with quality indicators in terms of pedobiological ecoclimatic of the year 2010 from the research stationary studied AF Maxim and A.F. Vavilov in vegetable ecosystems exploited in conventional system. The biological analysis of soil including the determination of soil physiological microflora (soil respiration and celulozoliza) and enzyme activity (catalase, invertase, urease and phosphatase).*

**Key words:** vital soil potential, enzymatic soil potential, biological soil analysis soil.

**Rezumat.** *Cercetările au fost desfășurate în cadrul ecosistemului legumicol al microzonei Târgu Frumos în 2010. Nivelul potențialului biotic ilustrează activitatea fiziologică a totalității microbiotei solului (microfloră, mezofaună edafică) care este implicată în procesele biochimice de transformare a materiei organice, a humusului și a materiei minerale din sol. Evaluarea potențialului biotic și enzimatic al solului din microzona Târgu Frumos a fost posibilă cu ajutorul indicatorilor de calitate pedobiologică în condițiile ecoclimatice ale anului 2010 din staționarele de cercetare luate în studiu A.F. Maxim și A.F. Vavilov în ecosisteme legumicole exploatate în sistem convențional. Analiza biologică a solului include determinarea activității fiziologice a microflorei solului (respirația solului și celulozoliza) și a activității enzimatice (catalaza, invertaza, ureaza și fosfataza totală).*

**Cuvinte cheie:** potențial vital al solului, potențial enzimatic al solului, analiza biologică a solului.

## INTRODUCTION

Fundamental quality of the soil in relation to known biological soil fertility factor (Stefanic, 1994, Stephanic et al., 2006; Birescu, 2001) is a fundamental feature that characterizes micropopulation vital activity of soil, plant roots, the enzyme accumulated and biochemical processes. The fertility

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup> Biological Research Institute of Iași, Romania

potential of the process depends on the accumulation and mineralization of soil organic matter specific, which in turn depends on the program and environmental conditions of the ecological subsystem development and anthropogenic influences (Gianfreda et al., 2005). The relationship between microbial diversity and the normal functioning of the soil is understood, on the one hand, the relationship between genetic diversity and microbial community structure and on the other hand, the relationship between microbial community structure and its function (Nannipieri et al., 2002).

The main goal of our research was to analyse the biotic and enzymatic potential of soil from Targu Frumos vegetable microzone, which could be extremely important in assessment of sustainability of vegetable production.

## **MATERIAL AND METHODS**

The research was conducted in 2010 - 2011 in the ecosystem of micro-area vegetable Târgu Frumos for two micro-area, A.F. Maxim and A.F. Vavilov, in order to evaluate potential biotic and enzyme land in this area (Munteanu, 2009).

Samples were taken 0-20 cm soil depth on plant row and the rows where the potential range of vital biological and enzyme can be strong, being the most active roots action.

To study the effects of human intervention and technological elements in the two ecosystems vegetable was determined experimentally, physiological potential of the soil, resulted in potential respiration (Stefanic, 1994, 1999) and cellulolytic potential of soil (Stefanic, 1994, 1999) and potential soil enzyme (catalase, sucrase, urease, phosphatase total).

The determination of soil respiration was possible using respirometry by Stefanic et al. (1998) able to replace oxygen consumed in the process of soil respiration and capture CO<sub>2</sub> released.

The method used for determining cellulolytic potential of soil (Stefanic, 1994, 1999) is based on replacement blade cotton cloth contains 50% cotton + 50% polyester, spun common thread that, following the celulozolisate to wash cloth, not to produce loss of cloth degraded and appear as an exaggerated celulozolisate.

Potential catalase activity is determined in the laboratory using a device called catalazometre. Principle is based on the fact that enzymatic and chemical reaction takes place simultaneously in the soil and therefore to get only the value will be determined separately catalase activity in soil samples inactivated enzyme catalytic activity (chemical) soil (Stefanic et al., 2006).

Sucrase activity in the soil analysis was made possible by the spectrophotometric method to determine the amount of reducing sugars (glucose and fructose, mg/100 g soil su) hydrolyzed enzymatically from sucrose.

Principle of determination of urease activity is that, ammonia result is determined quantitatively by colorimetric, with Nessler solution.

The principle method for determining the potential phosphatase activity is introduction, the enzyme mixture, a quantity of glucose act as "traps" for phosphate ions combine with free enzyme, determining the amount of glucose remaining uncombined. This can then be converted to equivalent phosphorus (P) with an index which is the quotient ratio combining phosphorus with glucose, determined experimentally by Stefanic (1999), within the limits of possible concentrations of phosphorus released free enzyme + phosphorus in soil and sugar added the reaction mixture.

## RESULTS AND DISCUSSIONS

### *Biotic potential (vital) of the soil - soil respiration*

Stationary Tg. Frumos-A.F. Maxim, the 0-20 cm depth, the values are the mean potential respiratory cultures among solar plant, varying slightly from 23.42 mg CO<sub>2</sub> for growing peppers - Maradona F1 to 26, 07 mg CO<sub>2</sub> from cucumber - Merengue.

The range of soil respiration values are lower rows nearly 50% due to summer hard consistency, low aeration porosity of soil compaction and lack of regular loose soil.

Stationary Tg. Frumos-A.F. Vavilov, the 0-20 cm depth, the values are of potential respiratory medium, comparable to those of tunnels from the solar A.F. Maxim crops among the plants, varying slightly from 24.81 mg CO<sub>2</sub> culture cucumbers, up to 26.14 mg CO<sub>2</sub>, the solarium planted with tomatoes.

The range of soil respiration values are lower rows nearly 50% due to consistency

### *Celulozolise*

For the vegetation samples for analysis at harvest time, cellulolytic potential values increase, similar to those of soil respiration, with somewhat higher values in A.F. Vavilov. These values vary among the plants A.F. Maxim between 24.53 to 27.16% cellulose and the range appear much lower values ranging from 12.65 to 15.44% cellulose.

These values vary among the plants A.F. Vavilov 25.33 to 27.18% cellulose and the range appear much lower values ranging from 13.24 to 18.27% cellulose.

### *Potential enzyme - catalase potential*

Pedoensimatic research on the physiological potential of certain specialized enzymes to catalyze decomposition of organic waste and organic fertilizers in conventional stationary vegetables, are presented in Table 1.

Stationary Tg. Frumos – A.F. Maxim, the depth of 0-20 cm, and the values are the mean potential of catalase to the cultures of greenhouses, row of plants that vary slightly from 317-351 cmc O<sub>2</sub>. The ranges of rows to 50% lower values.

Stationary Tg. Frumos – A.F. Vavilov, the depth of 0-20 cm, and the values are mean catalase medium potential slightly lower than those of greenhouses from A.F. Maxim values ranging from 303-322 cmc O<sub>2</sub>. The ranges of rows to 50% lower values.

### *Sucrase potential*

Stationary Tg. Frumos – A.F. Maxim, the depth of 0-20 cm, and the values are the mean sucrase potential plant row between 568-756 mg glucose, much higher than the beginning of the vernal season.

Table 1

**Biotic potential and enzymatic study of vegetable agroecopedotops  
Targu Frumos 2010**

| Stationary                         | Culture                       | Specification | BIOTIC AND ENZYMATIC POTENTIAL   |                            |                                   |                    |                                 |                                |
|------------------------------------|-------------------------------|---------------|----------------------------------|----------------------------|-----------------------------------|--------------------|---------------------------------|--------------------------------|
|                                    |                               |               | Respir.<br>(mg CO <sub>2</sub> ) | Celulozolis<br>(%celulose) | Catalase<br>(cmc O <sub>2</sub> ) | Sucrase<br>(mg gl) | Urease<br>(mg NH <sub>4</sub> ) | Total<br>Phosphatase<br>(mg P) |
| Tg.Frumos<br>A.F.Maxim<br>0-20cm   | Tomato<br><i>Granadero F1</i> | Row           | 25,86                            | 26,31                      | 317                               | 756                | 6                               | 4,2                            |
|                                    |                               | Interval      | 14,71                            | 15,44                      | 152                               | 273                | 3                               | 2,1                            |
|                                    | Tomato <i>Caliope F1</i>      | Row           | 24,31                            | 25,21                      | 336                               | 684                | 5                               | 3,7                            |
|                                    |                               | Interval      | 13,17                            | 14,36                      | 125                               | 315                | 2                               | 1,8                            |
|                                    | Pepper <i>Maradona</i>        | Row           | 23,42                            | 24,53                      | 351                               | 568                | 4                               | 4,6                            |
|                                    |                               | Interval      | 11,31                            | 12,65                      | 172                               | 276                | 2                               | 2,3                            |
|                                    | Cucumber<br><i>Merengue</i>   | Row           | 26,07                            | 27,16                      | 342                               | 618                | 3                               | 4,8                            |
|                                    |                               | Interval      | 13,51                            | 14,08                      | 171                               | 306                | 1                               | 2,1                            |
| Tg.Frumos<br>A.F.Vavilov<br>0-20cm | Tomato <i>Belle F1</i>        | Row           | 26,14                            | 27,18                      | 312                               | 754                | 7                               | 4,4                            |
|                                    |                               | Interval      | 14,05                            | 13,24                      | 165                               | 471                | 4                               | 3,0                            |
|                                    | Cucumber<br><i>Merengue</i>   | Row           | 24,81                            | 25,33                      | 322                               | 788                | 9                               | 3,7                            |
|                                    |                               | Interval      | 17,03                            | 18,27                      | 161                               | 442                | 4                               | 1,8                            |
|                                    | Pepper <i>Verdana</i>         | Row           | 25,13                            | 26,54                      | 303                               | 715                | 7                               | 4,8                            |
|                                    |                               | Interval      | 13,08                            | 14,41                      | 152                               | 407                | 3                               | 2,3                            |



*The ranges of rows to 50% lower values.*

Stationary Tg. Frumos – A.F. Vavilov, the depth of 0-20 cm, the values are mean sucrose potential, something greater than 715-788 mg glucose A.F. Maxim respectively.

The interval between rows of plants, compared with plants that are irrigated row and loose, the average decrease by 50%, in submijlociu.

*Potential urease*

Stationary Tg. Frumos – A.F. Maxim, the depth of 0-20 cm, the values are the mean potential urease row crops in greenhouses for plants, ranging from 3-6 mg NH<sub>4</sub> easy.

The interval between rows of plants, compared with plants that are irrigated row and loose, the average decrease by 50% in the small.

Stationary Tg. Frumos – A.F. Vavilov, the depth of 0-20 cm, and the values are the mean potential of urease, something greater than A.F. Maxim between 7-9 mg NH<sub>4</sub>.

The interval between rows of plants, compared with plants that are irrigated row and loose, the average decrease by 50%.

*Potential total phosphatase*

Stationary Tg. Frumos – A.F. Maxim, the depth of 0-20 cm, the values are the mean total phosphatase potential for growing vegetables in solar light among plants ranging from 3.7 to 4.8 mg P, higher than the vernal season.

The interval between rows of plants, compared with plants that are irrigated row and loose, the average decrease by 50%.

Stationary Tg. Frumos – A.F. Vavilov, the depth of 0-20 cm, the values are the average potential of solar phosphatase crops among the plants, comparable to those of A.F. Maxim 3.7 to 4.8 mg P.

The interval between rows of plants, compared with plants that are irrigated row and loose, the average decrease by 50%.

Biological activity is lower due to poor physical and mechanical properties (medium-fine texture soil, low aeration porosity and texture summer hard soil and the human impact of subsidence phenomenon and especially stressful and limiting the impact of risk factors conventional technology system.

## CONCLUSIONS

1. The biotic potential physiological activity illustrate the totality of soil microbiota (microflora, mezofaună edaphic) which is involved in biochemical transformation of organic matter, humus and mineral soil material;

2. Values as indicators of fertility and are influenced by specific environmental conditions and the microclimate of a technological nature;

3. Lower biological activity especially within the physical and mechanical properties due to faulty (medium-fine texture soil, low aeration porosity and consistency summer hard soil and human impact through phenomena due to subsidence and the effects of stressful and limiting factors risk to life in the soil.

4. These positive features are due to increased levels of some chemical

quality and especially the fertility and biological quality under stressful and mitigation of risk factors determined by the conventional system in which risk factors significant roles in limiting and stressful have.

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# EFFECT OF FUROSTANOL GLYCOSIDE TREATMENTS IN STRAWBERRY (*Fragaria x ananassa L.*) PLANTING MATERIAL PRODUCTION

## EFFECTUL TRATAMENTELOR CU FUROSTANOL GLICOZIDE ÎN PROCESUL DE PRODUCERE A MATERIALULUI SĂDITOR LA CĂPȘUN (*Fragaria x ananassa L.*)

**CĂULEȚ Raluca Petronela<sup>1</sup>, SUDITU Manuela<sup>1</sup>,  
MORARIU Aliona<sup>1</sup>, IUREA Dorina<sup>2</sup>, GRĂDINARU G.<sup>1</sup>**  
e-mail: raluca.petronela@yahoo.com

**Abstract.** The aim of this paper was to estimate the effect of two furostanol glycoside treatments (G1 and G2) on quality of the planting material in strawberry (*Fragaria x ananassa L.*). The experiments were carried on in U.A.S.V.M. greenhouse and were divided in two sections: the first one was focused on establishing the optimum concentration of treatment solution and its effect on rooting process and the second part has as aim the studying of the influence of furostanol glycoside treatments on foliar apparatus growth and development related with the treatment application method. The results showed that irrespective of the cultivar, both glycosides had a positive influence on rooting process, while vegetative growth was stimulated rather by G1 than G2 treatments. The treatment application method influenced the growth parameter in relation with the cultivar and glycoside type.

**Key words:** furostanol glycoside, rooting, strawberry, vegetative growth, application method.

**Rezumat.** Lucrarea are ca scop determinarea efectului tratamentelor cu două glicozide furostanolice (G1 și G2) asupra calității materialului săditor la căpșun (*Fragaria x ananassa L.*). Experimentul s-a desfășurat în sera U.S.A.M.V. Iași și a cuprins două etape: prima a vizat stabilirea concentrației optime de tratament și a influenței acestuia asupra procesului de înrădăcinare și a calității materialului săditor de căpșun, iar a doua a avut în vedere studierea efectului tratamentelor cu glicozide furostanolice asupra creșterii și dezvoltării aparatului foliar în funcție de metoda de aplicare. Rezultatele au evidențiat că ambele glicozide au avut influență pozitivă asupra procesului de înrădăcinare, iar creșterile vegetative au fost stimulate într-o mai mare măsură de tratamentele cu G1. Metoda de aplicare a tratamentelor a influențat indicatorii biometrici în funcție de soi și de produsul folosit.

**Cuvinte cheie:** glicozide furostanolice, înrădăcinare, căpșun, creșterivegetative, metoda de aplicare.

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup>Biological Institute Research Iasi, Romania

## INTRODUCTION

The effect of biostimulators in strawberry have been studied by many researchers showing that some of the products helps in improvement of fruit rot resistance (Washington et al. 1999), others increases the fruits nutrients content (Eşitken and Pirlak 2002), or fruit yield (Rohloff et al. 2002, Prokkola et al. 2003, Masny et al. 2004, Botta et al. 2009) and while others improves the number of runners and daughter plants (Abdel-Mawgoud et al. 2010). It was also demonstrated that some of the biostimulators increase the antioxidant enzymes activity in leaves (Špoljarević, 2010).

Furostanol glycosides are a new class of compounds which has recently being shown having a biostimulator effect on vines (Munteanu et al., 2008) and currants (unpubl. data) and also antioxidant, fungicidal, antiviral, bactericidal, nematocidal effect on tomatoes, cucumbers and potatoes, (Vasil'eva et al., 2000). It seems that furostanol glycoside plays an important role in the rate of pigment biosynthesis as well as in the biochemical systems of plant protection against oxidative damages (Vasileva et al., 2005).

In our paper the influence of two furostanol glycosides (G1 and G2) treatments on strawberry daughter plants rooting process and their furtherer behaviour in relation to the treatment application method.

## MATERIAL AND METHOD

The experiments were carried out in UASVM greenhouse during November 2011- January 2012. Young daughter strawberry plants (central bud and 2-3 leaves) were detached from the runners and maintained in glycoside solution for one hour and then were planted them in perlite for rooting. Treatment variants were: control (maintained for one hour in distillate water), and two glycoside solution (G1 and G2) in different concentration: 3 mM (V1), 0.3 mM (V2) and 0.03 mM (V3). G1 is an alcoholic extract from tomato seeds while G2 was obtained by alcoholic extraction of *Digitalis sp.* leaves. Each variant was represented by 10 plants. After one month the effect of treatments on rooting process has been evaluated by quantification of roots number/plant, the roots mean length, the new leaves mean number and their mean length.

For the second part of the experiment, only control plants and glycoside treated ones (G1V2 and G2V2) were kept and for the glycoside treatment was chosen only one variant of concentration (0.3mM). The treatment application method was varied: foliar (f) and foliar + radicular (f+r); so, before planting in pots, some of the young rooted strawberry plants were maintained in glycoside solution for 30 min, while the others were maintained in distillate water for the same period. Strawberries were planted in a mixture of soil-peat 4:1(v/v), in pots (500 ml capacity) and maintained in greenhouse for two months. During this time, treated variants were sprayed with glycosides solution (G1 and G2) while the control was sprayed with distillate water. After two months the effect of glycoside treatment and its application method was analysed by biometrical determinations

such as roots number/plant and their length; the new leaves number/ plant and their mean length. For each variant 10 plants were used.

## RESULTS AND DISCUSSIONS

In both cultivars rooting percentage was 100 % but differences have been seen between roots number/plant. Both in Real and Magic cultivars, glycoside treatments led to an increasing roots number/plant by 10-37% (tab.1). Irrespective of cultivar, G2 utilization seems to have a higher influence on rooting process, roots number being 8-10% higher than G1 treated variants. It can be also observed that V2 and V3 concentrations led to a better rooting than V1, which make us to suppose that 3mM is too high for root inducing in this specie.

*Table 1*

**Influence of glycoside treatment on rooting process of strawberry daughter plants (Mean±SE; n = 10)**

| Treatment | Cultivar | Roots mean number/plant | Roots mean length (cm) | New leaves number/plant | Leaves mean length (cm) |
|-----------|----------|-------------------------|------------------------|-------------------------|-------------------------|
| Untreated | REAL     | 10,60±0,18              | 9,47±0,44              | 3,20±0,14               | 4,38±0,44               |
|           | MAGIC    | 11,00±1,00              | 8,76±0,53              | 2,33±0,15               | 4,43±0,53               |
| G1V1      | REAL     | 12,00±0,47              | 10,68±0,69             | 3,45±0,30               | 4,97±0,89               |
|           | MAGIC    | 11,67±0,58              | 9,97±0,89              | 2,52±0,58               | 4,95±0,89               |
| G1V2      | REAL     | 13,20±0,17              | 11,89±0,38             | 3,94±0,15               | 5,93±0,38               |
|           | MAGIC    | 13,35±0,53              | 10,90±1,32             | 2,85±0,15               | 5,57±1,32               |
| G1V3      | REAL     | 12,87±0,36              | 11,67±0,55             | 3,78±0,26               | 5,19±0,55               |
|           | MAGIC    | 12,80±0,87              | 10,92±0,29             | 2,77±0,58               | 5,10±0,29               |
| G2V1      | REAL     | 11,68±0,61              | 10,79±0,44             | 3,53±0,08               | 4,25±0,44               |
|           | MAGIC    | 12,29±0,85              | 10,02±0,12             | 2,55±0,20               | 4,22±0,02               |
| G2V2      | REAL     | 14,50±0,54              | 12,17±0,66             | 3,75±0,44               | 5,05±0,66               |
|           | MAGIC    | 14,67±1,04              | 11,06±0,76             | 2,67±0,38               | 4,92±0,76               |
| G2V3      | REAL     | 13,55±0,43              | 11,62±0,81             | 3,62±0,46               | 4,80±0,81               |
|           | MAGIC    | 13,57±1,09              | 10,84±0,61             | 2,65±0,58               | 4,39±0,61               |

Beside roots number, the mean length of the roots is another important indicator used in strawberry plant material production, knowing that a good developed rooting system will provide better water and nutrients absorption, as well as a better drought resistance. Our experiment showed that glycoside treated variants had 25-28% bigger values of roots length that control (tab.1). No major differences between the two glycoside treatments had been observed either Real or Magic cultivar. Regarding the treatment concentration influence on root length the same pattern as in roots number can be observed. The roots were 12-14% longer in V2 and V3 variants than V1.

Another quality parameter of strawberry planting material is the morphological state of foliar apparatus. In our experiments glycoside

treatment induced an increasing by 10-20% in the leaves number. In both cultivars, G1 treated variants recorded higher values of this parameter than G2 treated ones, especially when V2 concentration was used (tab.1).

The leaves mean length was also influenced by the glycoside treatments. 20-35 % higher values of this parameter were determined at G1 treated variants, while in those treated with G2 leaves mean length increased only by 10-15 % than control (tab.1). It seems that contrary of G2, G1 stimulates the foliar growth in both Real and Magic cultivar.

Due to the higher values obtained in all studied parameters 0,3 mM was the concentration chosen to be used for studying the influence of glycoside treatment application method on growth and development of strawberry plants.

The young plans behaviour after transplantation in pots has been studied in the aim of determining the influence of furostanol glycoside treatment on vegetative growth after a longer treatment period. Therefore two months after planting, the root and leaves growth parameters were measured again. The results showed an increasing of root mean number only in G2 treated variants (values being 20% higher than control), the G1 treated ones having similar values with control (tab. 2).

Table 2

**Influence of glycoside treatment and application method on growth and development of strawberry plants (Mean±SE; n = 10)**

| Treatment | Cultivar | Roots mean number/plant | Roots mean length (cm) | Leaves mean number/plant | Leaves mean length (cm) |
|-----------|----------|-------------------------|------------------------|--------------------------|-------------------------|
| Untreated | REAL     | 17,24±0,17              | 7,10±0,07              | 7,43±0,08                | 10,60±0,11              |
|           | MAGIC    | 20,28±0,21              | 10,14±0,10             | 6,41±0,05                | 10,54±0,11              |
| G1f       | REAL     | 18,28±0,21              | 7,44±0,10              | 9,62±0,07                | 12,18±0,12              |
|           | MAGIC    | 19,96±0,26              | 10,49±0,19             | 7,89±0,05                | 13,00±0,12              |
| G1f+r     | REAL     | 18,09±0,30              | 7,35±0,08              | 9,59±0,09                | 11,61±0,12              |
|           | MAGIC    | 19,27±0,20              | 10,12±0,12             | 7,90±0,09                | 12,48±0,13              |
| G2f       | REAL     | 21,01±0,23              | 8,14±0,10              | 7,73±0,07                | 10,27±0,11              |
|           | MAGIC    | 24,34±0,25              | 11,17±0,10             | 6,40±0,06                | 10,15±0,12              |
| G2f+r     | REAL     | 20,31±0,15              | 8,02±0,09              | 7,06±0,09                | 9,41±0,11               |
|           | MAGIC    | 23,34±0,11              | 10,85±0,17             | 5,48±0,08                | 9,67±0,11               |

Comparing the two ways of product application, it has been observed that in both cultivars foliar and radicular application of G2 led to a slightly decrease of roots mean number/plant, while in G1 treated variants this fact had been observed only in Magic (tab. 2).

Almost the same trend, but with higher differences between G1 and G2 treatments, has been observed in analysis of roots mean length. G2

treated variants had 12-15% higher values of this parameter than control, while in G1 treated ones the differences were only about 3-5% (tab. 2).

The influence of the application method was observed only in G2 treated variants. In case of the mixed treatments (leaves and roots – G2f+r) a lower increase of roots length has been observed comparing with foliar treatment (G2f). This may be due to an accumulation of the product in plant, in which, above some level, cannot be used anymore or becomes inhibitory for roots growth.

Irrespective of the cultivar and the application method G1 treatments led to an increasing of leaves mean number/plant by 20-30% than control (untreated). In case of G2 treatments, foliar application (G2f) did not influence this parameter (values being similar to control), on the contrary it decreased it (by 10-15%), in case of mixed application (G2f+r) (tab. 2). Similar results had been obtained by some other researchers (Keltling et al. 1997) which shown that bio stimulators have not always act an improvement of plant growth.

In both Real and Magic, the leaf mean length was influenced rather by glycoside type than the application method. G1 treated variants had 14-23% longer leaves than control, while in G2 treated variants the values of this parameter were even smaller (9-12%) than control. Moreover, in case of mixed treated variants (G1f+r and G2f+r) the leaves length values were a little bit lower than those recorded in foliar treated ones (G1f and G2f).

Taking into account the higher values obtained in Magic cultivar in almost all of the studied parameters we can conclude that this cultivar responded better on glycosides treatments (especially to G1) than Real which is a quite normal behaviour knowing that plants can react differently on the same bio stimulators and this kind of variation has been reported by many other authors at several species (Laugale and Daugavietis, 2009).

## CONCLUSIONS

1. Furostanol glycoside treatments improved the rooting process by increasing of roots number and their length (especially G2) and determined a better growth and development of foliar apparatus (G1).

2. Magic cultivar responded better than Real on glycoside treatments after transplantation in soil, recording higher values in almost all of the studied parameters.

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# EFFECT OF FUROSTANOL GLYCOSIDE TREATMENTS IN PLANT MATERIAL PRODUCTION IN CURRANTS (*Ribes sp.*)

## EFFECTUL TRATAMENTELOR CU FUROSTANOL GLICOZIDE ÎN PROCESUL DE PRODUCERE A MATERIALULUI SĂDITOR LA COACĂZ (*Ribes sp.*)

CĂULEȚ Raluca Petronela<sup>1</sup>, ONOFREI Oana<sup>1</sup>,  
MORARIU Aliona, IUREA Dorina<sup>2</sup>, GRĂDINARU G.<sup>1</sup>  
e-mail: ralucapetronela@yahoo.com

**Abstract.** *The influence of treatment with two furostanol glycosides (G1 and G2) on the rooting process and shoot development of cuttings was analysed relative with a usual commercial rooting product (RADI-STIM) at three currants cultivars (Deea, Abanos and Ronix). Although both glycosides treatments induced a better rooting than RADI-STIM, G2 treatment induced higher number of roots and a stronger development of root system (with more and longer roots) while G1 treatment had a higher influence on foliar apparatus growth and development.*

**Key words:** furostanol glycoside, biostimulators, rooting, currants, vegetative growth

**Rezumat.** *Influența tratamentelor cu două glicozide furostanolice (G1 și G2) asupra înrădăcinării și a dezvoltării aparatului foliar a fost studiată comparativ cu influența unui produs comercial (RADI-STIM) la trei soiuri de coacăz (Deea, Abanos și Ronix). Deși tratamentul cu ambele glicozide a stimulat înrădăcinarea într-o măsură mai mare decât RADI-STIM, tratamentul cu G2 a determinat formarea unui sistem radicular mai puternic (cu rădăcini mai multe și mai lungi) decât RADI-STIM, în timp ce tratamentele cu G1 au influențat într-o măsură mai mare creșterea și dezvoltarea aparatului foliar.*

**Cuvinte cheie:** glicozide furostanolice, biostimulatori, înrădăcinare, coacăz, creșteri vegetative.

## INTRODUCTION

A key step in vegetative propagation is adventitious root formation and high losses can occur because of the poor quality of the root system (De Klerk et al., 1999). Often growth regulators are used for improving cuttings rooting or its uniformity. Indole-3-butyric acid (IBA) is the primary root hormone which promotes the induction of adventitious roots, being in many cases more efficiently than IAA (Indole-acetic-acid) (Epstein and Ludwig-Müller, 1993) due to its higher stability (Nordström et al., 1991). Beside that there are many others products which can be used to improve cuttings rooting.

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup>Biological Institute Research Iasi, Romania

In the last decade, a number of products referred to generically as biostimulant have appeared on the market (Boehme et al. 2005). Some of them has been show to increase root and leaf growth, (Ertani et al. 2009), nutrient uptake (Eşitken and Pirlak 2002), chlorophyll content (Neri et al. 2002) and enhance resistance to biotic and abiotic stress (Boehme et al., 2008, Botta et al. 2009, Marfà et al. 2009) helping plants to cope with pest and diseases (Washington et al. 1999, Prokkola et al. 2003).

Furostanol glycosides are a new class of natural compounds which can act like bioactive compound shaving a wide range of biological activities: antioxidant, fungicidal, antiviral, bactericidal, nematocidal etc. (Vasil'eva et al., 2005). The adaptive effect of furostanol glycosides was first discovered at tomatoes and cucumbers affected by the gull nematode (Vasil'eva et al., 2000). Subsequently their influence on antioxidant enzymes (Volkova et al., 2007), assimilator pigments biosynthesis (Vasil'eva et al., 2005) and grapevines plants growth and development (Munteanu et al., 2008) has been studied.

Our experiments were focused on furostanol glycoside effect on rooting process and the furtherer growth and development of rooted cuttings of three currant cultivars.

## MATERIAL AND METHOD

The experiments were carried out in UASVM greenhouse during November 2011- January 2012. Currants cuttings from Deea, Abanos and Ronixcultivars were cut at the end of vegetation period (November 2011).

Treatment variants were represented by untreated cuttings (Control), cuttings treated with a commercial rooting product (RADI-STIM), and treated with glycoside 1 (G1) and glycoside 2 (G2) solution in different concentrations: 3mM (V1), 0.3mM (V2) and 0.03mM (V3). G1 is an alcoholic extract from tomato seeds while G2 was obtained by alcoholic extraction of *Digitalis sp.* leaves.

RADI-STIM treatments were made by dipping of cuttings in RADI-STIM solution 2% for 2-3 seconds, and glycoside treatments were made by maintaining the cuttings in glycoside solution for 1 hour. Control was maintained for the same period in distillate water.

After treatment cuttings were put for rooting in perlite assuring the necessaries temperature (22-25<sup>0</sup>C) and humidity (80-90%) conditions. After one month the effect of treatments on rooting process has been determined by quantification of rooting percentage of cuttings, roots number/cutting and the roots mean length.

In the second part experiment was conducted only for control plants and the best glycoside concentration treatment (0.3mM). Hence, after rooting, the cuttings were planted in pots with soil/peat 3:1 (v/v) mixture and maintained in greenhouse for two months while the treatment with G1 and G2 were carried on by foliar spraying weekly. Control plants were sprayed with distillate water.

The influence of the glycoside treatments on vegetative growth was determined by the shoots length, leaves number/cutting and leaf area determination.

Leaf area measurements were made by scanning the leaves and the images were analysed with ImageJ software at 300 dpi resolution.

## RESULTS AND DISCUSSIONS

Rooting percentage varied in a range of 66-81% relative with cultivar and treatment variant. In Ronix and Abanos both furostanol glycoside treatments and RADI-STIM led to an increasing of rooting percentage with 15-20%, and only with 10% in Deea cultivar (Tab. 1), withno significant differences between the usual commercial product (RADI-STIM) and furostanol glycoside treatments. However, the highest values of the rooting percentage were recorded on G2 treated variants, especially at the 0.3mM concentration.

Table 1

**Influence of furostanol glycoside treatments on rooting process**(Mean±SE; n = 10)

| Treatment variant | Cultivar | Rooting %  | Roots number/cutting | Roots mean length (cm) |
|-------------------|----------|------------|----------------------|------------------------|
| Untreated         | DEEA     | 66,40±0,72 | 8,34±0,04            | 6,74±0,11              |
|                   | ABANOS   | 68,72±1,09 | 12,06±0,03           | 6,83±0,08              |
|                   | RONIX    | 67,56±0,91 | 10,20±0,04           | 6,78±0,09              |
| RADI-STIM         | DEEA     | 70,57±0,81 | 9,13±0,04            | 6,78±0,12              |
|                   | ABANOS   | 75,41±0,70 | 12,64±0,06           | 7,74±0,09              |
|                   | RONIX    | 75,49±0,75 | 9,78±0,05            | 7,29±0,11              |
| G1V1              | DEEA     | 72,70±1,13 | 9,33±0,03            | 6,67±0,13              |
|                   | ABANOS   | 73,31±0,71 | 12,96±0,03           | 8,56±0,09              |
|                   | RONIX    | 74,00±0,92 | 10,34±0,03           | 7,48±0,11              |
| G1V2              | DEEA     | 73,81±0,07 | 11,35±0,11           | 7,65±0,16              |
|                   | ABANOS   | 81,43±0,22 | 14,62±0,04           | 8,86±0,05              |
|                   | RONIX    | 79,12±0,14 | 11,04±0,07           | 8,48±0,11              |
| G1V3              | DEEA     | 71,77±0,83 | 10,19±0,03           | 7,37±0,20              |
|                   | ABANOS   | 71,78±0,71 | 13,92±0,05           | 8,52±0,14              |
|                   | RONIX    | 73,78±0,77 | 10,75±0,04           | 8,54±0,17              |
| G2V1              | DEEA     | 72,51±0,84 | 9,73±0,04            | 6,59±0,20              |
|                   | ABANOS   | 75,18±0,73 | 12,78±0,05           | 7,40±0,14              |
|                   | RONIX    | 76,85±0,78 | 9,66±0,04            | 10,01±0,17             |
| G2V2              | DEEA     | 75,81±1,04 | 11,36±0,06           | 7,71±0,20              |
|                   | ABANOS   | 81,95±0,36 | 15,39±0,03           | 9,11±0,13              |
|                   | RONIX    | 80,88±0,70 | 11,44±0,04           | 9,32±0,16              |
| G2V3              | DEEA     | 70,54±0,85 | 11,40±0,07           | 7,12±0,21              |
|                   | ABANOS   | 77,46±0,73 | 14,52±0,04           | 8,52±0,14              |
|                   | RONIX    | 78,50±0,79 | 10,71±0,05           | 8,34±0,17              |

It has been showed that biostimulators treatments improve roots growth (Liu and Cooper, 2000). In our experiments furostanol glycoside acted like biostimulators increasing roots number in all of the three cultivars, by 10-30% at Deea and Abanos and by 10% at Ronix (tab 1).

Comparing with RADI-STIM variants, the results showed that glycoside treatment led to an increasing of roots number 13-15% at G1 and 20-25% at G2 treated ones.

It was shown that the intensity of rooting is determined by the type and concentration of the growth stimulators (Novickiene et al., 2004). In our experiments glycoside concentration influenced the roots number, the results showing that in V2 concentration (0.3mM) values of roots number/cutting were 10-15% higher than those recorded at V1 (3mM) and V3 (0.03mM).

Root length is an important indicator for a potential uptake of water and nutrients. Irrespective of the chemical used, treated variants recorded higher values than untreated ones (tab. 1).

In all of the three cultivars G1 and G2 treatment induced formation of by 10-20% longer roots than RADI-STIM treatment, especially when the concentration was 0.3mM.

Biostimulators can influence shoots length by stimulation or inhibition of cells division and elongation. Our experiments showed a decreasing in shoots length in G1 treatment, while in G2 treated variants shoots length was close to control. (tab. 2).

*Table 2*

**Influence of furostanol glycoside treatment on vegetative growth (Mean±SE; n = 10)**

| Treatment variant | Cultivar | Shoots mean length (cm) | Mean number of leaves/plant | Mean leaf area |
|-------------------|----------|-------------------------|-----------------------------|----------------|
| Untreated         | DEEA     | 13,24±0,54              | 7,40±0,15                   | 53,25±0,20     |
|                   | ABANOS   | 14,80±0,58              | 7,80±0,16                   | 47,93±0,20     |
|                   | RONIX    | 17,30±0,60              | 8,60±0,14                   | 58,45±0,53     |
| G1 Treatment      | DEEA     | 11,00±0,54              | 8,60±0,29                   | 86,79±0,54     |
|                   | ABANOS   | 10,28±0,80              | 9,10±0,25                   | 80,11±0,47     |
|                   | RONIX    | 13,56±0,67              | 11,00±0,21                  | 85,15±0,33     |
| G2 Treatment      | DEEA     | 13,55±0,54              | 8,15±0,23                   | 46,66±0,23     |
|                   | ABANOS   | 15,08±0,68              | 8,45±0,21                   | 40,88±0,16     |
|                   | RONIX    | 17,65±0,42              | 9,30±0,13                   | 50,22±0,18     |

In all of the three cultivars glycoside treatments led to an increasing of leaves number/plant, by 15-20% in G1 treated variants and only by 10% in G2 treated ones. This may be due to inhibition of shoot growth by G1 treatments and diversion of metabolites for bunch development thereby reducing availability of metabolites for shoot elongation (Bhat, 2011).

Leaf area was also influenced by glycoside treatments (tab. 2). In all of the three cultivars G1 treatment led to an increase this parameter with 40-60%, while at the variants treated with G2 the values were 10-15% lower than control,

which leads us to the supposition that G2 treatments has an inhibitory effect on leaves growth.

## CONCLUSIONS

1. In all of the three currants cultivars, G1 treatment induced a higher development of vegetative growth, while G2 treatments had a bigger influence on rooting process.

2. Irrespective of the cultivar, all parameters related with root system and foliar apparatus growth and development recorded similar or higher values in furostanol glycoside treated variants than those of the RADI-STIM treated ones

3. Furostanol glycosides treatments were not indispensable for root formation and growth, but their application resulted in more qualitative currants cuttings in all of the three cultivars.

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# RESEARCH ON THE ORNAMENTAL TREE SPECIES SANOGEN IMPACT UPON URBAN ECOSYSTEMS

## CERCETARI PRIVIND IMPACTUL SANOGEN AL SPECIILOR POMICOLE ORNAMENTALE ASUPRA ECOSISTEMELOR URBANE

*NEGREA Roxana*<sup>1</sup>, *ZLATI Cristina*<sup>1</sup>, *GRĂDINARIU G.*<sup>1</sup>

e-mail: roxana.acfrance@gmail.com

**Abstract:** *Meta studied landscape spaciousness throughout this paper, can be configured as bringing closer the artistic essence of urban space and industrial space in which most urban areas are planted with “sad” shrubs, “ornamental” trees that are not native to these areas and which need too much water, and annual flowers planted in organized rows. Therefore this paper presents the positive influence on the population that a city may have, city in which apple, pear, cherry, walnut trees and shrubs grow along the streets and create a well-deserved shade around parking spaces and in parks but also, offering fruits to those who wish to collect thus, combining the local gastronomic movement with the beauty of the neighborhood, consolidating communities and feeding them at the same time.*

**Key words:** landscape, artistic essence, urban space, ornamental trees.

**Rezumat:** *Meta spațialitatea peisajului studiat de-a lungul acestei lucrări, se poate configura ca un marș de apropiere a esenței estetice a spațiului citadin și a celui industrial în care majoritatea zonelor urbane sunt plantate cu arbuști “trști”, pomi “ornamentali” care nu sunt nativi zonelor respective și care necesită prea multă apă, și flori anuale plantate în șiruri organizate. Prin urmare lucrarea de față prezintă influența pozitivă asupra populației, pe care o poate avea un oraș în care merii, perii, nucii, cireșii și arbuștii fructiferi cresc de-a lungul străzilor și creează o bine-meritată umbră în preajma locurilor de parcare și în parcuri oferindu-și în același timp fructele celor care doresc să le culeagă combinând astfel mișcarea gastronomic-locală cu înfrumusețarea cartierelor, consolidând comunitățile și hrănindu-le în același timp.*

**Cuvinte cheie:** peisaj, esență estetică, spațiu citadin, pomi ornamentali.

### INTRODUCTION

In figurative terms, we can define the concept of introduction of the ornamental tree plants in city landscaping, as the process by which people admit that people have used too much natural capital and that – for our own sake – now it’s the time to offer something back to nature, because we depend on it and its functions. The introduction of these species in the urban landscape aims to increase the existent natural capital by complementing the efforts to preserve nature outside the delimited enclaves, where this natural capital has become dangerously reduced.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

This new way to approach of landscape, at the crossroad between project and use, as it can be seen in figure 1, determines a new polarity between the studies that see landscape as the integral result of a political construction and those for which landscape is the result of a vernacular construction, connected to the logics of everyday life. While the first studies regard the construction of the landscape from a political perspective, “from top to bottom”, the second one is more anthropological and “from bottom up” oriented.

This double perspective is applied to landscape both as spatial reality, in situ, and as a way to view and reproduce space. This means that we can talk about different ways to build landscape using a larger variety of species with the purpose of complementing the space and its need, as well as about different ways to view and use it by using ornamental tree species, that appeal to different instruments and belong to various social segments or groups. (Tudora, 2009)

## MATERIAL AND METHOD

In selecting some tree species to create landscaping compositions it is mandatory to know their biological characters in order to make the landscape more harmonious with the help of different colours that have the ability to generate emotions; these colours often receive epithets like cold or warm, calming or irritant.

The harmony of colours in a landscaping theme can be influenced by the line and habitus of the trees as well as by the shape of the grounds which you can see in figure 2.

This, association of colours on a paper is one thing, and reproducing it by using vegetal material can be an entirely different thing. Thus, the following species have been considered, depending on their decorative elements:

- Species which decorate through sprouts: *Cornus alba*, *C. stolonifera*, *C. sanguinea*, *Corylus avellana*, *Prunus lusitanica*, *Hippophae rhamnoides*;
- Species which decorate through flowers: *Corylus maxima*, *Cornus officinalis*, *Lonicera japonica*, *Malus coccinella*, *Prunus serrulata*, *Prunus tenella*;
- Species which decorate through fruits: *Cornus kousa*, *Cornus mas*, *Crataegus monogyna*, *Prunus persica*, *Prunus cerasus*, *Sambucus nigra*.

As working method, we have observed the decorative species, described and studied the psychological effect of colours on the psyche.

We would also like to mention that the secret, charm and soul of the composition depends on the colours' effect.

In nature it can be found in the branches of *Cornus alba* "Sibirica", *Cornus sanguinea*, *Prunus lusitanica*, in the flowers of *Chaenomeles japonica*, *Lonicera*, *Malus coccinella* and the fruits of *Malus sargentii*, *Prunus cerasus*, *Rubus idaeus* and others. (Connan, 1999)

In nature, it is most often met as the colour of the flowers for species: *Cornus kousa*, *Cornus floribunda*, *Prunus glandulosa*, *Prunus laurocerasus*, *Pyrus elaeagrifolia*, *Sambucus nigra*, *Mespilus germanica*, *Lonicera japonica* but it can also be found as the colour of young sprouts of *Pyrus nivalis* or the fruits of *Cornus glabrata*. (Pollak, 2004)





**Fig. 1** - Bacău center before introducing the fruit species in the landscape in two diferent compositions of the same square. (original)

## RESULTS AND DISSCUSIONS

With the present paper we will focus only on the ornamental tree plants of real esthetic and functional interest, because the project we propose focuses on a landscaping formation of intentional nature and not on natural landscape. Still, we must also consider the fact that landscaping can receive some conditionings or influences from the natural landscape it is surrounded by.

Consequently, the species taken in for the study besides their special aesthetic characteristics also have a high ecologic plasticity, since our country is one of the European countries where the culture of fruit-bearing trees is well represented through a high diversity of species and varieties, that find here the favourable soil and weather conditions for growth and fruit yield, also insuring an echeloned fruit consumption throughout the year.

As it is well known, the distribution of vegetation from one area depends on the modification of the climate from one region to another. Among the climate elements, temperature and humidity play an important part in this respect. Even if they can be controlled and artificially induced, it is recommended to predominantly use the species with a high rusticity degree that are also decorative in the landscaping compositions of a certain area, since they are much easier to use in arrangements and easier to care for on the long run, in comparison with the exotic species that in this case involve certain maintenance and care measures. (Grigorovschi, 2012).



**Fig. 2** - Chromatic effect of *Vaccinium* spp fruit, the green of the *Cornus mas* , the purple of the *Prunus cerasifera*'s leaves and the cherry of *Prunus laurocerasus*'s flowers compared with the dull landscape of Bacău center after introducing the fruit species in the landscape of the square. (original)

Thus, the present paper aims to make a presentation of ornamental fruit bearing trees that create such a diversity of the landscape in public areas that it's impossible to conceive an arrangement without them; they are highly decorative due to their varied shape, and the different colour of the leaves during the vegetation period as it can be seen in figure 3, the blossoms, that are often pleasantly fragrant and last but not least, due to the beauty of the fruit and the forms and colours they have, and the taste and health they offer through the vitamins they contain.

The species with small fruit will be placed closer to alleys, without creating any prejudices once they fall after maturation.

The ones with larger fruit that perhaps last after the leaves fall can be placed further away from the beholder.

Though most species have average or slow growth rate, the species with rapid growth rates are the most requested, needed to a rapid shape-up of the vegetal landscape.

Some tree species which you can see in figure 2, especially those belonging to the *Prunoideae* family have a high sucker ability, which must be taken into consideration for the emplacement and association of species, for a proper setting of planting distances. Sucking can become an inconvenience for the associated species, and a cause of sidewalks, alleys and construction elements deterioration.



**Fig. 3** - Psychological effect of different colours of the ornamental trees species in the urban landscape of Bacău center, presented in two different compositions of the same space. (original)

Green, yellow, orange are warm, active colours, and blue, green and violet are considered passive, asthenic. The warm tones harmoniously go together with the cold ones. (Miller, 2001).

Besides the colour effect, we mustn't ignore the different degree of compactness of the crowns, or the texture of the leafage.

In selecting some tree species for the creating of landscaping compositions it is mandatory, that in addition to the understanding of the decorative characters we have a biological and ecopedological understanding in

order to be able to create a more harmonious design from an aesthetic and practice point of view, combining the local gastronomic movement with the beauty of the neighborhood, consolidating communities and feeding them at the same time.

## CONCLUSIONS

1. The harmony of colours in a landscaping composition is obtained by skilfully combining more colours.

2. Meta studied landscape spaciousness throughout this paper, can be configured as bringing closer the artistic essence of urban space and industrial space in which most urban areas are planted with “sad” shrubs, “ornamental” trees that are not native to these areas and which need too much water, and annual flowers planted in organized rows.

3. This paper presents the positive influence on the population that a city may have, city in which apple, pear, cherry, walnut trees and shrubs grow along the streets and create a well-deserved shade around parking spaces and in parks but also, offering fruits to those who wish to collect thus.

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# ASPECTS CONCERNING THE NUTRITIONAL QUALITY OF SOME FRESH CHERRIES GROWN IN THE MOLDAVIAN REGION OF ROMANIA

## INVESTIGAREA CALITĂȚILOR NUTRITIVE ALE UNOR FRUCTE DE CIREȘ CULTIVATE ÎN ZONA MOLDOVEI A ROMÂNIEI

**PAȘCU D. D.<sup>1</sup>, GRĂDINARIU G. <sup>1</sup>, CIOBOTARI Gh.<sup>1</sup>**

e-mail: dragos\_daniil@yahoo.com

**Abstract.** *In this paper, we have determined the soluble carbohydrates, fruit acidity, total flavanoids and total content of minerals of the fruit from 20 cherry-tree varieties, cultivated in the Moldavian area. The purpose of this paper has been the identification of the varieties with high quality value regarding the content of these compounds, substances and properties. Following the analysis carried out, it has been identified that the soluble carbohydrates content varied between 10.8 and 17.77 %, the fruit acidity registered values between 3.9 and 10 g/L (expressed in citric acid), the flavanoids content was between 21.8 – 73.69 mg/100 g FW, and the total mineral content has been between 0.27 and 2.4 %.*

**Key words:** soluble carbohydrates, acidity, flavanoids, total minerals

**Rezumat.** *În această lucrare am determinat glucidele solubile, aciditatea fructelor, flavanoli totali și conținutul total de minerale la 20 de varietăți de cireș cultivate în regiunea Moldovei. Scopul lucrării a constat în identificarea soiurilor cu fructe de calitate superioară din punct de vedere al conținutului acestor compusi, substanțe și proprietăți. În urma analizelor efectuate s-a constatat ca conținutul de glucide solubile a variat între 10.8 și 17.77 %, aciditatea fructelor a înregistrat valori cuprinse între 3.9 și 10 g/L (exprimat în acid citric), conținutul de flavanoli s-a încadrat între 21.8 – 73.69 mg/100 g FW, iar conținutul de minerale totale a fost cuprins între 0.27 și 2.4%.*

**Cuvinte cheie:** glucide solubile, aciditate, flavanoli, minerale totale

### INTRODUCTION

The cherry tree is one of the most important fruit bearing trees from the temperate area. Its fruit is highly appreciated due to their quality (sugar, vitamins, mineral content and various bio-active compounds), as well as due to the fact that in some areas these are the first fresh fruit of the year.

The colour of the fruit is its most important commercial quality and an indicator of the maturity of the cherries that fully depends on the total antocians content. (Esti et al., 2002). It is known that cherries have an important content of polyphenols and antocians, that contribute to their antioxidant activity (Goncalves et al., 2004; Khanizadeh et al., 2007; Serrano et al., 2005; Vangdal et al., 2007; Vursavuset al., 2006).

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

The objective of our researches has been to quantify the main qualitative properties for 20 varieties of cherry-trees, of different origin and tree characteristics.

## MATERIAL AND METHODS

The fruit have been harvested on their commercial maturity directly in liquid nitrogen, afterwards they were stored at -80°C until the analysis were carried out. From the 20 varieties taken for the study, 17 had red epidermis (Ponoare, Cerna, Precoce della Marca, Viva, Iasirom, Daria, Rosii de Bistrita, Ramon Oliva, Rainbow, Timpurie Franceza, Victor, Bing, Lapins, Colina, Scorospelka, Bigarreau Burlat, Timpurii de Bistrita), one variety had yellow epidermis (Bigarreau Donissen) and two varieties were sour cherries, used mainly for industrialization (Amar Costuleni and Amar Boiste).

In order to determinate the soluble carbohydrates we have used the *School Method*, modified by Vlad Artenie. The dry soluble substance from the fruit juice has been determined through the *refracto-metric method*.

The total acidity from the fruit juice has been established through the *potentiometric method*. The diluted juice samples have been titred with a sodium hydroxide 0.1 N up to pH 7 which has been potentiometrically determined. Previously, the carbon dioxide has been removed from the sample.

The total flavanoids (TFA) have been determined using the *DMACA method* (Li, Tanner, Larkin, 1996). In a 10 mL test tube 0.2 mL extract has been mixed with 3 mL DMACA solution (0.1% HCl 1 mol/L in MeOH). The mixture was agitated and then 10 minutes later the absorbance was read at a wave length of 640 nm in comparison with the blind sample without DMACA. The TFA content has been established according to an echelon curve with catechin (6.25–200 mg/L).

In order to determine the total content of mineral from fruit, these have been weighed in porcelain crucible, then entered in the calcinations oven at 600°C for 6 hours, and after cooling, the crucibles were weighed.

## RESULTS AND DISCUSSIONS

The early fruition of the species *Prunus avium* L. suggests that a substantial part of the carbohydrates used in the incipient phase of the culture development comes from reserves (Roper and Kennedy, 1986).

Carbohydrates are the most important compounds of wooden plants, totalling three quarters of their dry substance (Pallardy, 2008).

Some authors (Serrano et al., 2005; Usenik et al., 2005) think that the predominant sugar in cherry-tree fruits is glucose, and for this reason we have resumed to determining the content of soluble carbohydrates.

As it can be observed from the results of our analysis, (tab. 1) the quantity of soluble carbohydrates from fruit varies between 10.8% at the Scorospelka variety and 17.77% at the Timpurie Franceza variety. Very similar values to the maximum one were also registered for the Amar Costuleni (17.33%) variety and Timpurii de Bistrita (17.32%).

Table 1

**Soluble dry substance content and soluble carbohydrates for the fruit of several cherry tree varieties**

| Variety              | S.U.S. (%) | Soluble carbohydrates (%) |
|----------------------|------------|---------------------------|
| Ponoare              | 15.50      | 13.95                     |
| Cerna                | 13.75      | 12.38                     |
| Precoce della Marca  | 14.49      | 13.04                     |
| Amar Costuleni       | 19.25      | 17.33                     |
| Viva                 | 14.56      | 13.11                     |
| Daria                | 15.26      | 13.74                     |
| Iasirom              | 17.67      | 15.90                     |
| Rosii de Bistrita    | 14.54      | 13.09                     |
| Ramon Oliva          | 14.76      | 13.28                     |
| Bigarreau Donissen   | 15.75      | 14.17                     |
| Rainbow              | 14.71      | 13.23                     |
| Timpurie Franceza    | 19.75      | 17.77                     |
| Victor               | 15.00      | 13.50                     |
| Bing                 | 16.50      | 14.85                     |
| Lapins               | 14.51      | 13.06                     |
| Colina               | 14.97      | 13.47                     |
| Scorospelka          | 12.00      | 10.80                     |
| Amar Boiște          | 16.26      | 14.63                     |
| Bigarreau Burlat     | 14.00      | 12.60                     |
| Timpurii de Bistrita | 19.25      | 17.32                     |

The fruit of the superior plants contain a large variety of organic acids, among which the most important is the malic, oxalic and critic acids that together total 80% of the titled acidity.

The titled acidity of the fruit juice, the leaves infusion etc measures the concentration of titled hydrogen ions that are contained in the analyzed samples, by neutralization with a strong base solution at fixed pH fix. Its value contains all substances of acid nature such as: free hydrogen ions, organic acids, acids and cations. Since the organic acid is the main component from the sample that reacts with a basic solution, the titled acidity is expressed as g/L or g/100 mL from the main acid. Usually, in fruit, the predominate acid is either the citric or the malic acid, or both.

The total acidity of the cherry fruit has been expressed in g L<sup>-1</sup> citric acid (C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>).

The results of the analysis carried out for the fruit of the varieties presented in fig. 1 presented acidity values of the total acidity between 3.9 g L<sup>-1</sup> C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> at the Victor variety and 10.5 g L<sup>-1</sup> C<sub>6</sub>H<sub>8</sub>O<sub>7</sub> la at Iasirom variety.

High values have also been registered for the varieties: Timpurie Franceza (8.51 g L<sup>-1</sup> C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>), Ramon Oliva (7.42 g L<sup>-1</sup> C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>) and Amar Costuleni (7.3 g L<sup>-1</sup> C<sub>6</sub>H<sub>8</sub>O<sub>7</sub>)

Some authors (Serrano et al., 2005; Usenik et al., 2005) state that in cherries the malic acid is the predominant one.

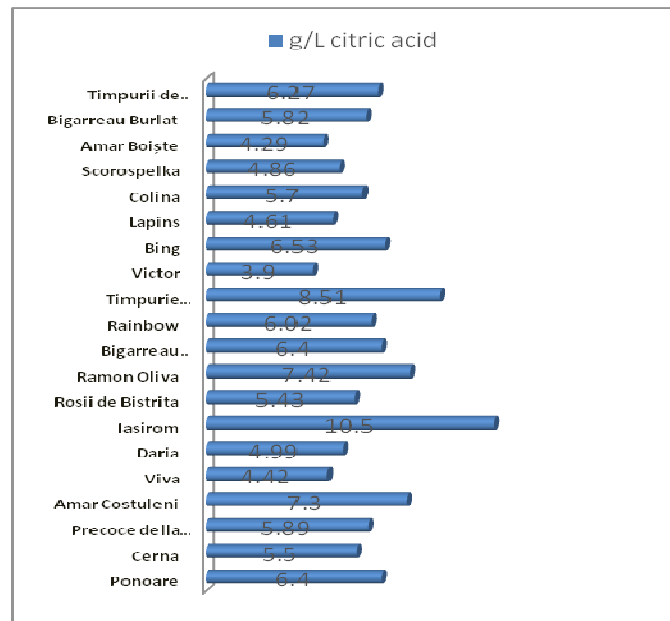


Fig. 1 – Fruit acidity

For total flavanoids (fig. 2), we have registered values between 21.8 mg/100 g FW for Iasirom variety and 73.69 mg/100 g FW for Amar Boiste variety. High values were also obtained for the Lapins (61.03 mg/100 g FW) and Daria (56.68 mg/100 g FW) varieties.

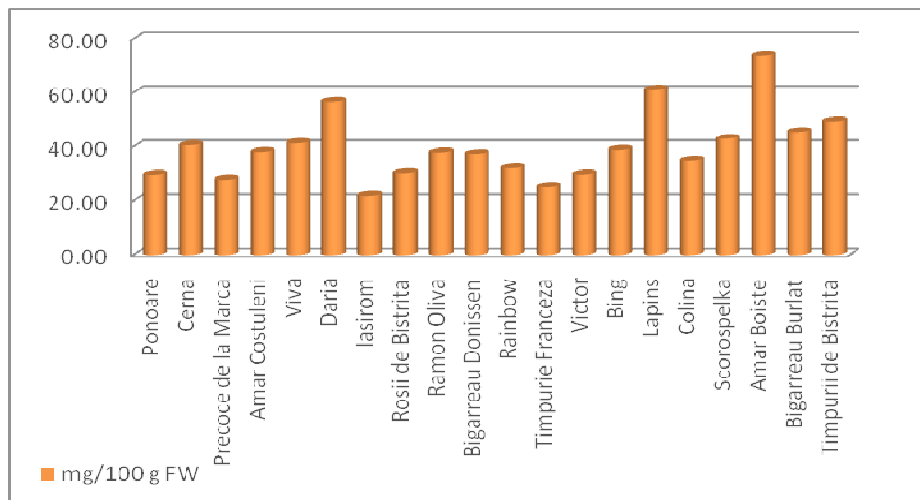


Fig. 2 – Total flavanols



Regarding the total mineral content (table 2), we have registered the highest values for Timpurie Franceza (1.12 %) and Ramon Oliva (1.05 %) varieties, and the lowest for Rainbow (0.32%) and Precoce della Marca (0.33%) varieties.

Table 2

**Dry weight and total mineral content for the fruit of several cherry tree varieties**

| Variety              | Dry weight (%) | Total minerals (%) |
|----------------------|----------------|--------------------|
| Ponoare              | 14.50          | 0.72               |
| Cerna                | 15.68          | 0.27               |
| Precoce della Marca  | 15.42          | 0.33               |
| Amar Costuleni       | 17.59          | 0.67               |
| Viva                 | 14.17          | 0.78               |
| Daria                | 15.61          | 0.43               |
| Iasirom              | 17.10          | 0.64               |
| Rosii de Bistrita    | 19.17          | 0.94               |
| Ramon Oliva          | 14.99          | 1.05               |
| Bigarreau Donissen   | 19.18          | 0.86               |
| Rainbow              | 13.88          | 0.32               |
| Timpurie Franceza    | 17.51          | 1.12               |
| Victor               | 16.43          | 0.47               |
| Bing                 | 17.40          | 0.82               |
| Lapins               | 15.80          | 0.54               |
| Colina               | 16.67          | 0.41               |
| Scorospelka          | 11.52          | 0.37               |
| Amar Boiste          | 17.10          | 0.65               |
| Bigarreau Burlat     | 17.58          | 0.39               |
| Timpurii de Bistrita | 17.86          | 0.49               |

## CONCLUSIONS

The results of the analysis carried out show the existence of some significant differences on the bio-chemical composition of the fruit from the cherry-tree varieties taken for the study. The cultivar Timpurie Franceza has stood out through its high content of sugar, citric acid and minerals, but also had one of the lowest quantities of flavonoids. For the Scorospelka variety we have obtained the lowest values at all the analysis

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# WATER DEFICIT AND LIGHT INTENSITY EFFECTS ON THE ACCUMULATION OF TOTAL PHENOLICS AND ANTHOCYANINS IN SEVERAL RASPBERRY AND BLACKBERRY CULTIVARS

## INFLUENȚA DEFICITULUI HIDRIC ȘI A INTENSITĂȚII RADIAȚIEI SOLARE ASUPRA ACUMULĂRII COMPUSILOR FENOLICI TOTALI ȘI A ANTOCIANILOR LA UNELE SOIURI DE ZMEUR ȘI MUR

PASCU D.D.<sup>1</sup>, MORARIU Aliona<sup>1</sup>, CAULET Raluca Petronela<sup>1</sup>,  
EFROSE Rodica<sup>1</sup>, SFICHI-DUKE Liliana<sup>1</sup>  
e-mail: alionamorariuu@yahoo.co.uk

**Abstract.** *In field conditions, high light intensities often accompanied drought, which may significantly affect plant growth and development. Both water and light may decrease the capacity of plants for carbon assimilation by stomatal closure and photoinhibition. Angiosperms can prevent excessive light absorption by spatial leaves repositioning or photoprotective pigments synthesis such as anthocyanins. The aim of this work was to investigate the effects of water stress and light intensity on the accumulation of total phenolics and anthocyanins in several raspberries (Opal, Cayuga și Ruvi) and blackberry (Thornfree și Lochness) cultivars. The experiments were performed from June to October 2011. Plants have been subjected to two water conditions, irrigated and non-irrigated, and two light intensities 100% sunlit and 25% sunlit. In all cultivars, total phenolics content was sensitive to light conditions and tolerant to poor water conditions. A combination of water stress and low light intensities decreased the anthocyanin content in raspberry cultivars. Contrary, the accumulation of anthocyanins increased in all blackberry cultivars when exposed to water stress, irrespective of light conditions. The relative importance of these phenolic compounds to the protection of raspberry and blackberry plants against abiotic stress is discussed.*

**Key words:** *Rubus* sp., phenolics, anthocyanins

**Rezumat.** *Adeseori, în cazul cultivării plantelor horticole în câmp, deficitul hidric este însoțit de intensități luminoase mari iar această combinație de factori abiotici poate conduce la scăderea capacității fotosintetice datorită închiderii stomatelor și fotoinhibiției. Angiospermele pot preveni absorbția în exces a luminii de care frunzele prin re poziționarea lor spațială sau prin sinteza unor pigmenți fotoprotectori de tipul carotenoizilor sau a antocianilor. În această lucrare ne-am propus determinarea conținutului în polifenoli totali și antociani din frunze la unele soiuri de zmeur (Opal, Cayuga și Ruvi) și mur (Thornfree și Lochness), cultivate în câmp în condiții de apă și lumină diferite. Experimentul s-a desfășurat în perioada iunie-octombrie 2011. Plantele au fost cultivate în regim irigat și neirigat cu 100% expunere la lumină și umbră la 25% lumină. Conținutul de polifenoli a fost influențat în mod semnificativ de condițiile de lumină și mai puțin de regimul hidric la toate soiurile luate în*

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

*studiu. În schimb, interacțiunea celor doi factori abiotici influențează diferit acumularea de antociani, astfel încât în condiții de stres hidric conținutul de antociani în frunze scade la zmeur doar la plantele menținute la umbră. În schimb, la mur există o acumulare mai mare a antocianilor la plantele neirigate indiferent de condițiile de lumină.*

**Cuvinte cheie:** *Rubus* sp., polifenoli, antociani

## INTRODUCTION

In field conditions, water stress is often accompanied by excessive light which can significantly alter plant growth and development. The light energy that exceeds the assimilation abilities of the leaves may be caused by the high incidence of the solar radiation, as well as by the decline in photosynthetic carbon assimilation, resulting from stomatal closure. This excess energy causes an increase in the production of active oxygen species (ROS) (Mittler, 2006) which may lead to the inhibition of photosynthesis and growth processes (Lawlor and Cornic, 2002; Chaves et al., 2003).

In order to prevent damage, plants have developed different protection mechanisms of the photosynthetic apparatus against excess light associated with tissue dehydration (Tuba et al., 1996). Angiosperms can prevent the excess absorption of light through spatial repositioning of leaves or synthesis of some photoprotective pigments such as carotenoids or anthocyanins (Sherwin and Farrant, 1998). On the other hand, the stimulation of the plant antioxidant capacity is a highly efficient protection mechanism against the harmful effects of oxygen radicals (Sherwin and Farrant, 1998). Phenols, mainly anthocyanins are chemical compounds with high antioxidative effects that play an important role in the adaptation of plants to abiotic stress factors (Smimoff and Cumbes, 1989; Apel and Hirt, 2004; Kruk et al., 2005).

## MATERIAL AND METHOD

The experiment has been carried out in the experimental field of SDCV Adamachi, in 2011. Plant material was represented by three varieties of raspberry (Opal, Cayuga and Ruvi) and two of blackberry (Thornfree and Lochness). Plants were cultivated continuously since June in two distinct sunlight conditions. One group was given full 100% sunlight and the second group had the sunlight reduced by 75%. The reduction was obtained by net covering. Both variants were maintained in well watered conditions by supplementary irrigation and natural water flow (non-irrigated). After two months, leaf samples were harvested from 3 plants / variant in 3 repetitions / plant. The leaves were ground in liquid nitrogen and kept at -80°C.

### *Determination of total phenolics:*

100 mg ground tissue was extracted with 1 ml 60% acetone for 60 min at 25 °C on shaker. Total phenolics were quantified in extracts using the Folin–Ciocalteu protocol, as modified by Singleton and Rossi (1965). Gallic acid was used as a standard, and results were calculated as gallic acid equivalent (GAE) (mg/100 g dry weight basis).

### *Determination of anthocyanin content:*

Anthocyanins were extracted by incubating 100 mg of grounded tissue overnight in 150 µL of methanol acidified with 1% HCl (v/v). Total anthocyanins were

determined by measuring the A530 and A657 of the aqueous phase using a spectrophotometer. By subtracting the A657 from the A530, the relative amount of anthocyanin was calculated as OD/g.fw. (Neff & Chory, 1998).

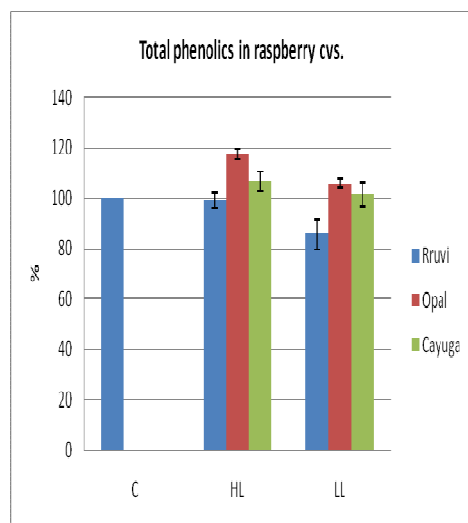
All spectrophotometric assays were carried out with a T70 UV /VIS spectrophotometer (PG Instruments LTD)

## RESULTS AND DISCUSSIONS

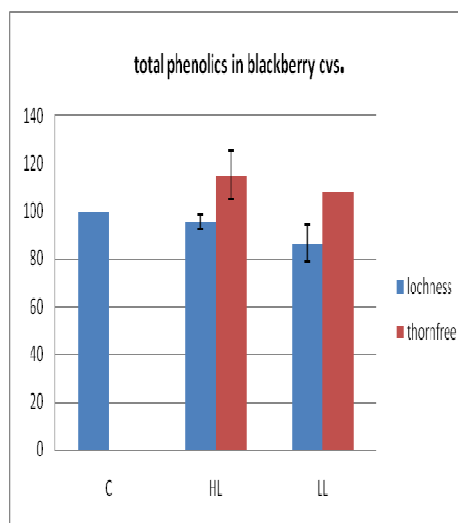
Plants can accumulate phenolic compounds under various stress conditions such as light, low temperature, hydric deficit (Sakihama et al. 2002).

Our data show that total phenolics are relatively stable among the experimental categories. However, we noticed an increase in the total phenolics content under water deficit in Opal and Cayuga plants cultured in 100% sunlight conditions. The water deficit did not alter total phenolics in Opal and Cayuga cultured in shade and water deficit conditions but it induced their decrease in Ruvi (fig. 1).

Under water deficit conditions, the accumulation of total phenolics was slightly stimulated in Thornfree, mainly in plants cultured in full sunlight conditions. Contrary, total phenolics decreased in Lochness, mainly in shaded plants (fig. 2).



**Fig. 1** – Influence of the water deficit on the total phenol content in 3 raspberry varieties cultivated under two light conditions

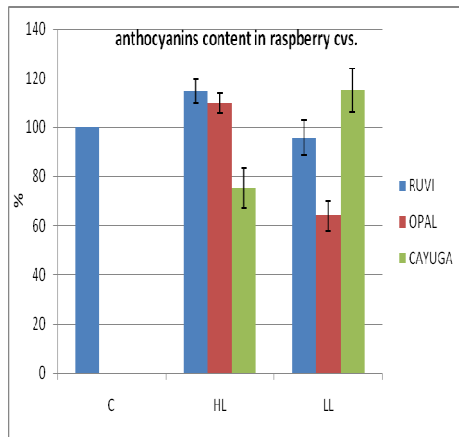


**Fig. 2** – Influence of the water deficit on the total phenol content in 2 blackberry varieties cultivated under two light conditions

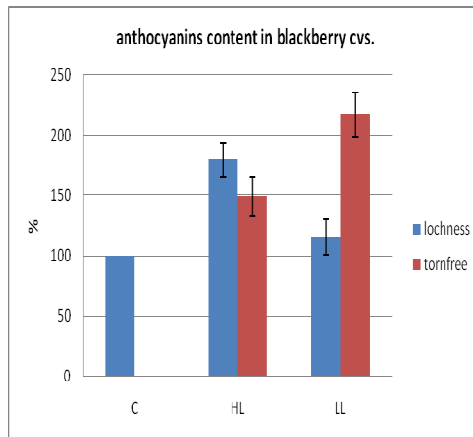
Anthocyanins are water-soluble pigments that belong to the family of flavonoids. They are also involved in defence against environmental stresses such as ultraviolet radiation, herbivores, drought and chilling (Chalker-Scott 1999, Hatier and Gould 2008). Close & Beadle (2003) have reviewed and discussed the ecophysiological roles of anthocyanins as antioxidants, compatible solutes in osmotic regulation, and photoprotectants against ultraviolet radiation and visible light.

Plant tissues containing anthocyanins are usually resistant to drought (Chalker-Scott 1999). For example, the drought tolerant resurrection plants highly accumulate anthocyanins during dehydration (Sherwin and Farrant 1998). A possible mechanism of anthocyanin-induced drought resistance is related to anthocyanins ability to stabilize the water potential (Choinski and Johnson 1993, Chalker-Scott 2002).

Under water deficit conditions, we found an increase in anthocyanins in Ruvi (18%) and Opal (10%) plants exposed to 100% sunlight, while it decreased in the plants maintained in shade conditions. Contrary, anthocyanins increased in Cayuga (18%) plants that received only 25% solar light. Our data suggest that light intensity is a factor that influences the sensitivity of these cultivars to water stress. Both Opal and Ruvi cultivars were less sensitive to water deficit when exposed to 100% sun light conditions, while Cayuga is more tolerant to water deficit when exposed to low light intensities (fig. 3).



**Fig. 3** - The influence of the water deficit on the anthocyanin content for some raspberry varieties cultivated under two light conditions



**Fig. 4** - The influence of the water deficit on the anthocyanin content for some blackberry varieties cultivated under two light conditions

Konczak-Islam et al. (2003) reported at sweet potato that anthocyanin constituent was correlated with phenolic compounds content and their antioxidant activity. In our experiments case this challenge has been confirmed only for the blackberry species. For the raspberry the anthocyan content varies independently to that of the polyphenols.

In blackberry the accumulation of anthocyanins under water deficit conditions was influenced by light intensity and genotype. Under full sun light, both cultivars increased their anthocyanin content by 50-60%. Under low light conditions, the anthocyanin accumulation increased by 110% in in Thornfree and only 10% in Lochness (fig. 4).

The role of anthocyanins in vegetative tissues such as leaves is still under debate. The basic question is whether they are directly involved in stress

responses or just assist in plant defense (Hatier and Gould 2008). For instance, anthocyanins are suggested to protect chloroplasts from excess irradiance due to their ability to absorb light between 400 and 600 nm, although carotenoids are more effective in this respect (Gould et al. 2000). Our data demonstrate that anthocyanins accumulation increased under water deficit conditions mainly in high light conditions. This increase suggests that anthocyanins can be involved in photoprotection under direct drought stress, which is in accordance with previous investigations (Gould et al. 2000, Hoch et al. 2001, Close and Beadle 2003, Merzlyak et al. 2008).

## CONCLUSIONS

1. The water deficit leads to the accumulation of total polyphenols in raspberry and blackberry cultivars under high light conditions.
2. Under water stress, there are genotypic variations in anthocyanin accumulation in response to light intensity.
3. The accumulation of anthocyanins under water deficit and high light condition suggests that these compounds may play a photoprotective role.

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# STUDY OF BIOLOGICAL FEATURES OF SOME NEW SWEET CHERRY CULTIVARS

## STUDIUL PARTICULARITĂȚILOR BIOLOGICE LA UNELE SOIURI NOI DE CIREȘ

*SÎRBU Sorina<sup>1</sup>, IUREA Elena<sup>1</sup>, CORNEANU Margareta<sup>1</sup>*

e-mail: sorinas66@gmail.com

**Abstract.** *Biological properties of 4 new sweet cherry cultivars ('Alex', 'Andrei', 'Ludovic' and 'Mihai', homologated in 2011), grafted on Prunus mahaleb L. rootstock, were studied in the North Eastern Romania region during a five-years period (2007-2011). Investigation has included phenological properties (flowering and ripening time), pomological properties and biochemical composition of fruits. The highest fruit weight was found in 'Alex' (over 10 g). 'Alex' is also the earliest as the flowering time, whereas 'Mihai' is the latest flowering cultivar. All cultivar taken under study has a medium ripening time (8-25 June). The soluble solids content ranged from 16,8 °Brix ('Ludovic') and 17,9 °Brix ('Andrei'). The highest reducing sugars content was found in 'Alex' and 'Andrei' (16 g/100 g fruit weight) and the lowest in 'Ludovic' (14 g/100 g fruit weight). The research performed and results obtained suggest that new studied sweet cherry cultivars have shown good results in agroecological conditions of Iași area and could have significant place in the structure of assortment of sweet cherry in the region of North Eastern Romania.*

**Key words:** sweet cherry, cultivar, phenological properties, fruit quality.

**Rezumat.** *Particularitățile biologice la 4 soiuri noi de cireș (Alex, Andrei, Ludovic și Mihai, omologate în anul 2011), altoite pe mahaleb (Prunus mahaleb L.) au fost studiate în NE României, pe o perioadă de 5 ani (2007-2011). Investigațiile au inclus stadii fenologice (perioada înfloritului și perioada de maturare a fructelor), particularitățile pomologice și compoziția biochimică a fructelor. Cea mai mare greutate a fructului s-a înregistrat la soiul Alex (10,36 g). Soiul Alex este și cel mai timpuriu (ca perioadă de înflorire), în timp ce soiul Mihai are o perioadă de înflorire mai târzie. Toate soiurile au perioada medie de maturare a fructelor (8-25 iunie). Conținutul în substanță uscată solubilă a fost între 16,8 °Brix (Ludovic) și 17,9 °Brix (Andrei), iar conținutul în zaharuri reducătoare a înregistrat valorile cele mai mari la Alex și Andrei (16 g/100 g fruct) și cel mai redus la Ludovic (14 g/100 g fruct). Cercetările realizate și rezultatele obținute arată că soiurile luate în studiu au demonstrat bune rezultate în condițiile ecologice din zona Iași și pot ocupa un loc bun în structura sortimentului de cireș din zona de NE a României.*

**Cuvinte cheie:** cireș, soi, proprietăți fenologice, calitatea fructului.

### INTRODUCTION

In the last three decades the sweet cherry quality criteria have changed, special accent were to obtaining cultivars with large fruits (Albertini et al., 2001; Apostol, 2005; Beceanu and Sîrbu, 2007).

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<sup>1</sup> Research and Development Station for Fruit Tree Growing of Iași, Romania

In Europe, there have been many new sweet cherry cultivars in countries with tradition in the culture of this species (Edin, 1996, Godin et al., 1996, Millan and Charlot, 2005; Nikolić and al., 1996, Saunier, 1996). Also, Canada and United States are permanent sources of new sweet cherry cultivars (Lugli, 2006; Kappel and MacDonald, 2000).

Breeding program at sweet cherry began to SCDP Iasi in 1981 and was initiated by PhD. eng. Ioachim Bodi, then continued by PhD. eng. Ludovic Petre and now by PhD. eng. Sorina Sirbu and PhD. stud. Eng. Elena Iurea.

The main method used were controlled hybridization (Cociu and Oprea, 1989) and the aim was to obtain new sweet cherry cultivars with high quality features (Webster and Looney, 1996; Budan and Gradinariu, 2000) and promoted them into assortment.

Following the breeding works carried out during 1980 - 2000 were obtained 24 new sweet cherry cultivars at SCDP Iasi. This paper presents four new sweet cherry cultivars obtained at SCDP Iasi and approved during 2010-2011.

## MATERIAL AND METHOD

In our research, we have made observations and measurements at four new sweet cherry cultivars ('Alex', 'Andrei', 'Ludovic' and 'Mihai') approved during 2010 - 2011 compared with 'Van' which is a very extensive cultivar in orchards (table 1). Trees are planted in 2002, on *Prunus mahaleb* L. as rootstock, at a distance of 3.5 x 4m, with free palmata shaped crown without support and irrigation system in the experimental polygon of SCDP Iasi.

Table 1

Sources of biological material used

| Cultivar      | Authors  | Homologation year | Hybrid combination                  |
|---------------|--|-------------------|-------------------------------------|
| Alex          | Corneanu Margareta, Petre Ludovic, Corneanu Gelu               | 2010              | Open pollination of Lijana          |
| Andrei        | Corneanu Margareta, Petre Ludovic, Corneanu Gelu               | 2011              | HC27/4 x Boambe de Cotnari          |
| Ludovic       | Petre Ludovic, Iurea Elena                                     | 2010              | Van x Boambe de Cotnari             |
| Mihai         | Corneanu Margareta, Petre Ludovic, Corneanu Gelu, Sirbu Sorina | 2011              | Pietroase de Geoagiu x NY 9801      |
| Van (control) | A.J. Mann  | 1944              | Open pollination of Empress Eugenia |

We made observations and measurements on: phenology and abundance of flowering, fruit ripening period, fruit mass (g) and fruit width (mm), percentage of stone. As the main biochemical properties were determined soluble solids content (%) by refractometry, reducing sugar content by the Luff-Schoorl method, titratable acidity (%) by titration with 0.1 N NaOH until the equivalence point and soluble solids content and titratable acidity ratio.

## RESULTS AND DISCUSSIONS

Flowering period at studied sweet cherry cultivars was variable during 2007-2011, ranging between April 1 at 'Ludovic' and May 6 at 'Alex'. As average over the studied period, the cultivars 'Andrei', 'Van' and 'Ludovic' has the earlier flowering period than 'Alex' and 'Mihai', which the start of flowering period occurs later (tab. 2). Flowering abundance recorded maximum values at 'Ludovic', which are the cultivar most valuable in this regard compared with the all others studied cultivars.

Table 2

**Phenology and abundance of flowering of some sweet cherry cultivars**  
(average 2007-2011)

| Cultivar | Phenology          |            |              | Maturation period | Days between end of bloom until | Flowering abundance* |
|----------|--------------------|------------|--------------|-------------------|---------------------------------|----------------------|
|          | Beginning of bloom | Full bloom | End of bloom |                   |                                 |                      |
| Alex     | 12- 24 IV          | 15 -27 IV  | 24 IV- 6 V   | 8 - 20 VI         | 51                              | 4.5                  |
| Andrei   | 8 - 23 IV          | 10 - 20 IV | 16 IV- 4V    | 8 - 20VI          | 50                              | 4.5                  |
| Ludovic  | 1- 22 IV           | 3 - 20 IV  | 16 IV- 4 V   | 10 - 25VI         | 53                              | 5                    |
| Mihai    | 10-23 IV           | 12 - 22 IV | 21 IV -3 V   | 8 - 16VI          | 48                              | 4.5                  |
| Van      | 3-22 IV            | 10 - 20 IV | 18-26 IV     | 9 - 15VI          | 51                              | 4.5                  |

Note: excellent (5), very good (4), good (3), poor (2), bad (1), without flowers (0)  
(Radicevic et al., 2011 a)

**Maturation period** was different at studied cultivar and depending on climatic conditions during the growing season and during 2007 - 2011 ranged from June 8 to 'Mihai', 'Alex' and 'Andrei' and June 25 at 'Ludovic' (tab. 2).

**Tree vigor** has not been different values from one cultivar to another, all five of studied sweet cherry cultivars being medium (tab. 3).

**Fruit production** recorded as average during 2007-2011 ranged between 12.8 kg / tree at 'Mihai' and 14.6 kg / tree at 'Ludovic' (table 3). Average yield in t / ha recorded the highest value to the cultivar 'Ludovic' with 10.43 t / ha. Compared with 'Van', the cultivar 'Mihai', 'Alex', 'Andrei' and 'Ludovic' are more productive, but these values should be verified in the next years, those being cultivated only in experimental plantations currently.

**Fruit mass** recorded high values to 'Alex' (10.4 g), 'Andrei' (9 g) and 'Ludovic' (10 g), values which expressing a very valuable dimension of the sweet cherry fruits that being according with the latest international trends in quality. 'Andrei' showed the highest values of the fruit's width (26.5 mm) but the percentage of mesocarp ratio of 'Van' proved to be the most valuable (95.89%) (table 4 ). Cultivar 'Van' showed the lowest peduncle length (25 mm) while the new sweet cherry cultivars created at SCDP Iasi have stalk length ranging from

28 to 31 mm. When using fruits for processing into syrup or jams, mechanical work of stalk remove are difficult at cultivars with short stalks (Sirbu, 2011).

Table 3

Fruit yield at some sweet cherry cultivars

| Cultivar | Tree vigor* | Yield (average 2007-2011) (kg/tree) | Yield (average 2007-2011) (t/ha) | Differences from 'Van' (%) |
|----------|-------------|-------------------------------------|----------------------------------|----------------------------|
| Alex     | 5           | 13.77±0.71                          | 9,83±0.50                        | 107,55                     |
| Andrei   | 5           | 13.53±0.65                          | 9,63±0.45                        | 114,06                     |
| Ludovic  | 5           | 14.60±0.82                          | 10,43±0.57                       | 105,73                     |
| Mihai    | 5           | 13.57±1.27                          | 9.70±0.92                        | 105,9                      |
| Van      | 5           | 12.80±0.66                          | 8,71±0.63                        | -                          |

\*UPOV test: 1= very low; 3=low; 5=medium; 7=high; 9=very high

Also, 'Van' has the disadvantage that when are at full ripening time the stalk falls causing crop losses.

Table 4

Morphological features of some sweet cherry cultivars (average, 2007-2011)

| Cultivar | Fruit dimensions |            |                |             | Stalk length (mm) | Stone weight (g) | Mesocarp ratio (%) |
|----------|------------------|------------|----------------|-------------|-------------------|------------------|--------------------|
|          | Fruit weight (g) | Width (mm) | Thickness (mm) | Length (mm) |                   |                  |                    |
| Alex     | 10.4             | 24.6       | 19.7           | 23.8        | 28                | 0.6              | 95.23              |
| Andrei   | 9                | 26.5       | 22.5           | 25.6        | 29                | 0.6              | 93.33              |
| Ludovic  | 10               | 25.3       | 21.8           | 25.4        | 28                | 0.5              | 95.00              |
| Mihai    | 7.3              | 20.7       | 18.1           | 21.6        | 31                | 0.6              | 91.78              |
| Van      | 7.3              | 21.3       | 18.7           | 22.5        | 25                | 0.3              | 95.89              |

Biochemical properties of the studied new sweet cherry cultivars are presented in Table 5. Soluble solids content showed values between 16.8% ('Ludovic') and 17.9% ('Andrei') and reducing sugars content recorded values between 15% ('Mihai') and 16% ('Andrei', 'Alex' and 'Ludovic'). Total acidity recorded values between 0.52% ('Alex') and 0.74% ('Van'). The studied sweet cherry cultivars have dark red colour of fruits, with no visual differences between them.

A fruit firmness value was different for cultivars and 'Mihai' recorded the lowest, which has semi firm fruits. 'Alex' has firm fruits and cultivars 'Andrei' and 'Ludovic' has very firm same as the cultivar 'Van'.

A balance between soluble solids content and acidity is considered important for determining the sweet cherry taste (Webster et al., 1996, Apostol,

2005), therefore sugars-acidity ratio analytically describe the sweet taste and slightly tart of the fruit.

Table 5

**Biochemical and technological properties of new sweet cherry cultivars**  
(average, 2007-2011)

| Cultivar  | Soluble solids content (%) | Reducing sugars content (%) | Total acids (%) | Sweetness index | Fruit colour* | Fermity* |
|---|----------------------------|-----------------------------|-----------------|-----------------|---------------|----------|
| Alex  | 17.0                       | 16                          | 0.52            | 32.69           | 7             | 7        |
| Andrei  | 17.9                       | 16                          | 0.65            | 27.54           | 7             | 9        |
| Ludovic   | 16.8                       | 16                          | 0.63            | 26.67           | 7             | 9        |
| Mihai   | 17.4                       | 15                          | 0.72            | 24.17           | 7             | 5        |
| Van   | 16.9                       | 15                          | 0.74            | 13.64           | 7             | 9        |
| *UPOV test: fruit firmness: 3=soft; 5=medium; 7=firm; 9= very firm;<br>skin colour: 1=yellow; 2=half yellow half red; 5=red; 7=dark red |                            |                             |                 |                 |               |          |

Lugli et al., 2006 describes the new cultivars created at the University of Bologna, whose glucose-acidimetry index was recorded between 20.3 (at 'Durone dell'Anella tardive') and 27.5 ('Mora di Vignola') and Radicevic et al., 2011 b, describes six new sweet cherry cultivars introduced in Serbia with the values of this parameter ranging from 16.35 (at 'Burlat') and 28.3 (at 'Regina'). At the new sweet cherry cultivars taken in the study this parameter were between 24.17 (at 'Mihai') and 32.69 (at 'Alex') (tab. 5).

## CONCLUSIONS

'Ludovic', 'Andrei' and 'Alex' has been marked by valuable morphological features as follows: large size of fruit in accordance with current international requirements and high sugar - acidity ratio, destined especially for fresh consumption. 'Mihai', although the fruit is large, but is semifirm therefore particularly suitable for industrial as jams and dried.

Biochemical properties of the new sweet cherry cultivars showed a pronounced sweet, slightly acidity taste, very appreciated by consumers on fruit destined for fresh consumption.

The four new cultivars created at SCDP Iasi can be successfully promoted in the national assortment of sweet cherry cultivars along with 'Van'.

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# AQUATIC FLOATING PLANTS WITH POTENTIAL USE IN PHYTOREMEDIATION

## PLANTE ACVATICE PLUTITOARE CU POTENȚIAL DE UTILIZARE ÎN FITOREMEDIERE

**BUTA Erzsebet<sup>1</sup>, CANTOR Maria<sup>1</sup>,  
BUTA M<sup>1</sup>, HORT Denisa<sup>1</sup>**  
e-mail: ebuta2008@yahoo.com

**Abstract.** Fast industrialization, increasing urbanization, growing living standards, development of sustained way chemicals administration in the current century require a greater consumption of water and implicitly increased charging with different residues and contaminants. Therefore, studies concerning the treatment of contaminated water are presents interest and impetuous necessary. The contamination of waste waters with toxic heavy metals is a major environmental issue, much debated on nationally and internationally level. Many aquatic plants (hydrophytes) have the capacity to decontaminate water (called hyperaccumulators) through phytoremediation. For this purpose, the floating aquatic species are used with great success: *Azolla pinnata* R. Br., *Eichhornia crassipes* L., *Hydrocharis morsus ranae* L., *Lemna minor* L., *Lemna gibba* L., *Pista stratiotes* L., *Salvinia natans* Kunth. They are used in different aquatic landscapes for decorate ponds and lakes. It creates real floating islands to remediation of contaminated wastewater and biomonitoring. This paper presents some measures to filter wastewater with aquatic plants and the assortment of widely used aquatics for this purpose.

**Key words:** contaminated water, plants, floating islands.

**Rezumat.** Industrializarea rapidă, creșterea gradului de urbanizare, ridicarea standardului de viață, dezvoltarea în mod susținut a chimizării impun în secolul actual un consum mai ridicat de apă și implicit o încărcare mărită cu diferite reziduuri și contaminați. De aceea, studiile privind epurarea apelor contaminate sunt de actualitate și impetuos necesare. Contaminarea apelor reziduale cu metale grele toxice, este o problematică majoră de mediu, mult dezbătută pe plan național și internațional. Numeroase plante de apă (hidrofite) au capacitatea de a decontamina apele (numite hiperacumulatori), prin fitoremediere. În acest scop, dintre speciile acvatice plutitoare se utilizează cu mare succes: *Azolla pinnata* R. Br., *Eichhornia crassipes* L., *Hydrocharis morsus ranae* L., *Lemna minor* L., *Lemna gibba* L., *Pistia stratiotes* L., *Salvinia natans* Kunth. Acestea se folosesc și în diferite amenajări acvatice, pentru decorul ochiurilor de apă și a lacurilor. Se creează adevărate insule plutitoare (floating islands) pentru depoluarea și biomonitorizarea apelor reziduale și contaminate. Lucrarea de față prezintă câteva măsuri de filtrare a apelor uzate cu ajutorul plante și sortimentul de acvatice folosite pe scară largă în acest scop.

**Cuvinte cheie:** ape contaminate, plante, insule plutitoare.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

## INTRODUCTION

Phytoremediation is a process that uses different types of plants in association with microbes to remove, destruct, relocate, extract and absorb contaminants from wastewaters or soils. Plants can clean or remove many kinds of pollution including heavy metals, pesticides, explosives, and oil. This technology is an innovative, cost-effective alternative method the more utilized treatment ways used at cleanup the waste sites.

Plants can remediate organic compound and metal contaminations, but they are usually used for removing toxic metals from soil or aquatic sediments. When plants remove pollutants completely out of a contaminated site, the process is called phytoextraction (Maczulak, 2009).

Aquatic plants absorb heavy metals from the water; and those rooted ones also from the bed material. Generally, aquatic plants accumulate high amounts of heavy metals via roots, stems or leaves, and accumulate them in organs. They can absorb elements selectively. In this way, they reflect the toxicity of the water environment and may serve as a tool for the biomonitoring of contaminated waters (Wang, 1991; Sawidis et al., 1995; Ravera, 2001, Zurayk et al., 2001; Cardwell et al., 2002). Accumulation and distribution of heavy metals in the plant depends on the plant species, bioavailability, pH, cation exchange capacity, dissolved oxygen, temperature, and secretion of roots. Plants are employed in the decontamination of heavy metals from polluted water and have demonstrated high performances in treating mineral water and industrial effluents (Cheng, 2003).

Water contamination with heavy metals is a very important problem in the current world (Kaur et al., 2010). Since the dawn of civilization, metal pollutants have been a part of human history. However, toxic metal pollution of the biosphere has intensified rapidly since the onset of the industrial revolution, posing major environmental and health problems (Chandra, Srivastava, 2003).

One of specific methods for removal of metals from these environmental segments is use plant species which have the ability to accumulate and distribute heavy metals. The term, "hyperaccumulation" was first described by Jaffre et al. (1976), when they observed the New Caledonian plant (*Sebertia acuminata*) accumulated Ni in their above ground biomass (Chandra, Srivastava, 2003).

A plant can be named hyperaccumulator if it has capacity to concentrate the pollutants in a minimum percentage which varies according to the pollutant involved (for example: more than 1000 mg/kg of dry weight for nickel, copper, cobalt, chromium or lead; or more than 10,000 mg/kg for zinc or manganese) (Baker, 1989). This capacity for accumulation is due to hypertolerance, or phytotolerance: the result of adaptative evolution from the plants to hostile environments through many generations.

Biofiltration of the heavy metals (Pb, Zn, Cu, Cd, Co) has been successfully demonstrated using many aquatic plants such as: *Salvinia sp.* (Espinoza-Quinones et al., 2005), *Potamogeton crispus* (Aysel et al., 2010), *Potamogeton pectinatus* and *Potamogeton malaiianus* (Peng et al., 2008), *Eichhornia crassipes* (Buta et al., 2011), *Typha angustifolia* (Dilek and Ahmet, 2004),



*Myriophyllum heterophyllum* (Aysel et al., 2010), *Lemna minor* (Yeşim et al., 2003) and *Azolla caroliniana* (Bennicelli et al., 2004). In addition, Lavid et al., (2000) discovered that water lilies (*Nymphaea* variety) are endowed with anatomical and physiological traits that allow them to thrive on water with high concentrations of heavy metals such as Cd, Hg, Ni and Co.

The National Aeronautics and Space Administration/National Space Technology Laboratories (NASA/NSTL) have successfully used water hyacinths to remove organics and heavy metals from chemical wastes before their discharge (Wolverton et al., 1977).

Heavy metals can be clean, destruct or remove from waste or contaminated waters with grasses and other aquatic species. In Auckland region the reduction of Cu, Zn from urban storm water is made by floating wetlands.

Floating treatment wetlands (FTWs), consisting of rooted emergent wetland plants growing on a mat floating on the water surface of a pond, have the potential to combine the strengths of both conventional ponds and wetlands into one system, whilst overcoming some of the limitations of each. For this kind of waste water treatment can be used grasses as: *Carex dipsacea*, *Carex virgata*, *Cyperus ustulatus*, *Eleocharis acuta*, *Juncus edgariae*, and *Schoenoplectus tabernaemontani* (Headley, 2007).

Regarding the metal removal through floating wetlands (FTW), the first report found by Headley (2006) was the pilot FTW system constructed at Heathrow Airport in London for the treatment of glycol-laden de-icing water. Copper removal was approximately 20-30%, while removal of zinc was ineffective. However, the floating structure in this system was apparently constructed using galvanized steel materials which may have acted as a source of metals such as zinc, whilst the limited time frame of the study renders the information of limited value.

The ecosystems of floating wetland occur naturally in various locations around the world, such as the Danube Delta, Germany, New Zealand, The Netherlands, England, the lower reaches of the South in Africa, the Central Amazon, the Gulf Coast of the USA, and Tasmania in Australia.

The aim of this study was to assess the mode of action and role of aquatic plants in removing heavy metals from wastewater.

## MATERIAL AND METHODS

As was shown above many aquatic plants can be used in phytoremediation. In some of cases for wastewater treatment are utilized the free-floating aquatic plants which float either as thin layer on the water surface (e.g., *Lemna* and *Azolla*) or have specially adapted as floating leaf-bases (e.g., *Eichhornia*, *Pistia* and *Salvinia*).

Floating treatment wetlands are distinguished from free-floating aquatic plant systems by the fact that they utilize larger emergent wetland plants growing on a somewhat consolidated floating mat, as opposed to an unconsolidated mass of small, individual buoyant plants lacking any significant mat. Free-floating aquatic plant systems have been used to reduce particulate and organic loads in sewage and industrial wastewaters. Artificial wetlands has many other functions: increasing biodiversity,

recreational, esthetical and educational (Tomescu, 2009). The high growth and uptake rates of many free-floating plants can also result in significant removal of nutrients and metals if there is enough land area available and the plants are regularly harvested.

Floating treatment wetlands may be likened to a hydroponics system, as the plants acquire their nutrition directly from the water column in which their roots are suspended, rather than from the soil (Headley, 2006).

Natural floating wetlands typically consist of a 40 – 60 cm deep floating organic mat supporting plant growth, the upper portion of which is comprised of densely intertwined live, dead and decaying roots with some litter collection on the surface. Below the active root zone a layer of low-density decomposed peat and decaying plant detritus develops, the depth of which is usually dictated by the rooting depth of the plants. Beneath the peat layer a zone of relatively clear free-water exists, that varies in depth (0 – 2 m) with the lake or wetland water level. On the base of the wetland basin, beneath the free-water zone, a layer of organic sludge develops over the native subsurface material (Headley, 2006).

The most common approach to constructing floating wetlands is through the creation of a floating raft or frame supporting a mesh on which plants are grown. Coconut fiber or peat is often used as a growth medium. Constructed treatment wetlands typically involve flow of contaminated water through the root-zone of emergent species of sedges, rushes and reeds.

As biological material can be used: *Phragmites australis* (Common reed) has been very widely used throughout Europe, Asia, and Australia and is known to perform well in treatment wetlands, *Schoenoplectus tabernaemontani*, *Typha orientalis*, *Baumea articulata*, *Eleocharis sphacelata* and *Carex secta*. *Typha* species have effectively been used in floating treatment wetlands in Canada and the USA (Headley, 2006). Other species recommended for floating islands: *Alisma plantago-aquatica*, *Glyceria maxima*, *Mentha aquatica*, *Myosotis palustris*, *Sagittaria sagittifolia*, *Sium erectum*, *Sium latifolium*, *Sparganium erectum*, *Symphytum officinale*, *Thelypteris palustris*, *Typha laxmannii*, *Typhoides arundinacea*.

The selected growing media must provide suitable growth conditions, including porosity, water retention, capillarity, and fertility with pH between 5.0 and 6.5.

## RESULTS AND DISCUSSIONS

Phytoremediation process of contaminated waters usually uses green plants to remove pollutants from the environment, by up taking, concentrating, incorporating and distributing them in harvestable parts. Is a newly introduced field of science and technology to clean and remediate contaminated sites. This method is cost-effective, long lasting aesthetic solution and environmental friendly. Disadvantages of this process can be related to the climate and the phytotoxicity of some plants when the metal concentration is high (Liang, 2009). The role of aquatic plants in phytoextraction was established through numerous researches. Using hyperaccumulators (more than 1 g/mg heavy metals) as biofilter is frequently, because these plants not only tolerates high quantity of toxic elements, but also distribute them in their roots or leaves. More than 400 plants were reported as hyperaccumulators from 45 botanical families (Liang, 2009). Generally, for phytoextraction are selected plants with very high productivity, higher rate of metal removal, easy to harvest, easy to grow, safety to process by drying, ashing or composting for recycling (Lu, 2004). It is important to use native

plants for phytoremediation because these plants are grown and reproduce without stress conditions. The identification of hyperaccumulators is an important task as the key of phytoextraction.

Concerning the constructed wetlands, the advantages being easy and cheap to construct, and suitable for wastewater purification. They help to prevent the spread of heavy metals contamination from land to aquatic environment (Lu, 2004) and it is used to improve the water quality.

Heavy metal contamination of waters becomes serious and continuous problem on the whole world, which can attract the attention on implementation and development of different remediation methods.

## CONCLUSIONS

Phytoremediation can detoxify, remove, degrade, absorb, relocate, immobilize, or extract contaminants, depending on the nature of the pollution and the type of plant or microbe. It is an eco friendly method, is gentle to ecosystems and long lasting aesthetic solution.

These methods work slowly, however, and may not always be suitable for large cleanup tasks or cleanups that require fast action.

Hyperaccumulators has superior capacity to absorb contaminants from wastewaters and can remove and distribute high quantity of heavy metals in roots or leaves.

Floating islands are gentle to ecosystems and can be used not only to clean waste waters, but also to decorate lacks and ponds.

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# THE INFLUENCE OF ROOTING SUBSTRATE ON THE DEVELOPMENT OF ROSEMARY CUTTINGS

## INFLUENȚA SUBSTRATULUI DE ÎNRĂDĂCINARE ASUPRA DEZVOLTĂRII BUTAȘILOR DE ROZMARIN

*BUTA Erzsebet*<sup>1</sup>, *CANTOR Maria*<sup>1</sup>,  
*BUTA M.*<sup>1</sup>, *HORȚ Denisa*<sup>1</sup>  
e-mail: ebuta2008@yahoo.com

**Abstract.** *The increasing the demand on the market of aromatic species determine more detailed study of rosemary propagation. This species is not only a seasoning plant; it is used in alternative medicine, but also modern and rustic gardens, balconies, terraces and sills. This paper makes some recommendations on the best substrate for rooting of Rosmarinus cuttings. During the experiments were made some morphological observations, were recorded dates regarding the growth and development of Rosmarinus cuttings in different rooting substrates. The dates were statistically interpreted by LSD test (Least Significant Difference) to illustrate the significance of differences. The best results concerning the total length of the cuttings and root systems were recorded in the substrate consist in peat and perlite. The number offshoots per cuttings was influenced most favorable by the substrate consist in peat with sand. Perlite of 0.02 mm favorably influenced the diameter of rosette and the number of leaves.*

**Key words:** medicinal properties, perlite, peat, propagation, aromatic plants

**Rezumat.** *Cererea tot mai mare pe piață a speciilor aromatice a determinat studierea mai amănunțită a înmulțirii rozmarinului. Această specie nu este numai o plantă condimentară, ea se folosește și în medicina alternativă, dar și în decorul grădinilor moderne și rustice, balcoanelor, teraselor și pervazelor. Lucrarea de față aduce câteva recomandări cu privire la cel mai bun substrat de înrădăcinare a butașilor de Rosmarinus. Pe parcursul desfășurării experiențelor s-au efectuat observații morfologice, s-au înregistrat date cu privire la creșterea și dezvoltarea butașilor de Rosmarinus în diferitele substraturi de înrădăcinare. Datele obținute au fost interpretate statistic cu ajutorul analizei varianței. Cele mai bune rezultate cu privire la lungimea totală a butașilor și a sistemului radicular s-au înregistrat în substratul format din perlit cu turbă. Numărul de lăstari pe butași a fost influențat cel mai favorabil de substratul compus din turbă cu nisip. Perlitul de 0,02 mm a influențat favorabil diametrul rozetei plantelor și numărul de frunze.*

**Cuvinte cheie:** proprietăți medicinale, perlit, turbă, plante aromatice

### INTRODUCTION

The history of herbs begin around 1066 AD, the custodians of medicinal herbs were European monasteries. After their dissolution herbs became the province of the manor house garden, and by Elizabethan times herb gardens had

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

become very formal. In 1621, the first Botanic Garden in the UK opened at Oxford University, and in 1673 the Chelsea Physic Garden followed. By the 18th century, herbs became an informal part of the flower border and vegetable plot.

By the end of the 20th century, herbs were once again recognized for their own culinary and medicinal merit, and the dedicated herb garden plan has now undergone a huge revival (Jekka, 2010).

Rosemary, traditionally herb is a sweet-scented, shrubby, evergreen perennial that is usually grown as an annual in cooler climates. It is native to southern Europe, Morocco, and Tunisia (Small, 2001). Its scientific name *Rosmarinus* refers to its origins: “ros” is the Latin for “dew,” and “marinus” means “belonging to the sea.” Students in ancient Greece used to braid rosemary wreaths in their hair when taking examinations as the plant was thought to fortify the brain and refresh the memory.

In the Middle Ages, rosemary was believed to grow only in the gardens of the righteous, and so it was used as a magic charm to protect the wearer from the “evil eye.” Placing a sprig under the pillow was also believed to have the power to repel evil spirits and bad dreams. For centuries, rosemary has been a symbol of happiness, fidelity, and love, and it has long been held that a man indifferent to rosemary’s perfume is incapable of truly loving a woman.

Legend has it that when the Holy Family was fleeing from Herod’s soldiers to Egypt, the Virgin Mary hung her blue cloak one night on a rosemary bush that had white flowers. The next morning the flowers had turned blue, like Mary’s garment. From then on, the herb was known as “rose of Mary” (Small, 2001)

Rosemary is a woody evergreen with a height and spread of 80 cm - 1 m (Small, 2001; Jekka, 2010), 60-150 cm (Ardelean and Mohan, 2008). The flowers are small, pale blue, or bluish-lilac flowers (occasionally white or pink) that grow in clusters on the branches, it is appear in early spring and last until early summer, with, sometimes, a second flush in early fall. The leaves are short, needle-shaped, dark green and are highly aromatic when crushed. The leaves give off a pungent fragrance rather similar to tea or a combination of pine and nutmeg (Small, 2001). This is an important culinary and medicinal herb. Leaves, stems, and flowers can be used as culinary art.

Rosemary contains the antioxidants carnosic acid and rosmarinic acid and other bioactive compounds including camphor, caffeic acid, ursolic acid, betulinic acid, rosmaridiphenol, and rosmanol.

The plant has a very long tradition as a medicinal herb (Al-Sereiti, 1999). Hungary water was an infusion of flowering apices and wine that was allegedly formulated by Queen Elisabeth of Hungary in the eleventh century. The wine was used as a cordial for palpitations, to stimulate kidney function, or as a remedy against headache caused by insufficient circulation.

A steamy infusion of flowering apices is a good remedy for colic, colds, and nervous depression. It has a marked action on the nervous system, by reducing mental fatigue and stimulating memory and aids recovery from long-term stress and chronic illness (Jekka, 2010). Because of its antimicrobial activity, it can be used for acne and other skin infections (Burlando, 2010).

Fragrant rosemary enhances the flavor of any food – savory or sweet – to which it is added. Widely used to season meat, and is also added to jellies, jams, cakes and cookies. Can be adding to salads and vegetable dishes and is an essential ingredient in herb breads and biscuits, including “focaccia”, the classic Italian flat bread (Small, 2001). If dried rosemary is utilized to uncooked foods is necessary to introduce in hot water before adding it. The flowers, with their milder flavor, can be candied, preserved, or added to jellies, honey, vinegar, and wine. Flavor olive oil by adding a few sprigs of rosemary. The essential oil is a good insect repellent (Jekka, 2010).

In traditional folk medicine, rosemary tea was used to stimulate the heart, alleviate headaches, and induce sleep. This species is not used much in modern medicine, although the oil does have antibacterial properties. Researchers are presently studying the value of rosemary in treating indigestion, rheumatic disorders (Iserin, 2001), and circulatory problems, and there is some promise of new medicinal uses. Rosemary oil is used commercially in various personal care products, including shampoos for oily hair and conditioners to bring out the highlights in dark hair (Small, 2001).

Rosemary is used as a decorative plant in gardens for xeriscape landscaping, especially in regions of Mediterranean climate. It is considered easy to grow and pest-resistant. It is often used in topiary, that venerable craft in which shrubs are trained into ornamental shapes.

## MATERIAL AND METHODS

Experiences regarding the unilateral influence of substrate on the development of rosemary cuttings were placed in the greenhouse belonging to the Floriculture Department of UASVM - Cluj-Napoca. The greenhouse is old, flat glass, with semi-automatic equipment. Rooting was carried out on tables in substrate and growing trays.

The experiment was carried out during 2011 and was organized an experience with two factors and the analyzed factors were *Rosmarinus officinalis* and different rooting substrate.

The experience was monofactorial with four variants which were placed in randomized blocks, in three repetitions. The rooting substrate was consisting in the following: a<sub>1</sub> perlite; b<sub>2</sub> peat + perlite 1:1; b<sub>3</sub> peat + sand 1:1. The peat provides from Lithuania, was crushed, fertilized and neutralized (TS 3 peat).

The control of experience was the average of experience. For the propagation of *Rosmarinus*, on 15.06.2011 were collected 15 cuttings for each variant from the healthy mother plants, vigorous and free from diseases and pests. Cuttings were made from the middle-aged healthy stems. The prepared cuttings were treated with Radistim 1 for a better rooting.

Biological material prepared before had been planted in three rooting substrates, the cuttings being introduced in the substrate with at thickness of 8-10 cm. The rooting process was developed different according to the substrate: in perlite – 48 days, in peat+perlite – 37 days and peat+sand – 41 days.

## RESULTS AND DISCUSSIONS

The recorded data concerning the influence of substrate on the

development of rosemary cuttings were statistical interpreted with „LSD” test (Least Significant Difference) to illustrate the significance of differences.

In the table 1 are presents data concerning the influence of rooting substrate on the total length of *Rosmarinus* cuttings. Significant difference comparing with the control (average of experience) were registered at the substrates consist in peat + perlite 1:1, which excel the control with 2.80 cm. The third substrate (peat+sand 1:1) achieved the same difference as the control.

Table 1

**Unilateral influence of rooting substrate on the total length of *Rosmarinus* cuttings**

| Rooting substrates              | The total length of cutting |              | ±d (cm) | Signification of difference |
|---------------------------------|-----------------------------|--------------|---------|-----------------------------|
|                                 | Absolute (cm)               | Relative (%) |         |                             |
| Perlite                         | 18.90                       | 87.1         | -2.70   | -                           |
| Peat + perlite                  | 23.70                       | 113.4        | 2.80    | *                           |
| Peat + sand                     | 20.90                       | 100.0        | 0.00    | -                           |
| Average of experience (control) | 20.90                       | 100.0        | 0.00    | -                           |
| LSD (p 5%)                      |                             |              | 2.71    |                             |
| LSD (p 1%)                      |                             |              | 4.97    |                             |
| LSD (p 0.1%)                    |                             |              | 6.02    |                             |

Table 2

**Unilateral influence of rooting substrate on length of *Rosmarinus* cuttings**

| Rooting substrates              | The length of cutting |              | ±d (cm) | Signification of difference |
|---------------------------------|-----------------------|--------------|---------|-----------------------------|
|                                 | Absolute (cm)         | Relative (%) |         |                             |
| Perlite                         | 7.83                  | 69.9         | -3.37   | 00                          |
| Peat + perlite                  | 12.10                 | 108.0        | 0.90    | *                           |
| Peat + sand                     | 13.75                 | 122.8        | 2.55    | **                          |
| Average of experience (control) | 11.20                 | 100.0        | 0.00    | -                           |
| LSD (p 5%)                      |                       |              | 0.83    |                             |
| LSD (p 1%)                      |                       |              | 1.53    |                             |
| LSD (p 0.1%)                    |                       |              | 3.40    |                             |

Results concerning the unilateral influence of rooting substrate on the length of rosemary cuttings are presented in table 2. Data show that the best substrate was the mix of peat + sand 1:1, which has generated distinct significant differences, which exceeds the control with 2.55 cm. The second substrate (peat+perlite) registered significant positive differences comparing with the control (0.90 cm).

Regarding the influence of rooting substrate on the number of leaves at rosemary cuttings can conclude that in similar way perlite and the mix of peat+perlite registered significant positive differences (tab. 3).



Table 3

**The influence of rooting substrate on number of leaves of *Rosmarinus* cuttings**

| Rooting substrates              | The number of leaves |              | ±d    | Signification of difference |
|---------------------------------|----------------------|--------------|-------|-----------------------------|
|                                 | Absolute             | Relative (%) |       |                             |
| Perlite                         | 14.65                | 105.8        | 0.80  | *                           |
| Peat + perlite                  | 14.50                | 104.7        | 0.65  | *                           |
| Peat + sand                     | 12.50                | 90.3         | -1.35 | 000                         |
| Average of experience (control) | 13.85                | 100.0        | 0.00  | -                           |
| LSD (p 5%)                      |                      |              | 0.54  |                             |
| LSD (p 1%)                      |                      |              | 0.98  |                             |
| LSD (p 0.1%)                    |                      |              | 1.18  |                             |

In the case of *Rosmarinus officinalis*, the best results regarding the influence of rooting substrate on diameter of rosette was registered when was used the perlite. The difference which exceeds the control was 1.31 cm, distinct significant positive (tab. 4).

Table 4

**The influence of rooting substrate on diameter of rosette of *Rosmarinus* cuttings**

| Rooting substrates              | The diameter of rosette |              | ±d (cm) | Signification of difference |
|---------------------------------|-------------------------|--------------|---------|-----------------------------|
|                                 | Absolute (cm)           | Relative (%) |         |                             |
| Perlite                         | 10.60                   | 114.1        | 1.31    | **                          |
| Peat + perlite                  | 9.40                    | 101.2        | 0.11    | -                           |
| Peat + sand                     | 7.89                    | 84.9         | -1.41   | 00                          |
| Average of experience (control) | 9.26                    | 100.0        | 0.00    | -                           |
| LSD (p 5%)                      |                         |              | 0.62    |                             |
| LSD (p 1%)                      |                         |              | 1.14    |                             |
| LSD (p 0.1%)                    |                         |              | 2.52    |                             |

Statistical data shows that in the substrate composed of peat + sand 1:1 the number of offshoots exceed the control of experiences. The value of 2.50 determines a significant difference. The peat+perlite substrate has a positive influence on the number of offshoots, but not statistically assure. The substrate that generates negative influence, in this case, was perlite (tab. 5).

Table 5

**The influence of rooting substrate on the number of offshoots of *Rosmarinus* cuttings**

| Rooting substrates              | The number of offshoots |              | ±d    | Signification of difference |
|---------------------------------|-------------------------|--------------|-------|-----------------------------|
|                                 | Absolute                | Relative (%) |       |                             |
| Perlite                         | 4.50                    | 62.1         | -2.75 | 0                           |
| Peat + perlite                  | 7.50                    | 103.4        | 0.25  | -                           |
| Peat + sand                     | 9.75                    | 134.5        | 2.50  | *                           |
| Average of experience (control) | 7.25                    | 100.0        | 0.00  | -                           |
| LSD (p 5%)                      |                         |              | 2.43  |                             |
| LSD (p 1%)                      |                         |              | 4.46  |                             |
| LSD (p 0.1%)                    |                         |              | 5.88  |                             |

Analyzing the influence of rooting substrate on the length of rosemary roots, data presented in table 6 show that the first and second substrates (perlite and peat+perlite) registered distinct significant differences. At the first substrate the difference which exceeds the control was 1.45 cm, at the second 1.50 cm.

Table 6

The influence of rooting substrate on the length of roots of *Rosmarinus* cuttings

| Rooting substrates              | The length of roots |              | ±d (cm) | Signification of difference |
|---------------------------------|---------------------|--------------|---------|-----------------------------|
|                                 | Absolute (cm)       | Relative (%) |         |                             |
| Perlite                         | 10.95               | 115.2        | 1.45    | **                          |
| Peat + perlite                  | 11.00               | 115.7        | 1.50    | **                          |
| Peat + sand                     | 8.20                | 86.3         | -1.30   | 00                          |
| Average of experience (control) | 9.50                | 100.0        | 0.00    | -                           |
| LSD (p 5%)                      |                     |              | 0.45    |                             |
| LSD (p 1%)                      |                     |              | 0.83    |                             |
| LSD (p 0.1%)                    |                     |              | 1.84    |                             |

## CONCLUSIONS

Based on the results the following conclusions and recommendations:

1. The rooting process was the best in the second substrate (peat+perlite), cutting were rooted in 37 days.
2. The total length of cuttings was influenced positive by the second substrate (peat+perlite), which generate significant difference.
3. Regarding the length of cutting can conclude that peat+perlite determine significant difference and peat+sand achieved distinct significant difference.
4. The number of leaves was influenced favorable influenced by perlite and peat+perlite.
5. Concerning the diameter of rosette, best results were obtained in case of perlite.
6. Favorable influence was registered at peat+sand in case of number of offshoots.
7. Almost similar differences were registered at perlite and peat+perlite in case of length of roots.

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# PROSPECTS FOR THE USE OF SOME PLANT LIGNEOUS TO THE CONTAINER

## PERSPECTIVELE UTILIZĂRII UNOR PLANTE LEMNOASE LA CONTAINER

MITRĂ M.<sup>1</sup>, MITREA I.<sup>1</sup>, BOTU I.<sup>1</sup>

e-mail: mitramarian@yahoo.com

**Abstract.** *In the last two decades have appeared widely in our country, fruit and dendrological plants on the container. These species are used for decorative effects, fruit and even research. Research carried out along 10 years have proposed to establish the most effective types of containers, mixtures of nutrients and different types of fruit and dendrological plants. The variants with plastic containers of 90 liters capacity and nutrient composed of 1/3 peat, 1/3 sand and 1/3 ground celery with 3-4 kg barbel have achieved the highest growth and highest number of fruits at the studied plants. (Juglans regia cv. Student, Corylus avelana cv. Vâlcea 22, Prunus domestica cv. Stanley și Tuleu gras, Prunus cerasifera cv. Red Castle. Growth differences were recorded in all species where the containers were buried in the soil ( $SST_x = 53 \text{ cm}^2$  and crown volume =  $6,2 \text{ m}^3$ ) comparative with the aboveground container ( $SST_x = 17,1 \text{ cm}^2$  and crown volume =  $0,83 \text{ m}^3$ ) where the plants grew much less. For the fruit plants was found that fruit yield were from 0.5 to 7.6 kg / plant for containers buried in the soil. Benefits of plants fruit and dendrological grown in 90 liters containers and buried in the soil are superior to those grown in aboveground containers, also through increasing the quantities of fruit and the strength gives for the root systems during the winter frost ( $-12^\circ\text{C}$ ,  $-20^\circ\text{C}$ ) and even in summer ( $40^\circ\text{C}$ ). Multiannual plants grown in containers are successfully used in yards, gardens and even on street alignments.*

**Key words:** containers, woody plants, growing, bearing.

**Rezumat.** *În ultimele două decenii și-au făcut apariția pe scară largă, în România, unele plante pomicele la container. Scopul acestora este de a le cultiva în grădini, terase sau pentru conservarea resurselor genetice. Cercetările efectuate de-a lungul a 10 ani și-au propus să stabilească cele mai eficiente tipuri de containere, de amestecuri nutritive și a unor feluri de plante pomicele. Containerele de plastic cu capacitatea de 90 L și amestecul nutritiv format din 1/3 turbă, 1/3 nisip și 1/3 pământ de țelină cu 3-4 kg mraniță au realizat cele mai mari creșteri și cele mai multe fructe la plantele studiate, Juglans regia cv. Student, Corylus avelana cv. Vâlcea 22, Prunus domestica cv. Stanley, Tuleu gras și Prunus cerasifera cv. Red castle. Diferențe mari de creștere s-au înregistrat la toate speciile, în cazul în care containerele au fost îngropate în sol – Suprafața Secțiunii Trunchiului (SST), ( $SST_x = 53 \text{ cm}^2$  și volumul coroanei =  $6,2 \text{ m}^3$ ), comparativ cu containerele supraterane ( $SST_x = 17,1 \text{ cm}^2$  și volumul coroanei =  $0,83 \text{ m}^3$ ), în care plantele au crescut mult mai puțin. La plantele pomicele s-a constatat că se obțin producții de fructe de 0,5 la 7,6 kg/plantă, în cazul containerelor îngropate în sol. Avantajele oferite de*

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<sup>1</sup> University of Craiova, Romania

*plantele pomicele cultivate în containere de 90 L și îngropate în sol sunt superioare față de cele cultivate în containere neîngropate, atât prin creșterile și cantitățile de fructe realizate, cât și prin rezistența pe care o conferă sistemelor radiculare în cazul gerurilor din iarnă (-12; -20<sup>0</sup>C) și chiar în vară (+40<sup>0</sup>C). Plantele lemnoase cultivate la containere se folosesc cu succes în curți, grădini familiale și chiar pe aliniamente stradale.*

**Cuvinte cheie:** containere, plante lemnoase, creștere, fructificare.

## INTRODUCTION

Ornamental plant growing in containers made of wood (crates, boxes, tub, etc.) or clay (pottery, amphorae, vessels of different shapes) dates back to ancient times. Many paintings, frescoes, archeological artifacts are found throughout the ancient sites.

This type of ornamental plant cultivation has spread to this day remains the precursor of modern culture and plant in container in urban centers today (Dartigues et Lemaire, 1989; Stanica et al., 2002).

Introduction of exotic plants, including those fruit plants in temperate areas (citrus, fig, pomegranate, etc. Have given a new perspective to use plants in container. Gardens Palace of Versailles, built by Le Nôtre, after 1861 became among the most representative of the culture of exotic fruit and ornamental plants in container. Around the same time make their appearance on paths in parks, sidewalks in court, many containers with grown plants.

From the great palaces and museums of the world (Louvre, Vatican, Schönbrun, Leipzig, etc.) The cultivation of plants in container is ubiquitous in urban life, and in recent years even under field conditions, the purpose of research (gene banks, hybrid cultures etc.) (Bunt, 1984, Wright and Nemiera, 1987, Costea, 2001).

Through our research at the University of Craiova (SCDP Vâlcea) we proposed to establish the behavior of plants grown in containers of plastic fruit with capacity of 90 L.

## MATERIAL AND METHOD

The research was conducted on the following species: *Juglans regia cv. Student*, *Corylus avellana cv. Valcea 22*, *Prunus domestica cv. Stanley* and *Tuleu gras*, *Prunus cerasifera cv. Red castle*, *Malus domestica cv. Wjczik*.

In this paper we refer to results obtained in plastic containers with a capacity of 90 L and nutrient mixture consisting of 1/3 peat, 1/3 sand and 1/3 of fallow land and 3-4 kg manure.

Each year the container was fertilized with 25 g NH<sub>4</sub>NO<sub>3</sub>/container.

Containers with plants were used in two ways:

- containers buried in the soil (60-70 cm);

- containers maintained above ground (on film or concrete), with protection in solariums during winter.

Irrigation was performed every three days (containers above ground) and every six-seven days in the underground. Observations and measurements were made on trunk sectional area, crown volume, fruit production, ornamental appearance etc.

Some observations were processed by statistical methods (standard deviation and variance analysis), the experience using a number of five plants, each representing a repetition, a total of 5 repetitions.

## RESULTS AND DISCUSSIONS

Culture of fruit and ornamental plants in containers of 90 L was found to be dependent on plant genotype and the use of containers. The way the plants growth for a period of 10 years was distinguished by the characteristic features: trunk sectional area (SST) and crown volume (table 1 and 2).

Trunk section area (SST) has differentiated as value between genotypes. This ranged from 32.1 cm<sup>2</sup> (*Corylus avelana* cv. *Válcea 22*) and 63.6 cm<sup>2</sup> (*Juglans regia* cv. *Student*, fig. 1) at the plants grown in containers buried in the soil. At the containers above ground trunk sectional area values (SST) ranged from 7.1 cm<sup>2</sup> and 40.7 cm<sup>2</sup>.

Medium trunk sectional area (SST) in all plants grown in containers buried (50.9 cm<sup>2</sup>) were higher than those of ground containers (13.7 cm<sup>2</sup>), with a probability of transgression of P.005 = 6,8 cm<sup>2</sup>.

Coefficients of variation (s%) of trunk sectional area (SST) ranged between 14.9% (*Malus domestica* cv. *Wijcik.*) (fig. 2) and 20.4% (*Juglans regia* cv. *Student*) for buried containers and between 14, 2% (*Corylus avelana* cv. *Válcea 22*) and 20.2% (*Prunus domestica* cv. *Tuleu gras*) (fig.3), standing in groups with average variability (10-20%).

Table 1

Trunk section area at plants grown in plastic containers of 90 L (age 10 years)

| No.     | Species  | Containers buried      |      | Containers above ground |      | R = $\frac{SSTC_i}{SSTC_s}$ |
|---------|--|------------------------|------|-------------------------|------|-----------------------------|
|         |  | SST (cm <sup>2</sup> ) | s%   | SST (cm <sup>2</sup> )  | s%   |                             |
| 1.      | <i>Juglans regia</i> cv. <i>Student</i>        | 63,6                   | 20,4 | 12,5                    | 19,6 | 5,09                        |
| 2.      | <i>Corylus avelana</i> cv. <i>Válcea 22</i>    | 32,1                   | 16,2 | 8,7                     | 14,2 | 3,69                        |
| 3.      | <i>Prunus domestica</i> cv. <i>Stanley</i>     | 50,2                   | 17,1 | 15,2                    | 19,4 | 3,30                        |
| 4.      | <i>Prunus domestica</i> cv. <i>Tuleu gras</i>  | 53,2                   | 18,9 | 12,5                    | 20,2 | 4,25                        |
| 5.      | <i>Prunus cerasifera</i> cv. <i>Red castle</i> | 52,8                   | 16,4 | 15,2                    | 18,2 | 3,47                        |
| 6.      | <i>Malus domestica</i> cv. <i>Wijcik</i>       | 53,6                   | 14,9 | 18,2                    | 16,4 | 2,94                        |
| Average |  | 50,9                   |      | 13,7                    | -    | 3,71                        |

SST C<sub>i</sub> = Trunk section area at buried containers

SST C<sub>s</sub> = Trunk section area at above ground containers

P.005 = 6,8 cm<sup>2</sup>

Trunk sectional area ratio values (SST) of the same genotype grown in buried and unburied container varies between 2.94 and 5.09, for those buried.

Values recorded at crown volume (table 2) show an average of 5.53 m<sup>3</sup> at plants in buried containers comparative to 0.67 m<sup>3</sup> (with P.005 = 0.75 m<sup>3</sup>).



**Fig. 1** - *Juglans regia* cv. *Student*  
at above ground containers



**Fig.2** - *Malus domestica* cv. *Wijcik*  
at above ground containers



**Fig. 3** - *Prunus domestica* cv. *Tuleu gras*  
at buried containers



**Fig. 4** - *Prunus domestica* cv. *Stanley*  
at above ground containers

Table 2

**Crown volume at plants grown in  
plastic containers of 90 L (age 10 years)**

| No.     | Species  | Containers buried              |      | Containers above ground        |      | $R = \frac{V.cor.\hat{i}}{V.cor.s}$ |
|---------|--|--------------------------------|------|--------------------------------|------|-------------------------------------|
|         |  | Crown volume (m <sup>3</sup> ) | s%   | Crown volume (m <sup>3</sup> ) | s%   |                                     |
| 1.      | <i>Juglans regia</i> cv. <i>Student</i>        | 5,5                            | 19,5 | 0,40                           | 20,4 | 13,7                                |
| 2.      | <i>Corylus avelana</i> cv. <i>Vâlcea 22</i>    | 3,7                            | 18,5 | 0,39                           | 16,6 | 9,5                                 |
| 3.      | <i>Prunus domestica</i> cv. <i>Stanley</i>     | 6,9                            | 18,5 | 0,76                           | 19,6 | 9,1                                 |
| 4.      | <i>Prunus domestica</i> cv. <i>Tuleu gras</i>  | 7,2                            | 20,1 | 0,92                           | 20,8 | 7,8                                 |
| 5.      | <i>Prunus cerasifera</i> cv. <i>Red castle</i> | 7,5                            | 16,5 | 0,96                           | 19,1 | 7,8                                 |
| 6.      | <i>Malus domestica</i> cv. <i>Wijcik</i>       | 2,4                            | 10,2 | 0,63                           | 11,4 | 3,8                                 |
| Average |  | 5,53                           | -    | 0,67                           | -    | 8,6                                 |

V.cor.î = Crown volume at buried containers

V.cor.s = Crown volume at above ground containers

P.005 = 0,75 cm<sup>2</sup>

Table 3

**Fruit production at some plants grown  
in containers of 90 L (average 3 years)**

| No.     | Species  | Fruit production kg/pl |                         | Fruit production t/ha * |                         |
|---------|--|------------------------|-------------------------|-------------------------|-------------------------|
|         |  | Containers buried      | Containers above ground | Containers buried       | Containers above ground |
| 1.      | <i>Prunus domestica</i> cv. <i>Stanley</i>     | 4,3                    | 0,8                     | 7,1                     | 1,3                     |
| 2.      | <i>Prunus domestica</i> cv. <i>Tuleu gras</i>  | 3,7                    | 0,7                     | 6,1                     | 1,1                     |
| 3.      | <i>Malus domestica</i> cv. <i>Wijcik</i>       | 7,6                    | 3,9                     | 12,6                    | 6,5                     |
| 4.      | <i>Corylus avelana</i> cv. <i>Vâlcea 22</i>    | 0,5                    | 0,3                     | 0,8                     | 0,5                     |
| 5.      | <i>Prunus cerasifera</i> cv. <i>Red castle</i> | 0,6                    | 0,3                     | 1,0                     | 0,5                     |
| Average |  | 3,3                    | 1,2                     | 5,5                     | 2,0                     |

\* - Fruit production calculated for 1660 plants / ha

All plants in buried containers their crowns and heights were higher than those in unburied containers and this phenomenon is because the temperature and humidity in the container is very close to that of surrounding soil and the holes in the container allow to some roots have direct contact with the ground.

The crown volume of the plants in buried containers ranged from 7.5 m<sup>3</sup> (*Prunus cerasifera* cv. *Red Castle*) and 2.4 m<sup>3</sup> (*Malus domestica* cv. *Wijcik*), and for the plants in the unburied containers between 0.39 m<sup>3</sup> (*Corylus avelana* cv. *Vâlcea 22*) and 0.96 m<sup>3</sup> (*Prunus cerasifera* cv. *Red castle*).

Ratio between crown volumes from the same plant, ranged from 3.8 (*Malus domestica* cv. *Wijcik*) and 13.7 (*Juglans regia* cv. *Student*).

For a total of five varieties there was recorded the fruit production (table 3).

Plants grown in buried containers achieved an average production of fruit (3.3 kg/plant) comparative with those in containers above ground (1.2 kg/plant).

The most productive proved to be: *Malus domestica* cv. *Wijcik* (7.6 kg / plant), *Prunus domestica* cv. *Stanley* (4.3 kg / plant) (fig. 4) and *Prunus domestica* cv. *Tuleu gras* (3.7 kg / plant).

For a culture with 1660 plants/ha, buried containers, fruit yields are higher, *Malus domestica* cv. *Wijcik* (12.6 t / ha), *Prunus domestica* cv. *Stanley* (7.1 t / ha) and *Prunus domestica* cv. *Tuleu gras* (6.1 t / ha).

Variety of hazel *Vâlcea 22* and plum variety *Red Castle* have made very high productions (0.8 to 1.0 t / ha).

For the plants grown in containers above ground one variety, *Malus domestica* cv. *Wijcik* made a high production (6.5 t/ha), the rest of genotypes have produced between 0.5 and 1.3 t/ha.

## CONCLUSIONS

Cultivation of the fruit plants/ornamental plants in container 90 L with burial in soil is advantageous to the ornamental and scientific production (gene banks).

Plants grown in containers without soil burial, need protection from low temperatures in winter, but can be used in different locations.

Between plants grown in the two systems shows differences of vigor and fruition in favor of container buried in the soil.

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# RESEARCH ON *CANNA INDICA* L. SEED GERMINATION

## CERCETĂRI PRIVIND GERMINAREA SEMINTELOR DE *CANNA INDICA* L.

**POPESCU Liana<sup>1</sup>**

e-mail: popescu.liana@primariabistrita.ro

**Abstract.** *This study aims to test the influence of constant temperature and gibberellic acid GA<sub>3</sub>, on seed germination of five Canna indica L. cultivars. The seeds were exposed to constant temperature (30°C) for 24h and 72h. Both, variable (15-22°C) and constant temperature (30°C) provided the necessary heat to activate the embryo. In order to study the effect of gibberellic acid GA<sub>3</sub>, the seeds were treated with GA<sub>3</sub> solution in three levels: 150 ppm, 300 ppm, 450 ppm. The highest percentage of germination was obtained at 450 ppm concentration, on 'Tropical Bronze Scarlet' cultivar.*

**Key words:** *Canna indica* L., seeds, temperature, gibberellic acid

**Rezumat.** *Lucrarea urmărește influența temperaturii constante și a acidului giberelic GA<sub>3</sub>, asupra germinării semințelor a cinci cultivare de Canna indica L. Semințele au fost expuse la temperatură constantă (30°C) timp de 24h, respectiv 72h. Atât temperatura variabilă (15-22°C), cât și temperatura constantă (30°C), au asigurat necesarul de căldură pentru activarea embrionului. Pentru studierea efectului acidului giberelic asupra germinației semințelor, acestea au fost tratate cu soluție de GA<sub>3</sub>, în trei concentrații: 150 ppm, 300 ppm, 450 ppm. Cel mai mare procent de germinare s-a obținut la concentrația de 450ppm, pentru cultivarul 'Tropical Bronze Scarlet'.*

**Cuvinte cheie:** *Canna indica* L., semințe, temperatură, acid giberelic

### INTRODUCTION

Herbaceous plant, decorative by leaves and flowers both and through its size, *Canna indica* L. plays an important part in public and private landscape design. It is among the few flower plants that offer color to a space for such a long period (May - October), with relatively low maintenance requirements (Șelaru, 2007).

Generative propagation of *C. indica* L. plants is a relatively simple method, but it's not widely used yet, because many cultivars don't produce seeds or they have sterile seeds. *Canna* seeds are part of large seeds category (3-10 mm), spherical, ellipsoidal or intermediate shape, brownish black color, slightly glossy, smooth and very hard coat. With no special facilities for storage, they preserve their germination for 15-20 years, requiring minimal care during storage (Cantor, 2009). Facilitating the exit from dormancy of *Canna indica* L. seeds requires mechanical or chemical degradation of the coat. The result is ease of water and oxygen ingress, leading to enzymatic germination processes initiation (Geneve,

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<sup>1</sup> Bistrița City Hall – Public Services Department, Romania

2011). The purpose of this paper aims to establish the influence of heat and gibberellic acid treatments on *Canna indica* L. seed germination.

## MATERIAL AND METHODS

Experiments were conducted in the Public Services Department's greenhouses of the Bistrița City Hall, in 2011. The biological material used in experiments, was represented by the seeds belonging to five cultivars of *Canna indica* L. (fig.1).

The seeds of 'Sémaphore' and 'Firebird' cultivars were harvested in autumn 2010, from plants used in public green spaces of Bistrița city and they were manually scarified, by rubbing on sandpaper. The seeds of 'Tropical Rose', 'Tropical Bronze Scarlet' (introduced in 2010) and 'Tropical Yellow' cultivars, were bought from TakiiSeed Company and they were scarified by the producer, with the laser (1-3 holes of 0,8mm diameter), so that they didn't require other interventions for seed coat degradation.

Experiments with seeds placed to germinate under the influence of heat treatment were conducted by exposing the batches of seeds at a constant temperature of 30°C, the first 24 and 72 hours in a BINDER oven ED53 type, electric powered, with the temperature domain "ambient → 300°C", temperature fluctuation of ±0,3°C and heating time of 15 min. The material was placed on cardboard casing, on filter paper, for radicle appearance observation.

In order to study the effect of gibberellic acid on seed germination, they were treated for 24 hours with GA<sub>3</sub> solution, in three concentrations: 150 ppm, 300 ppm and 450 ppm. To complete the germination, the seeds were transferred to a peat substrate with pH 6-6,5 and fertilizers content (N-P<sub>2</sub>O<sub>5</sub>-K<sub>2</sub>O) of 1,5 kg/mc. They were placed in plastic trays, dimpled (4x4x3 cm), and the subsequent observations on the emergence dynamics were daily made.

Statistical analysis of data was based on the variance calculation on a bifactorial experiment that allowed the significance of differences interpretation between experimental variants (Ardeleanu, 2008). Each variant was found in the experiments in three repetitions.

## RESULTS AND DISCUSSIONS

Framing the experiments with heat – treated seeds, were used 54 seeds of each cultivar, meaning 18 seeds on each of the three repetitions.

From table 1 data, is shown that the differences statistically provided as very significant negative from control were recorded at V2, V7 and V12 while the very significant positive difference from control, has appeared at V4. Table 2 data shows that, between the limits of used temperatures, seed germination was not significantly influenced. Both, the ambient temperature that ranged between 15 and 22°C, and constant 30°C temperature provided the necessary heat for leaving the state of dormancy and activate the embryo. The results vary depending on cultivar (table 3), the differences from control being provided as very significant negative at 'Firebird' cultivar and very significant positive at 'Tropical Bronze Scarlet' cultivar.

Table 1

***Canna indica* L. seed germination, under the influence of temperature and cultivar**

| No. | Variant<br>Factor combination   | Number of fully germinated seeds |              | ± d  | Significance of difference |
|-----|---------------------------------|----------------------------------|--------------|------|----------------------------|
|     |                                 | Absolute (no.)                   | Relative (%) |      |                            |
| V1  | 15-22°C x Sémaphore             | 3,7                              | 38,5         | -5,9 | 00                         |
| V2  | 15-22°C x Firebird              | 1,0                              | 10,4         | -8,6 | 000                        |
| V3  | 15-22°C x Tropical Rose         | 14,3                             | 149,0        | 4,7  | **                         |
| V4  | 15-22°C x Tropical Br. Scarlet  | 16,3                             | 169,8        | 6,7  | ***                        |
| V5  | 15-22°C x Tropical Yellow       | 12,7                             | 132,3        | 3,1  | *                          |
| V6  | 24h/30°C x Sémaphore            | 8,0                              | 83,3         | -1,6 | –                          |
| V7  | 24h/30°C x Firebird             | 1,3                              | 13,5         | -8,3 | 000                        |
| V8  | 24h/30°C x Tropical Rose        | 9,7                              | 101,0        | 0,1  | –                          |
| V9  | 24h/30°C x Tropical Br. Scarlet | 12,7                             | 132,3        | 3,1  | *                          |
| V10 | 24h/30°C x Tropical Yellow      | 12,7                             | 132,3        | 3,1  | *                          |
| V11 | 72h/30°C x Sémaphore            | 9,0                              | 93,8         | -0,6 | –                          |
| V12 | 72h/30°C x Firebird             | 2,7                              | 28,1         | -6,9 | 000                        |
| V13 | 72h/30°C x Tropical Rose        | 13,0                             | 135,4        | 3,4  | *                          |
| V14 | 72h/30°C x Tropical Br. Scarlet | 11,7                             | 121,9        | 2,1  | –                          |
| V15 | 72h/30°C x Tropical Yellow      | 14,3                             | 149,0        | 4,7  | **                         |
|     | Average V1-V5, Control          | 9,6                              | 100,0        | -    | -                          |

DL 5% = 2,92 pieces      DL 1% = 4,35 pieces      DL 0,1% = 6,12 pieces

Table 2

***Canna indica* L. seed germination, under the influence of temperature**

| Factor A graduations<br>(temperature) | No. of fully germinated seeds |              | ± d  | Significance of difference |
|---------------------------------------|-------------------------------|--------------|------|----------------------------|
|                                       | Absolute (no.)                | Relative (%) |      |                            |
| 24h/30°C                              | 8,9                           | 121,2        | -0,7 | –                          |
| 72h/30°C                              | 10,1                          | 117,2        | 0,5  | –                          |
| Average V1-V5, Control                | 9,6                           | 100,0        | -    | -                          |

DL 5% = 1,97 pieces      DL 1% = 2,69 pieces      DL 0,1% = 3,60 pieces

Table 3

***Canna indica* L. seed germination, under the influence of cultivar**

| Factor B graduations<br>(cultivar) | No. of fully germinated seeds |              | ± d  | Significance of difference |
|------------------------------------|-------------------------------|--------------|------|----------------------------|
|                                    | Absolute (no.)                | Relative (%) |      |                            |
| Sémaphore                          | 6,9                           | 71,9         | -2,7 | 00                         |
| Firebird                           | 1,7                           | 17,7         | -7,9 | 000                        |
| Tropical Rose                      | 12,3                          | 128,1        | 2,7  | **                         |
| Tropical Bronze Scarlet            | 13,6                          | 141,7        | 4,0  | ***                        |
| Tropical Yellow                    | 13,2                          | 137,5        | 3,6  | **(*)                      |
| Average V1-V5, Control             | 9,6                           | 100,0        | -    | -                          |

DL 5% = 1,92 pieces      DL 1% = 2,63 pieces      DL 0,1% = 3,84 pieces

Organizing the experiments with GA<sub>3</sub> – treated seeds, were used 36 seeds of each cultivar, meaning 12 seeds on each of the three repetitions. From table 4 data, results that the differences statistically provided as very significant negative from control were recorded at V1, V2, V7, V12 and V17, while the very significant positive differences from control, have appeared at V3, V4, V8, V9, V14, V18 and V19.

Table 4

***Canna indica* L. seed germination,  
under the influence of gibberellic acid GA<sub>3</sub> and cultivar**

| Variant |  | No. of fully germinated seeds |              | ± d  | Significance of difference |
|---------|--|-------------------------------|--------------|------|----------------------------|
| No.     | Factor combination                           | Absolute (no.)                | Relative (%) |      |                            |
| V1      | Untreated x Sémaphore                        | 2,5                           | 42,2         | -3,9 | 000                        |
| V2      | Untreated x Firebird                         | 0,7                           | 10,9         | -5,7 | 000                        |
| V3      | Untreated x Tropical Rose                    | 9,5                           | 151,6        | 3,1  | ***                        |
| V4      | Untreated x Tropical Br. Scarlet             | 10,9                          | 167,2        | 4,5  | ***                        |
| V5      | Untreated x Tropical Yellow                  | 8,5                           | 129,7        | 2,1  | **                         |
| V6      | 150ppmGA <sub>3</sub> x Sémaphore            | 5,3                           | 82,8         | -1,1 | 0                          |
| V7      | 150ppmGA <sub>3</sub> x Firebird             | 1,7                           | 26,6         | -4,7 | 000                        |
| V8      | 150ppmGA <sub>3</sub> x Tropical Rose        | 10,0                          | 156,3        | 3,6  | ***                        |
| V9      | 150ppmGA <sub>3</sub> x Tropical Br. Scarlet | 10,3                          | 160,9        | 3,9  | ***                        |
| V10     | 150ppmGA <sub>3</sub> x Tropical Yellow      | 7,7                           | 120,3        | 1,3  | *                          |
| V11     | 300ppmGA <sub>3</sub> x Sémaphore            | 7,0                           | 109,4        | 0,6  | –                          |
| V12     | 300ppmGA <sub>3</sub> x Firebird             | 0,7                           | 10,9         | -5,7 | 000                        |
| V13     | 300ppmGA <sub>3</sub> x Tropical Rose        | 8,7                           | 135,9        | 2,3  | **                         |
| V14     | 300ppmGA <sub>3</sub> x Tropical Br. Scarlet | 10,0                          | 156,3        | 3,6  | ***                        |
| V15     | 300ppmGA <sub>3</sub> x Tropical Yellow      | 7,3                           | 114,1        | 0,9  | –                          |
| V16     | 450ppmGA <sub>3</sub> x Sémaphore            | 5,7                           | 89,1         | -0,7 | –                          |
| V17     | 450ppmGA <sub>3</sub> x Firebird             | 0,7                           | 10,9         | -5,7 | 000                        |
| V18     | 450ppmGA <sub>3</sub> x Tropical Rose        | 9,7                           | 151,6        | 3,3  | ***                        |
| V19     | 450ppmGA <sub>3</sub> x Tropical Br. Scarlet | 11,3                          | 176,6        | 4,9  | ***                        |
| V20     | 450ppmGA <sub>3</sub> x Tropical Yellow      | 7,7                           | 120,3        | 1,3  | *                          |
|         | Average V1-V5, Control                       | 6,4                           | 100,0        | -    | -                          |

DL 5% = 1,02 pieces      DL 1% = 1,94 pieces      DL 0,1% = 2,53 pieces

Analyzing the table 5 data is observed that the three GA<sub>3</sub> solution used concentrations, did not significantly influenced the seeds germination. The results vary depending on cultivar (table 6), the differences from control being provided as very significant negative at ‘Firebird’ cultivar and very significant positive at ‘Tropical Bronze Scarlet’ cultivar.

Table 5

***Canna indica* L. seed germination, under the influence of GA<sub>3</sub> treatment**

| Factor A graduations (GA <sub>3</sub> ) | No. of fully germinated seeds |              | ± d | Significance of difference |
|---|-------------------------------|--------------|-----|----------------------------|
|   | Absolute (no.)                | Relative (%) |     |                            |
| 150ppmGA <sub>3</sub>                   | 7,0                           | 109,4        | 0,6 | –                          |
| 300ppmGA <sub>3</sub>                   | 6,7                           | 104,7        | 0,3 | –                          |
| 450ppmGA <sub>3</sub>                   | 7,0                           | 109,4        | 0,6 | –                          |
| Average V1-V5, Control                  | 6,4                           | 100,0        | -   | -                          |

DL 5% = 0,98 pieces

DL 1% = 1,56 pieces

DL 0,1% = 2,93 pieces

Table 6

***Canna indica* L. seed germination, under the influence of cultivar**

| Factor B graduations (cultivar) | No. of fully germinated seeds |              | ± d  | Significance of difference |
|---------------------------------|-------------------------------|--------------|------|----------------------------|
|                                 | Absolute (no.)                | Relative (%) |      |                            |
| Sémaphore                       | 5,2                           | 81,3         | -1,2 | –                          |
| Firebird                        | 1,0                           | 15,6         | -5,4 | 000                        |
| Tropical Rose                   | 9,5                           | 148,4        | 3,1  | **                         |
| Tropical Bronze Scarlet         | 10,6                          | 165,6        | 4,2  | ***                        |
| Tropical Yellow                 | 7,8                           | 121,9        | 1,4  | *                          |
| Average V1-V5, Control          | 6,4                           | 100,0        | -    | -                          |

DL 5% = 1,36 pieces

DL 1% = 2,15 pieces

DL 0,1% = 3,67 pieces

After analyzing the results, a comparison was made between the two types of treatment with their graduation for each cultivar. The percentage values regarding complete seed germination of the five cultivars used in the experiment are presented in table 7 and table 8 presents data regarding complete germination duration.

Table 7

**Comparative germination capacity of *Canna indica* L. seeds, under the influence of seed treatments**

| Cultivar             | Fully germinated seeds (%) |          |          |                         |                         |                         |
|----------------------|----------------------------|----------|----------|-------------------------|-------------------------|-------------------------|
|                      | Untreated                  | 24h 30°C | 72h 30°C | 150 ppm GA <sub>3</sub> | 300 ppm GA <sub>3</sub> | 450 ppm GA <sub>3</sub> |
| Sémaphore            | 20,6                       | 44,4     | 50,0     | 44,2                    | 58,3                    | 47,5                    |
| Firebird             | 5,6                        | 7,2      | 15,0     | 14,2                    | 5,8                     | 5,8                     |
| Tropical Rose        | 79,4                       | 53,9     | 72,2     | 83,3                    | 72,5                    | 80,6                    |
| Tropical Br. Scarlet | 90,6                       | 70,6     | 65,0     | 85,8                    | 83,3                    | 94,2                    |
| Tropical Yellow      | 70,6                       | 70,6     | 79,4     | 64,2                    | 60,8                    | 64,2                    |

Maximum germination values for each cultivar are presented as follows: ‘Sémaphore’ – 58,3% (300 ppmGA<sub>3</sub>), ‘Firebird’ – 15% (72 h/30°C), ‘Tropical Rose’ – 83,3% (150 ppmGA<sub>3</sub>), ‘Tropical Bronze Scarlet’ – 94,2% (450 ppmGA<sub>3</sub>), ‘Tropical Yellow’ – 79,4% (72 h/30°C).

**Comparative complete germination duration  
of *Canna indica* L. seeds, under the influence of seed treatments**

| Cultivarul           | Complete germination time (no. of days) |             |             |                            |                            |                            |
|----------------------|---|-------------|-------------|----------------------------|----------------------------|----------------------------|
|                      | Untreated                               | 24h<br>30°C | 72h<br>30°C | 150 ppm<br>GA <sub>3</sub> | 300 ppm<br>GA <sub>3</sub> | 450 ppm<br>GA <sub>3</sub> |
| Sémaphore            | 15                                      | 28          | 17          | 22                         | 18                         | 19                         |
| Firebird             | 18                                      | 33          | 29          | 19                         | 29                         | 17                         |
| Tropical Rose        | 26                                      | 28          | 16          | 24                         | 20                         | 22                         |
| Tropical Br. Scarlet | 20                                      | 20          | 25          | 28                         | 14                         | 17                         |
| Tropical Yellow      | 26                                      | 25          | 15          | 23                         | 23                         | 22                         |

## CONCLUSIONS

1. Both, heat and GA<sub>3</sub> treatments, significantly influenced seed germination of two cultivars, as follows: ‘Sémaphore’ – from 20,6% (untreated) to 50,0% (72h/30°C) and 58,3% (300 ppmGA<sub>3</sub>); ‘Firebird’ – from 5,6% (untreated) to 15,0% (72h/30°C) and 14,2% (150 ppmGA<sub>3</sub>).

2. Maximum percentages of complete germinated seeds were obtained at ‘Tropical Bronze Scarlet’ (94,2%), treated with 450ppmGA<sub>3</sub> solution.

3. Among the five used cultivars, ‘Firebird’ presents the lowest germination, with very significant negative difference from control, in both experiments. The opposite is ‘Tropical Bronze Scarlet’ cultivar, very significant positive difference from control.

4. The shortest period of time necessary to complete the seed germination was observed at ‘Tropical Bronze Scarlet’ cultivar (14 days), on 300 ppmGA<sub>3</sub> treated seeds and the longest period of time was observed at ‘Firebird’ cultivar (29 days), in the same experiment (300 ppmGA<sub>3</sub>), but also under the influence of heat treatment (72h/30°C).



**Fig. 1** – *Canna indica* L. cultivars used in the experiments: a) ‘Tropical Rose’, b) ‘Sémaphore’, c) ‘Tropical Bronze Scarlet’, d) ‘Firebird’, e) ‘Tropical Yellow’

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# RESEARCH ON *CANNA INDICA* L. RHIZOMES SPROUTING, UNDER THE INFLUENCE OF STORAGE CONDITIONS AND CULTIVAR

## CERCETĂRI PRIVIND PORNIREA ÎN VEGETAȚIE A RIZOMILOR DE *CANNA INDICA* L., SUB INFLUENȚA CONDIȚIILOR DE PĂSTRARE ȘI A CULTIVARULUI

**POPESCU Liana**<sup>1</sup>

e-mail: popescu.liana@primariabistrita.ro

**Abstract.** *It was studied the influence of storage conditions of Canna indica L. rhizomes, on the start of their vegetation. Were selected two locations, characterized by different temperature and humidity conditions: a warehouse and a greenhouse. Four cultivars were used in the experiment: 'Sémaphore', 'Tropical White', 'Austria' and 'Firebird'. The best results regarding the number of highlighted shoots per rhizomes were obtained at 'Austria' and 'Firebird' cultivars, stored in the greenhouse.*

**Key words:** *Canna indica* L rhizomes, dormancy, storage conditions.

**Rezumat.** *A fost studiată influența condițiilor de depozitare pe perioada repausului, a rizomilor de Canna indica L., asupra pornirii în vegetație a acestora. Au fost alese două locații caracterizate prin condiții diferite de temperatură și umiditate: o magazie neîncălzită și o seră. În organizarea experienței s-au utilizat rizomi proveniți de la 4 cultivare: 'Sémaphore', 'Tropical White', 'Austria' și 'Firebird'. Cele mai bune rezultate privind numărul de lăstari formați pe rizom, au fost obținute la cultivarele 'Austria' și 'Firebird', depozitate în seră.*

**Cuvinte cheie:** *Canna indica* L., rizomi, repaus, condiții de depozitare.

### INTRODUCTION

Genus *Canna* is native to tropical regions, the West Indies and South America, where it grows spontaneously in the mountain areas and in the plains. As ornamental plants, *Canna* species have been used in temperate regions of Europe since XVI century, where they were introduced thru Spain (Sonea și colab., 1979). In the last three decades there have been many debates regarding infragenerică classification of Cannas, currently two opinions being outlined: one according to which *Canna* genus includes 19 species (Tanaka, 2001) and the other, that supports the existence of 10 species (Kamer and Maas, 2008), of which *Canna indica* L. has the highest degree of complexity. Cannas are often used in landscape design as focal points of a group if planted solitary, but the tropical look is revealed when they are planted in floral massive, in association with other herbaceous plants. Semi hardy geophytes, *Canna* plants are vegetative propagated by rhizomes dividing. They have elongated shape, relatively short internodes,

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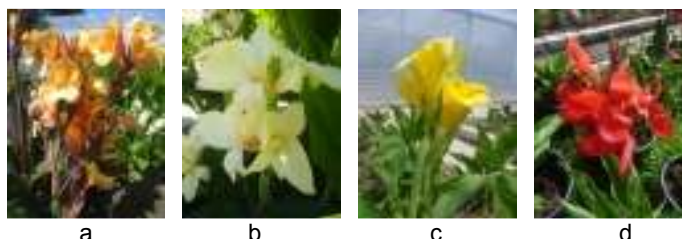
<sup>1</sup> Bistrița City Hall – Public Services Department, Romania

adventitious roots at nodes and buds in the axils of rudimentary leaves (squama). The rhizomes grow horizontally; they are sympodially branched and have terminal buds for annual shoot growth (Toma, 2005). Division is made after a storage period, in spaces providing the necessary conditions for rhizomes vegetative dormancy.

This paper aims to establish the influence of storage conditions of *Canna indica* L. rhizomes during dormancy period, on the start of their vegetation.

## MATERIAL AND METHODS

The present research was performed in the Public Services Department's production yard, of the Bistrița City Hall, in 2011. The biological material used in experiments, was represented by the rhizomes belonging to four cultivars of *Canna indica* L. (fig.1) and harvested from field (public green spaces of Bistrița city), in November 2010: 'Sémaphore' 'Tropical White', 'Austria' and 'Firebird'.



**Fig.1.** *Canna indica* L. cultivars used in the experiments: a) 'Sémaphore', b) 'Tropical White', c) 'Austria', d) 'Firebird'

Plants were entirely removed from the field at the beginning of November, after aerial parts having been cut to approx. 15 cm. The rhizomes were stratified in a mixture of compost and sawdust and they were stored in two locations (a warehouse and a greenhouse), with different temperature and humidity conditions. During the dormancy period (November 11 – January 25), environmental condition were monitored in both locations (table 1).

Table 1

**Values of environmental factors,  
registered in rhizomes storage locations (November-January)**

| Location   | Air temperature (°C) | Soil temperature (°C) | Rel. air humidity (Rh%) | Soil humidity (%) |
|------------|----------------------|-----------------------|-------------------------|-------------------|
| Warehouse  | 7,5                  | 6,7                   | 42,6                    | 7,8               |
| Greenhouse | 16,9                 | 14,7                  | 60,8                    | 12,2              |

After the storage period (75 days), warehouse rhizomes were brought into the greenhouse and those stored in the greenhouse were uncovered. The material was weight in order to determine how much turgidity the rhizomes have lost during storage and then it was planted in 22 cm diameter pots, into an exclusively compost substrate. The start of rhizomes vegetation was daily observed, watching the shoots emergence at the soil surface and their number, considering the two factors (fig.3).

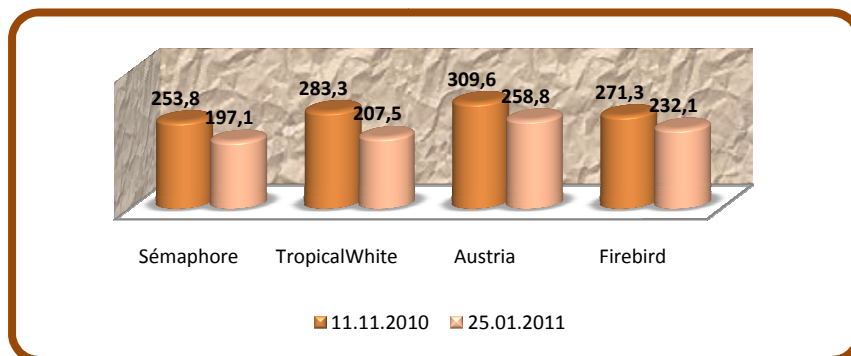
Statistical analysis of data was based on the variance calculation on a bifactorial experiment that allowed the significance of differences interpretation



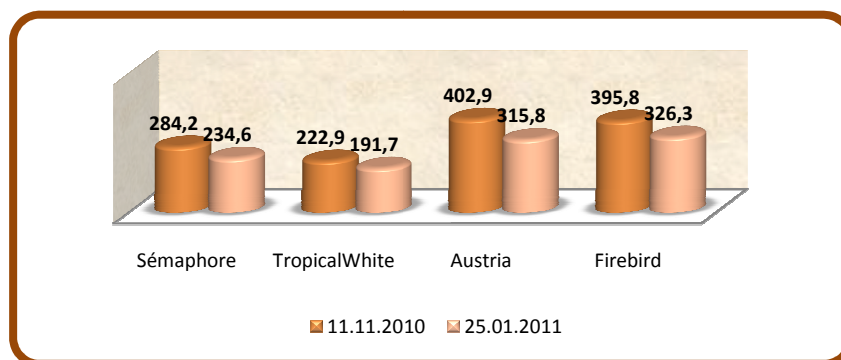
between experimental variants (Ardeleanu, 2008). Each variant was found in the experiments in three repetitions.

## RESULTS AND DISCUSSIONS

In order to organize the experiment were used 12 rhizomes from each cultivar, meaning 4 rhizomes on each of the three repetitions. Following storage, it was found that all rhizomes have lost some of their mass (fig. 2, fig. 3), depending of storage conditions and the cultivar.



**Fig. 2** - Rhizomes average mass (g), before and after storage in the warehouse



**Fig. 3** - Rhizomes average mass (g), before and after storage in the greenhouse

The highest loss (26,8%), occurred at ‘Tropical White’ cultivar stored in the warehouse, and the lowest, at the same cultivar (14,0%), with rhizomes stored in the greenhouse (tab. 2). In table 3 are presented the average results (absolute and percentage) regarding shoots emergence at the soil surface until May 17, when the plants were transferred to field. In order to comparing the

results, it was calculated the average of variants 1-4, where the rhizomes were maintained for dormancy in an unheated warehouse.

Table 2

Percentage mass loss during rhizomes storage

| Specification |            | Cultivar         |                       |                |                 |
|---------------|------------|------------------|-----------------------|----------------|-----------------|
|               |            | <i>Sémaphore</i> | <i>Tropical White</i> | <i>Austria</i> | <i>Firebird</i> |
| Mass loss (%) | warehouse  | 22,3             | 26,8                  | 16,4           | 14,4            |
|               | greenhouse | 17,5             | 14,0                  | 21,6           | 17,6            |

Table 3

Experimental results regarding *Canna indica* L. rhizomes sprouting, under the influence of storage conditions and cultivar

| Variant                |                                    | Shoots emergence at soil surface |            | Period (no. of days) | Initial number of buds on the rhizome | Shoots / rhizome formed until May 17 |      |
|------------------------|------------------------------------|----------------------------------|------------|----------------------|---------------------------------------|--------------------------------------|------|
| No.                    | Factor combination                 | first shoot                      | last shoot |                      |                                       | (no.)                                | (%)  |
| V1                     | Warehouse x <i>Sémaphore</i>       | Feb.18                           | May 10     | 81                   | 7,7                                   | 3,2                                  | 41,7 |
| V2                     | Warehouse x <i>Tropical White</i>  | Feb.27                           | May 17     | 79                   | 8,3                                   | 1,3                                  | 15,8 |
| V3                     | Warehouse x <i>Austria</i>         | Feb.03                           | May 17     | 103                  | 5,8                                   | 5,3                                  | 90,9 |
| V4                     | Warehouse x <i>Firebird</i>        | Feb.10                           | May 17     | 96                   | 6,3                                   | 4,2                                  | 66,3 |
| V5                     | Greenhouse x <i>Sémaphore</i>      | Feb.03                           | May 09     | 95                   | 8,5                                   | 5,2                                  | 61,2 |
| V6                     | Greenhouse x <i>Tropical White</i> | Feb.10                           | May 16     | 95                   | 7,8                                   | 5,4                                  | 69,7 |
| V7                     | Greenhouse x <i>Austria</i>        | Jan.28                           | May 17     | 109                  | 6,6                                   | 6,0                                  | 91,1 |
| V8                     | Greenhouse x <i>Firebird</i>       | Jan.28                           | May 17     | 109                  | 7,9                                   | 5,7                                  | 72,0 |
| Average V1-V4, Control |                                    |                                  |            | 89,8                 | 7,0                                   | 3,5                                  | 53,7 |

Analyzing the results, it is observed that the shoots were revealed in different number and percentage, depending on the storage conditions during dormancy and the cultivar. Thus, minimum values are registered at ‘Tropical White’ stored in the warehouse (15,8%), and the maximum at ‘Austria’ stored in the greenhouse (91,1%) – reporting at initial number of buds clearly defined on each rhizome, at the storage moment. Comparison to control value shows that two variants (V1 and V2) are placed under it. The period between the first and the last shoot appearance (until May 17), varies between 79 days (V2) and 109 days (V7 and V8). The results from table 4 show that a single variant (V2), records values under the control, difference being statistically assured at distinctly negative significant. Variant V7 shows a very positive significant

difference, followed by variants V5, V6 and V8, with distinctly significant positive difference from control.

Table 4

**Summary of results on *Canna indica* L.  
rhizomes sprouting, under the influence of storage conditions and cultivar**

| Variant |                        | Visible shoots at soil surface |              | ± d  | Significance of difference |
|---------|------------------------|--------------------------------|--------------|------|----------------------------|
| No.     | Factor combination     | Absolute (nr.)                 | Relative (%) |      |                            |
| V1      | Warehouse x Sémaphore  | 3,2                            | 91,4         | -0,3 | –                          |
| V2      | Warehouse x Tr. White  | 1,3                            | 37,1         | -2,2 | 00(0)                      |
| V3      | Warehouse x Austria    | 5,3                            | 151,4        | 1,8  | **                         |
| V4      | Warehouse x Firebird   | 4,2                            | 120,0        | 0,7  | –                          |
| V5      | Greenhouse x Sémaphore | 5,2                            | 148,6        | 1,7  | **                         |
| V6      | Greenhouse x Tr. White | 5,4                            | 154,3        | 1,9  | **                         |
| V7      | Greenhouse x Austria   | 6,0                            | 171,4        | 2,5  | ***                        |
| V8      | Greenhouse x Firebird  | 5,7                            | 162,9        | 2,2  | **(*)                      |
|         | Average V1-V4, Control | 3,5                            | 100,0        | -    | -                          |

DL 5% = 0,86 pieces      DL 1% = 1,62 pieces      DL 0,1% = 2,26 pieces

Table 5 data shows that the rhizomes sprouting, meaning the number of shoots formed from the existing number of buds on the rhizomes at the storage moment, was distinctly significant influenced by the storage conditions during dormancy.

Table 5

**Summary of results on *Canna indica* L.  
rhizomes sprouting, under the influence of storage conditions**

| Factor A graduations (storage location) | Visible shoots at soil surface |                  | ± d | Significance of difference |
|---|--------------------------------|------------------|-----|----------------------------|
|   | Average no. of shoots /var.    | (%) from control |     |                            |
| a1 = warehouse, Control                 | 3,5                            | 100,0            | -   | -                          |
| a2 = greenhouse                         | 5,6                            | 160,0            | 2,1 | **(*)                      |

DL 5% = 0,78 pieces      DL 1% = 1,47 pieces      DL 0,1% = 2,12 pieces

Table 6

**Summary of results on *Canna indica* L.  
rhizomes sprouting, under the influence of cultivar**

| Factor B graduations (cultivar) | Visible shoots at soil surface |                  | ± d  | Significance of difference |
|---------------------------------|--------------------------------|------------------|------|----------------------------|
|                                 | Average no. of shoots /var.    | (%) from control |      |                            |
| b1 = Sémaphore                  | 4,2                            | 120,0            | 0,7  | –                          |
| b2 = Tropical White             | 3,4                            | 97,1             | -0,1 | –                          |
| b1 = Austria                    | 5,7                            | 162,9            | 2,2  | ***                        |
| b1 = Firebird                   | 5,9                            | 168,6            | 2,4  | ***                        |
| Average V1-4, Control           | 3,5                            | 100,0            | 0    | -                          |

DL 5% = 0,82 pieces      DL 1% = 1,63 pieces      DL 0,1% = 2,08 pieces

Depending on cultivar (tab. 6), differences from control are statistically assured at very significant positive at ‘Austria’ and ‘Firebird’ cultivars. The values of ‘Sémaphore’ and ‘Tropical White’ cultivars are close to control, differences being insignificant. From the date of rhizomes planting in pots and start watering them (meaning actual removal from dormancy) to the date of first shoot appearance at the soil surface, there was a different period, influenced by the storage conditions and the cultivar (table 7).

The greenhouse stored rhizomes started their vegetation earlier (7,8 days) than those stored in the warehouse (20,5 days). The shortest time was registered at rhizomes stored in the greenhouse – ‘Austria’ and ‘Firebird’ cultivars (3 days), and the longest time was registered at ‘Tropical White’ cultivar (33 days), with rhizomes stored in the warehouse.

Table 7

**Period (days) between rhizomes planting time and shoots emergence, depending on storage conditions and cultivar**

| Specification  |            | Cultivar         |                       |                |                 |      | Average |
|--|------------|------------------|-----------------------|----------------|-----------------|------|---------|
|  |            | <i>Sémaphore</i> | <i>Tropical White</i> | <i>Austria</i> | <i>Firebird</i> |      |         |
| No. of days from rhizomes planting to shoots emergence | warehouse  | 24               | 33                    | 9              | 16              | 20,5 |         |
|  | greenhouse | 9                | 16                    | 3              | 3               | 7,8  |         |

## CONCLUSIONS

1. During storage time in the dormancy period, the largest mass losses were registered at ‘Tropical White’ cultivar (26,8%), stored in the warehouse.

2. The best results regarding the number of shoots formed on the rhizome were obtained at ‘Austria’ and ‘Firebird’ cultivars, stored in the greenhouse, showing very significant positive difference from control.

3. The rhizomes stored in the greenhouse started their vegetation 12 days earlier than the rhizomes stored in the warehouse.

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# RESEARCH REGARDING THE INFLUENCE OF THE DRAINAGE AND THE FERTILIZATION ON *SEMPERVIVUM* PLANTS CULTIVATED IN POTS

## CERCETĂRI PRIVIND INFLUENȚA DRENAJULUI ȘI A FERTILIZĂRILOR ASUPRA PLANTELOR DE *SEMPERVIVUM* CULTIVATE ÎN GHIVECE

ZAHARIA A.<sup>1</sup>, BALCĂU Simina<sup>1</sup>, BUTA Erszebet<sup>1</sup>, ZAHARIA D.<sup>1</sup>  
e-mail: adrian.zaharia@usamvcluj.ro

**Abstract.** *The research has been focused on the behaviour of Sempervivum montanum plants, grown in pots, to which were applied three graduations of fertilization and drainage. The purpose of the research was to determine the need for an asset drainage that ensures that the excess water is eliminated. On the other hand it was aimed at establishing the effect of fertilizer application to ensure normal growth and development of crops in a limited substrate volume and nutrient reserves. The results established that the variants which the drainage were unsatisfactory, water accumulated in excessive quantities, so the plants had suffered and eventually died, but the plants couldn't be saved even if they were taken from the harmful culture medium. The second factor applied proved to be beneficial, but the effect did not lead to significant differences. These results lead to the conclusion that Sempervivum plants are not demanding of the physico-chemical characteristics of the soil if drainage is performed properly.*

**Keywords:** sempervivum, excess water, drainage, fertilization

**Rezumat.** *Cercetările au vizat comportarea plantelor de Sempervivum montanum cultivate în ghivece la care s-au aplicat trei graduări de fertilizare și de drenaj. Scopul cercetărilor a fost de a stabili necesitatea realizării unui drenaj activ, care să asigure eliminarea excesului de apă. Pe de altă parte s-a urmărit stabilirea efectului aplicării unor fertilizanți care să asigure o creștere și o dezvoltare normală a plantelor cultivate într-un substrat limitat ca volum și rezerve ale elementelor nutritive. Rezultatele au stabilit că variantele la care drenajul a fost nesatisfăcător, apa s-a acumulat în cantități devenite în exces, iar plantele au suferit și în final au pierit, fără a putea fi salvate nici dacă au fost schimbate din mediul de cultură nociv. Al doilea factor aplicat s-a dovedit a fi benefic, dar efectul nu a condus la realizarea de diferențe semnificative. Aceste rezultate conduc la concluzia că plantele de Sempervivum nu sunt pretențioase la însușirile fizico-chimice ale solului, dacă este realizat drenajul corespunzător.*

**Cuvinte cheie:** Sempervivum, exces umiditate, fertilizare

### INTRODUCTION

In many parts of the world especially in western europe *Sempervivum*

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

plants are known and appreciated for their herbal qualities (Abram et al., 1995, Blozovics et al., 1994), but especially as ornamental plants (Coste, 1937, Murfitt, 1998), due to their varied but pleasant aspect as well as their high resistance to environmental factors, which facilitates the use of these plants in different places, many unsuitable for other plants (Zaharia, 2010).

The studies concerning these plants target especially their therapeutical qualities and on a much smaller scale the technological factors used in their cultivation. Considering these facts it has been considered a good opportunity to study and establish the effect of some technological factors on *Sempervivum* plants (Zaharia, 2007, Zaharia et al., 2010, Zaharia, 2010). In this study water drainage and fertilization were taken into consideration.

## MATERIAL AND METHODS

The biological material used in the experience consisted in plants of *Sempervivum montanum*. The plants had the same size and age.

The culture substrate consisted of soil low in nutrients, with a sandy texture. Depending on the experimental variant the soil was enriched with Multicare, a complex fertilizer that contains macroelements as well as microelements (S, B, Fe, Mn, Zn etc.).

Table 1

**Data concerning the characteristics of the experimental variants and the plant rosettes used in organizing the *Sempervivum montanum* experience**

| Variants |  | Quantity of fertilizer kg/m <sup>3</sup> | Rosettes |               |      |         |
|----------|--|--|----------|---------------|------|---------|
| Nr.      | Content  |  | Nr.      | Diametre (cm) |      |         |
|          |  |  |          | Min.          | Max. | Average |
| 1.       | Drainage with fine sand + unfertilized soil ( <b>control</b> ) | -  | 35       | 2,5           | 4,2  | 3,6     |
| 2.       | Drainage with fine sand + fertilized soil 1/2 dose             | 3,0                                      | 35       | 2,8           | 4,4  | 3,9     |
| 3.       | Drainage with fine sand + fertilized soil 1 dose               | 6,0                                      | 35       | 3,0           | 4,2  | 3,8     |
| 4.       | Without drainage + unfertilized soil                           | -  | 35       | 2,9           | 4,6  | 3,6     |
| 5.       | Without drainage + fertilized soil 1/2 dose                    | 3,0                                      | 35       | 2,7           | 4,4  | 3,5     |
| 6.       | Without drainage + fertilized soil 1 dose                      | 6,0                                      | 35       | 3,1           | 4,5  | 3,9     |
| 7.       | Gravel drainage + unfertilized soil                            | -  | 35       | 2,9           | 4,2  | 4,0     |
| 8.       | Gravel drainage + fertilized soil 1/2 dose                     | 3,0                                      | 35       | 3,0           | 4,1  | 3,8     |
| 9.       | Gravel drainage + fertilized soil 1 dose                       | 6,0                                      | 35       | 3,5           | 4,1  | 3,6     |

The pots used for plant cultivation were in a shape of a truncated cone. Their dimensions are 5 cm top diametre and 3 cm lower diametre, with a hight of 5 cm and

a volume of 64 cm<sup>3</sup>. The organized experience had two factors. In the experience the effect on plant behavior under the influence of fertilizer and drainage quality was studied.

After the organization of the experience conditions of excess water were created to highlight the effect of drainage. This was done by a daily application of 10 ml of water to each plant.

The experience lasted 21 days. After this period several observations were made concerning plant viability and size.

## RESULTS AND DISCUSSIONS

Throughout the research it was determined that some plants modified their appearance by becoming yellowish in color, and eventually lose their plant structure and become motherly. This phenomenon appeared differently for each variant. The determinations concerning the number of plants that were not affected by excess water, and maintained their viability are presented in table 2.

Table 2

**Experimental results concerning *Sempervivum montanum* plant rosettes under the influence of drainage and basic fertilization after 21 days**

| Variants |  | Viable rosettes at the end of the experience |      |                  | ± d No. | Significance of difference |
|----------|--|--|------|------------------|---------|----------------------------|
| Nr.      | Content  | Nr.  | %    | % to the control |         |                            |
| 1.       | Drainage with fine sand + unfertilized soil ( <b>control</b> ) | 27,2   | 77,7 | 100,0            | -       | -                          |
| 2.       | Drainage with fine sand + fertilized soil 1/2 dose             | 30,1   | 86,0 | 110,6            | 2,9     | -                          |
| 3.       | Drainage with fine sand + fertilized soil 1 dose               | 29,4   | 84,0 | 108,0            | 2,2     | -                          |
| 4.       | Without drainage + unfertilized soil                           | 3,1  | 8,8  | 11,3             | -24,1   | 000                        |
| 5.       | Without drainage + fertilized soil 1/2 dose                    | 2,3  | 6,5  | 8,4              | -24,9   | 000                        |
| 6.       | Without drainage + fertilized soil 1 dose                      | 4,6  | 13,1 | 16,9             | -22,6   | 000                        |
| 7.       | Gravel drainage + unfertilized soil                            | 32,4   | 92,5 | 119,1            | 5,2     | *                          |
| 8.       | Gravel drainage + fertilized soil 1/2 dose                     | 33,5   | 95,7 | 123,1            | 6,3     | **                         |
| 9.       | Gravel drainage + fertilized soil 1 dose                       | 32,7   | 93,4 | 120,2            | 5,5     | *                          |

LSD<sub>5%</sub> = 4,17

LSD<sub>1%</sub> = 5,76

LSD<sub>0,1%</sub> = 6,52

Analyzing the results in table 2 it can be observed that plants that were compromised in a large percent (30-32 plants) came from the variants with no

drainage ( $V_4$ ,  $V_5$ ,  $V_6$ ) regardless of substrate fertilization. The results of these variants compared to the control ( $V_1$ ) register negative differences between 22,6 and 24,9, values that are statistically ensured as very significant. Comparing the results of  $V_7$  and  $V_9$ , both with a gravel drainage, with the control it can be observed that the differences are positive, statistically ensured as significant ( $V_7$  and  $V_9$ ) and distinctly significant ( $V_8$ ).

The results presented in table 2 were obtained by statistic processing typical for the study of a combined influence of the 2 factors.

The results of the statistic process of the unilateral influence of the factors are presented in the following.

Table 3

**The synthesis of experimental results concerning the viability of *Sempervivum montanum* plant rosettes grown in pots under the influence of drainage**

| Variants |                              | Viable rosettes at the end of the experience |       | ±d No. | Significance of difference |
|----------|------------------------------|--|-------|--------|----------------------------|
| Nr.      | Content                      | Nr.  | %     |        |                            |
| 1.       | Fine sand drainage (control) | 28,9   | 100,0 | -      | -                          |
| 2.       | Without drainage             | 3,3  | 1,1   | - 25,6 | 000                        |
| 3.       | Gravel drainage              | 32,9   | 113,8 | 4,0    | *                          |

LSD<sub>5%</sub> = 3,8

LSD<sub>1%</sub> = 4,9

LSD<sub>0,1%</sub> = 6,2

The experimental results of the unilateral effect of drainage are presented in table 3. The results have very different values, especially the variant without drainage, which registered heavy plant loss compared to the variants with drainage.

The difference between the control and the variant without drainage is 25,6, statistically ensured as significantly positive. The difference between the variant with gravel drainage and the one with fine sand drainage is of 4 plants, a value statistically ensured as significantly positive.

Based on these results the conclusion that can be drawn is that the lack of drainage lead to an exces of water, to which the *Sempervivum montanum* plants showed a high sensibility, a fact that lead to the death of the plants in 5-7 days. The statistical analysis of the results of the unilateral influence of fertilization on *Sempervivum montanum* plants resistance to moisture excess lead to the conclusion that the supply of nutrients, regardless the administered amount, does not influence significantly the plant behavior concerning plant resistance to moisture excess.

The viable rosettes were biometrically measured to establish the possible changes. The diametre and hight of rosettes were measured. The results confirmed that in the experimental period changes occured concerning rosette



size, changes that were different for each variant. Analyzing the 2 variables (height and diameter) it was considered that they were insufficiently relevant for the determination of plant growth and so the measurement was continued by establishing the mass of the plants. The obtained results are presented in table 4.

Table 4

**Experimental results concerning rosette size of *Sempervivum montanum* plants grown in pots under the combined influence of drainage and fertilization**

| Variants |  | Rosettes size |       | ±d<br>g | Significance<br>of difference |
|----------|--|---------------|-------|---------|-------------------------------|
| Nr.      | Content  | g             | %     |         |                               |
| 1.       | Drainage with fine sand + unfertilized soil ( <b>control</b> ) | 10,3          | 100,0 | -       | -                             |
| 2.       | Drainage with fine sand +fertilized soil 1/2 dose              | 12,5          | 121,3 | 2,2     | *                             |
| 3.       | Drainage with fine sand + fertilized soil 1 dose               | 19,8          | 192,2 | 9,5     | ***                           |
| 4.       | Without drainage + unfertilized soil                           | 7,4           | 71,8  | -2,9    | 0                             |
| 5.       | Without drainage + fertilized soil 1/2 dose                    | 9,5           | 92,2  | -0,8    | -                             |
| 6.       | Without drainage + fertilized soil 1 dose                      | 12,1          | 117,4 | 1,8     | -                             |
| 7.       | Gravel drainage + unfertilized soil                            | 11,2          | 108,7 | 0,9     | -                             |
| 8.       | Gravel drainage + fertilized soil 1/2 dose                     | 14,3          | 138,8 | 4,0     | **                            |
| 9.       | Gravel drainage + fertilized soil 1 dose                       | 20,1          | 195,1 | 9,8     | ***                           |

LSD<sub>5%</sub> = 2,17  
LSD<sub>1%</sub> = 3,86  
LSD<sub>0,1%</sub> = 5,28

To establish the plant mass the rosettes were cut at ground level and weighed.

Analyzing the results from the table and comparing them with the control it can be observed that the variants without drainage, unfertilized or fertilized with half a dose have a lower mass. The other variants, with drainage and fertilized, show an increase in mass, the differences compared to the control being positive and statistically ensured as significant ( $V_2$ ), distinctly significant ( $V_8$ ) and very significant ( $V_3$  and  $V_9$ ).

Based on these results the conclusion that can be drawn is that the plants of *Sempervivum montanum* are not indifferent to the culture conditions especially to the nutrient supply of the soil.

## CONCLUSIONS

The undertaken research established that:

1. *Sempervivum* plants show a high sensitivity to water excess (the soil is

soaked in water, wet to marshy).

2. In conditions of water excess the plants suffer from biodegradation that affects all the leaves from the rosette and the plants eventually die.

3. The duration of biodegradation and eventually death is of 5 to 7 days from the infliction of water excess.

4. The application of fertilizers on a soil that lacks nutrients influences the evolution of *Sempervivum* plants in a positive way. The quantity of fertilizer applied to the substrate is of 3 to 6 kg for 1 m<sup>3</sup> of culture substrate.

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# INFLUENCE OF THE RIZOGENE SUBSTANCES ON ROOTING AND ON BIOCHEMICAL COMPOSITION OF *PELARGONIUM PELTATUM* PLANTS

## INFLUENȚA UNOR SUBSTANȚE RIZOGENE ASUPRA ÎNRĂDĂCINĂRII ȘI ASUPRA COMPOZIȚIEI BIOCHIMICE LA PLANTELE DE *PELARGONIUM PELTATUM*

VÂȘCĂ ZAMFIR Diana<sup>1</sup>, BĂLAN Daniela<sup>1</sup>, LUȚĂ Gabriela<sup>1</sup>,  
GHERGHINA Evelina<sup>1</sup>, DRĂGHICI Elena<sup>1</sup>  
e-mail: dianazamfir2000@yahoo.com

**Abstract.** *The present researches were focused on the effect of stimulating products on the rooting process of the cuttings on various specific substrates and on post rooting morphological development in Pelargonium peltatum plants. Also, some biochemical parameters were analyzed to show if the substances used to stimulate rooting influence the biochemical composition of leaves formed on shoots. Rooting percentage values and morphological indicators were minimal in untreated cuttings (control plants). The maximum percentage of rooting cuttings and the highest number of leaves on the shoot was determined in stimulated variants. The stimulation of the roots growth determined an increased accumulation of dry matter in the leaves, so that also the content in proteins and lipids registered high values in the analyzed leaves.*

**Keywords:** rooting stimulators, *Pelargonium*, rooting substrate, biochemical composition

**Rezumat.** *Prin prezentele studii s-a urmărit identificarea comparativă a efectului unor produse stimulative (rizogene) asupra procesului de înrădăcinare pe diferite substraturi specifice și evoluția postînădăcinare la butașii de Pelargonium peltatum. De asemenea, au fost analizați unii parametri biochimici pentru a evidenția dacă stimulatorii de înrădăcinare influențează compoziția chimică a frunzelor nou formate pe lăstari. Valorile procentului de înrădăcinare și ale indicatorilor morfologici determinați au fost minime în cazul butașilor netratați (plantele martor). Cel mai mare procent de înrădăcinare și cel mai mare număr de frunze pe lăstar a fost determinat în cazul variantelor tratate cu stimulatori. Stimularea creșterii rădăcinilor a determinat o creștere a acumulării de substanță uscată în frunzele formate, deci și a conținutului în proteina brută și în lipide totale.*

**Cuvinte cheie:** stimulatori de înrădăcinare, substrat de înrădăcinare, *Pelargonium*, compoziție biochimică

## INTRODUCTION

Plant propagation is through flower, seeds and vegetative. Currently known and used many types of vegetative propagation, but the most common is the

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Bucharest, Romania

cuttings method, based on the ability of vegetative organs (or parts of organs) for delivering roots and form a new individual.

Ability propagation by cuttings depends on the species, stock type, and environmental factors (Bhekithemba, 2010). To improve the training process there are used some different rooting stimulators that enhances and speed the rootedness process. By using these substances to enhance the penetration of water and treated portions become centers of attraction of nutrients. Products on the market are different forms: powder, gel, solution.

Genus *Pelargonium* includes over 170 species, originating in South Africa. One of the most decorative species is *Pelargonium peltatum*, known as „the flowing geranium”, much appreciated thanks to the spectacular flowering in many colors. Very popular, it provides the setting balconies, terraces, windows and gardens throughout the summer (Amăriuței and Zamfir-Vâșcă, 2010).

The present researches were focused on the effect of stimulating products on the rooting process of the cuttings on various specific substrates and post rooting morphological development of the new plants.

Also, some biochemical parameters were analyzed to show if the substances used to stimulate rooting influence the biochemical composition of leaves formed on shoots. Thus, were determined: dry matter content accumulated in the leaves, crude protein content and total lipid and assimilatory pigments content in leaves (chlorophylls and carotenes).

## MATERIALS AND METHODS

In order to estimate the effect of stimulating products on the rooting process and after rooting of *Pelargonium peltatum* were studied plants from the cuttings cultivated on different specific substrates in the greenhouses USAMV Bucharest, during February-March 2011.

As rooting stimulators, which constituted the experimental variants, were used: V<sub>1</sub>-Clonex (gel, powerful formula of hormones, vitamins and mineral nutrients); V<sub>2</sub>-Coralite (powder containing vitamin A, B complex, vitamins C, D, E, amino acids, trace elements); V<sub>3</sub>-Radistim (powder, rooting stimulant). The control variant (Vm) cuttings were not treated.

The biological material consisted of the shoot tip cuttings with standard sizes of 6-7 cm long, harvested from mother plants of *Pelargonium peltatum*.

After trimming, the cuttings were treated with listed rizogene substances. Thus prepared cuttings were distributed on two types of classical rooting substrates: peat (100%) and peat mixed with perlite (50% +50%). For each experimental variant were selected 10 rooting cuttings by for each type of substrate.

Throughout the rooting cuttings were maintained in greenhouse multiplier, where they were provided the same conditions in terms of environmental factors (temperature, humidity, light).

After rooting were made observations and measurements on rooted cuttings on: the percentage of rooting, main root length, main shoot length, number of leaves on the vine.

Also, measurements of the biochemical parameters were made in the *Pelargonium* leaves using proper biochemical methods (Iordachescu, 1988):

- The investigations of *chlorophyll and carotenoid pigments* were performed spectrophotometrically, after extraction in 80% acetone, at 663 nm, 646 nm and 470 nm wavelength. The results were calculated with Mackiney formula and were expressed in mg/100 g fresh weight.
  - *Dry mass content* was gravimetrically determined with a thermoscales.
  - Determination of the content in *crude protein* was made after the digestion of the vegetal material by Kjeldahl method. The content in total nitrogen was measured by volumetrical method and converted in crude protein content.
  - Content in *total lipid* was determined using the Soxhlet method: total fat was extracted in petroleum ether; then, the solvent was evaporated at 35°C using a rotary evaporator; finally, the lipid content of samples was determined gravimetrically.
- All measurements were related to the dry matter content.

## RESULTS AND DISCUSSION

### 1. Study of rooting process and of morphological development post rooting

The rooting percentage for the control variant (V<sub>m</sub>), untreated cuttings, was between 50% on peat and 66,67% on peat+ perlite, which are the lowest amounts (tab. 1).

Table 1

Summary of the experimental results

| Experimental variants | Rooting substrates     |                       |                        |                               |                        |                       |                        |                               |
|-----------------------|------------------------|-----------------------|------------------------|-------------------------------|------------------------|-----------------------|------------------------|-------------------------------|
|                       | Peat+ perlite          |                       |                        |                               | Peat                   |                       |                        |                               |
|                       | Rooting percentage (%) | main root length (cm) | main shoot length (cm) | number of leaves on the shoot | Rooting percentage (%) | main root length (cm) | main shoot length (cm) | number of leaves on the shoot |
| V <sub>m</sub>        | 66,67                  | 12,33                 | 8,40                   | 3,70                          | 50,00                  | 6,50                  | 9,40                   | 4,16                          |
| V <sub>1</sub>        | 100,0                  | 18,40                 | 9,66                   | 8,00                          | 100,00                 | 7,50                  | 11,60                  | 6,40                          |
| V <sub>2</sub>        | 100,0                  | 14,50                 | 8,66                   | 5,30                          | 83,33                  | 7,00                  | 12,50                  | 5,20                          |
| V <sub>3</sub>        | 100,0                  | 20,20                 | 13,0                   | 6,20                          | 83,33                  | 19,50                 | 13,00                  | 6,25                          |

The best results (100%) were registered in V<sub>1</sub> (Clonex), on both cultivation substrates. In V<sub>2</sub> (Coralite) and V<sub>3</sub> (Radistim) the results were identical, 100% in peat+perlite substrate and 83,33% in peat substrate. Regarding rooting substrate, the best results were obtained for peat+perlite.

The main shoot length of control plants also had the lowest (8,4 cm) on peat+ perlite and 9,4 cm on peat. Cuttings treated with Radistim had the highest length of shoots (13 cm) the same for both substrates. The Clonex variant registered values ranged from 9,66 cm (peat + perlite) to 11,6 cm (peat). Values of 8,66 cm (on peat+perlite) and 12,5 cm (on peat) were recorded at Radistim

variant. Lengths of shoots were higher for cuttings rooted in peat than those rooted in peat+ perlite.

Number of leaves per shoot for the control variant was 3,7 in peat+perlite and 4,16 in cuttings rooted on peat. The highest number of leaves per shoot was observed in Clonex variant, both on peat+perlite (8) and on peat (6,4). The values reached by the Coralite and Radistim variants were similar for both rooting substrates.

## 2. Study of biochemical parameters in the *Pelargonium* leaves in all experimental variants

The leaves content in chlorophyll is maximal in growth period of the plants because its role played in the process of photosynthesis.

The researches performed indicated that the content in chlorophyll in the leaves was not influenced by the rooting substrate, nor by the used stimulators. So, the obtained results (fig. 1) showed that content in chlorophyll *a* registered similar values at the control plants (70,02 mg/100 g chlorophyll *a* on perlite+peat as rooting substrate and 68,17 mg/100 g chlorophyll *a* on peat) comparing to the plants which received stimulatory treatments with Clonex and Coralite (between 65,07–71,05 mg/100 g chlorophyll *a*).

Generally, the values of the assimilatory pigments content determined in the leaves of stimulated plants were comparable in all the variants except for the plants treated with Radistim, which registered lower values of the content in chlorophyll *a* (55,88 and 54,51 mg/100 g) on both rooting substrates

In all the studied variants the amount of chlorophyll *a* was higher than chlorophyll *b* in the leaves tissues, the ratio chlorophyll *a*/chlorophyll *b* varied between 2,5–3,4 at the plants cultivated on peat+perlite and between 2,6–3,7 at the plants cultivated on peat.

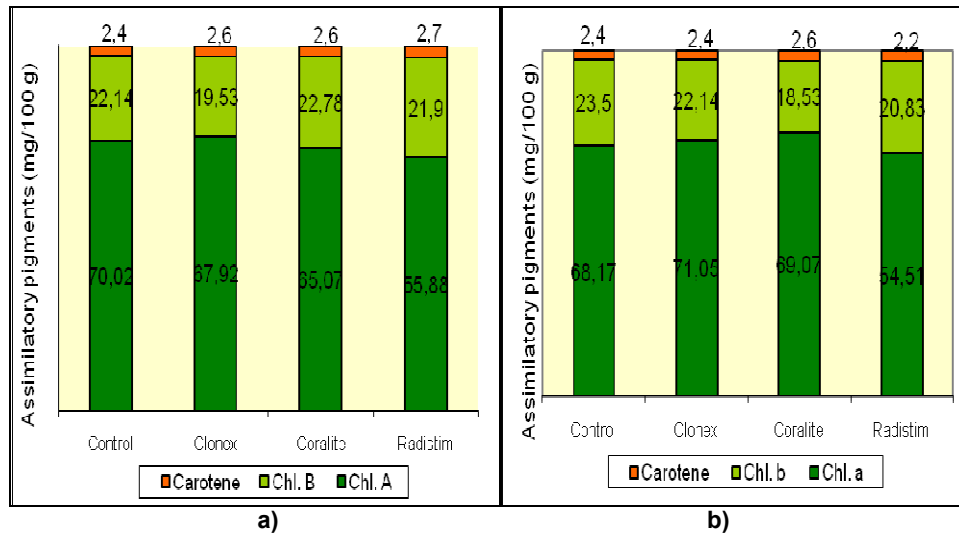


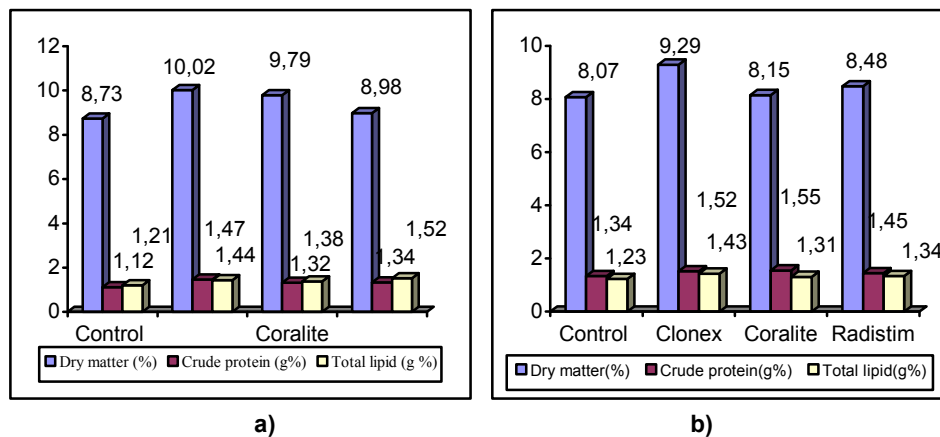
Fig. 1 - Content in assimilatory pigments at the variants cultivated on peat +perlite (a) and on peat (b)

Also the synthesis of the carotenes was not influenced by the stimulatory treatments of the roots. The differences between values of carotenes registered at the plants treated with roots stimulators and at the control plants were not significant: in average 2,4 mg/100 g carotenes registered at the control plants compare with 2,5 mg/100 g carotenes in the stimulated plants.

According to the literature, the plants use water and mineral compounds absorbed by the roots from the soil in order to biosynthesize its own organic compounds (Burzo, 2005). Thus, the amount of biochemical compounds in leaves may be influenced by the developing stage of the plant roots (Denny, 2001).

The results of the researches indicated that the stimulation of the roots growth determined an increased accumulation of dry matter in the leaves, so that also the content in proteins and lipids registered high values in the analyzed leaves (fig. 2).

Although the differences between the stimulated variants were not significant, the plants treated with Clonex registered the highest values of the analyzed parameters. At the plants cultivated on peat+perlite 1,47 g% crude proteins and 1,44 g% total lipids were determined in the Clonex variant compare with 1,32 g% crude protein and 1,38 g% total lipids in the Coralite variant. Also at the variants that used peat as rooting substrate were registered better results at the Clonex variant: 1,52 g% crude protein and 1,43 g% total lipids.



**Fig. 2 -** Content in biochemical parameters at the variants cultivated on peat+perlite (a) and on peat(b)

The accumulation of biochemical compounds was also influenced by the rooting substrate, so that the plants cultivated on peat+perlite registered a higher content in dry matter in all analyzed variants. In the leaves of control plants was determined 8,73% dry matter at the variant cultivated on peat+perlite compare with 8,07 % dry matter at the variant cultivated on peat. The plants treated with stimulators reached 9,59% dry matter in average on peat+perlite and 8,64% dry matter in average on peat.

## CONCLUSIONS

The results obtained in the researches performed indicated that the treatment with rooting stimulators of the *Pelargonium peltatum* plants influenced the rooting process and the morphological and biochemical features of the plants in post rooting period:

- Rooting percentage values and morphological indicators were minimal in untreated cuttings (control plants); the maximum percentage of rooting cuttings and the highest number of leaves on the shoot was determined in Clonex variants.
- The main root length was highest in Radistim variants.
- Treatment with Clonex influenced the growth of shoots: the highest values were noticed on peat+perlite as rooting substrate; on peat the treatment with Coralite determined the best results.
- Treatment of the plants with root stimulators did not influenced the content in assimilatory pigments of the leaves.
- The stimulation of the roots growth determined an increased accumulation of dry matter in the leaves, so that also the content in proteins and lipids registered high values in the analyzed leaves. However, the differences between the stimulated variants were not significant.
- The accumulation of biochemical compounds was also influenced by the rooting substrate: the plants cultivated on peat+perlite registered a higher content in dry matter in all analyzed variants.

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# PRELIMINARY STUDIES ON THE INFLUENCE OF THE SOIL MAINTENANCE SYSTEM ON THE BEHAVIOUR OF GROS SAUVIGNON VARIETY IN VINEYARD ODOBESTI

## CERCETĂRI PRELIMINARE PRIVIND INFLUENȚA SISTEMULUI DE ÎNTREȚINERE A SOLULUI ASUPRA COMPORTĂRII SOIULUI GROS SAUVIGNON ÎN CONDIȚIILE PODGORIEI ODOBESȚI

*BOSOI Marioara*<sup>1</sup>, *MIHU G.*<sup>1</sup>, *BOSOI Ionica*<sup>1</sup>, *STOLIAN Ileana*<sup>1</sup>  
e-mail: mioara\_bosoi@yahoo.com

**Abstract.** *This paper aims to address a comparative study concerning the response of the variety Gros Sauvignon on some agro biological and productive characteristics, by application of the various soil maintenance systems in the conditions of vineyard Odobesti. To ensure an optimal balance between growth and production in the conditions to achieve optimal quality, in the period 2009 - 2011, at SCDVV Odobesti has experienced the influence of the soil maintenance system on characteristics and productive agro biological of Gros Sauvignon variety.*

**Key words:** grassing, fertility coefficients, soil maintenance system

**Rezumat.** *Lucrarea de față își propune să abordeze un studiu comparativ privind reacția de răspuns a soiului Gros Sauvignon ca și caracteristici agrobiologice și productive, la aplicarea diferitelor sisteme de întreținere a solului, în condițiile podgoriei Odobești. În vederea asigurării unui echilibru optim între creștere și producție în condițiile realizării unei calități optime, în perioada 2009 – 2011, la S.C.D.V.V. Odobești s-a experimentat influența sistemului de întreținere a solului asupra caracteristicilor agrobiologice și productive ale soiului.*

**Cuvinte cheie:** înierbare, coeficienti de fertilitate, sistem de intretinere a solului

### INTRODUCTION

Gros Sauvignon is one the oenological valuable varieties, part of the basic assortment of quality white wines (DOC). The comparative with Petit Sauvignon variety show greater vigor, lower sugar concentration and higher grape production (Oslobeanu et al., 1980, Constantinescu et al., 1960). Under cultivation on soils with high fertility combined with ordering a medium or high vigor rootstock, the variety show force of increasing in detrimental productivity and high production quality. Optimizing quality wine production is a major goal of viticulture millennium, by those not incidentally stated today that "*great wines are made in the vineyard plantation*" (Fergoni, 2003), or "concept of quality starts in the vineyard" (Schlamp, 2001).

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<sup>1</sup> Research and Development Station for Viticulture and Vinifications Odobesti, Romania

## MATERIAL AND METHOD

The research works has been conducted in the experimental polygon agrotechnical of SCDVV Odobesti. Biological material is represented by Gros Sauvignon variety grafted on Berlandieri x Riparia - Sel. Oppenheim 4.

The plantation was established in 2004, with the following characteristics:

- planting distance of 2,0 / 0,9 m (density of 5555 vines / ha); the training on half steam; pruning system – mixed; type of pruning – Cazenave Cordon;
- fruit loading - 15.5 eyes/sq.m (28 eyes per vine), spread over four units consisting of one short cane of 5 eyes and one spur of 2 eyes;

The soil is a medium leached chernozem with favorable fertility for vine culture, the humus content in middle (2.0 to 2.3%), and a good supply of nutrients. The slope is 1-2%, with exhibition southeast.

Were studied three soil maintenance system: field black, permanently grassing with *Lolium perenne* and permanently grassing with *Medicago sativa*. The three experimental variants were located in the field by the method of subdivided parcels. Each experimental variant was three repetitions, each set comprising a total of 21 plants of the vine.

Were made observations, measurements and determinations of the elements of fertility (fertile shoots, the coefficients of fertility) and the quantity and quality of grapes. The measurements on grape production was done by weighing individual of grapes per vine and the determinations of the grape production quality, that the sugar content and acidity were performed at full maturity of the grapes.

## RESULTS AND DISCUSSION

The climatic conditions during the study are presented in tables 1 and 2.

Table 1

Climatic conditions during the study

| Year<br>Month             | Average temperature (°C ) |               |               |               | Sunlight (hours) |               |               |               |
|---------------------------|---------------------------|---------------|---------------|---------------|------------------|---------------|---------------|---------------|
|                           | Multi<br>annual           | 2008/<br>2009 | 2009/<br>2010 | 2010/<br>2011 | Multi<br>anuala  | 2008/<br>2009 | 2009/<br>2010 | 2010/<br>2011 |
| X                         | 11,2                      | 11,8          | 12,0          | 8,1           | 172,8            | 184,9         | 131,2         | 107,0         |
| XI                        | 5,2                       | 5,7           | 6,7           | 10,1          | 106,9            | 76,2          | 94,5          | 109,5         |
| XII                       | 0,3                       | 2,2           | -0,6          | -2,3          | 78,3             | 75,0          | 67,9          | 68,8          |
| I                         | -1,7                      | -0,5          | -4,2          | -2,5          | 84,5             | 66,0          | 61,5          | 60,0          |
| II                        | 0,1                       | 1,5           | -0,1          | -1,6          | 103,1            | 89,7          | 96,7          | 133,8         |
| III                       | 4,4                       | 5,2           | 5,2           | 4,6           | 149,8            | 158,5         | 186,0         | 191,1         |
| IV                        | 11,1                      | 12,4          | 11,6          | 10,6          | 187,8            | 245,1         | 183,5         | 196           |
| V                         | 16,6                      | 17,5          | 17,1          | 16,6          | 250,3            | 240,6         | 236,1         | 265           |
| VI                        | 20,0                      | 21,0          | 20,1          | 20,9          | 274,3            | 260,3         | 209,5         | 262,5         |
| VII                       | 22,0                      | 23,8          | 23,3          | 22,4          | 299,2            | 322,8         | 277,0         | 285           |
| VIII                      | 21,2                      | 22,9          | 24,7          | 22,1          | 285,2            | 289,1         | 295,5         | 332,3         |
| IX                        | 16,7                      | 18,9          | 16,7          | 20,0          | 218,9            | 243,3         | 189,0         | 293,5         |
| Average/<br>annual sum    | 10,6                      | 11,8          | 11,0          | 10,7          | 2211,1           | 2251,5        | 2028,4        | 2304,5        |
| In vegeta-<br>tion period | 17,9                      | 19,4          | 18,9          | 18,8          | 1515,7           | 1601,2        | 1390,6        | 1634,3        |

The data presented in Table 1 showed that in three years of study observations, annual average temperatures especially during the vegetation period were above the multi-values. Sunlight (amount of sunshine hours) recorded was higher values than multi-year value, except for year 2009. Compared to multiannual average, the precipitation during the vegetation period recorded a surplus in 2010 and 2011 and a substantial deficit in 2009, considered a dry year (table 2).

Table 2

**Dinamic of precipitation during the study**

| Year<br>Month        | Precipitation (mm) |           |           |           |
|----------------------|--------------------|-----------|-----------|-----------|
|                      | Multiannual        | 2008/2009 | 2009/2010 | 2010/2011 |
| X                    | 49,1               | 65,2      | 54,6      | 90,6      |
| XI                   | 31,6               | 25,2      | 49,2      | 23,8      |
| XII                  | 32,7               | 44,8      | 36,0      | 63,0      |
| I                    | 31,8               | 53,6      | 10,6      | 12,0      |
| II                   | 37,6               | 30,2      | 62,0      | 22,2      |
| III                  | 36,5               | 54,6      | 24,8      | 10,0      |
| IV                   | 47,5               | 17,6      | 24,4      | 35,6      |
| V                    | 72,3               | 35,8      | 117,0     | 80,2      |
| VI                   | 83,4               | 75,2      | 93,4      | 103,8     |
| VII                  | 73,8               | 30,8      | 139,4     | 147,0     |
| VIII                 | 59,1               | 5,0       | 47,2      | 34,8      |
| IX                   | 58,8               | 22,6      | 79,0      | 8,8       |
| Annual sum           | 614,2              | 460,6     | 737,6     | 631,8     |
| In vegetation period | 394,9              | 187,0     | 500,4     | 410,2     |

The influence of the soil maintenance system on fertility characters of Gros Sauvignon variety, is summarized in table 3, as follows:

- Percentage of fertile shoots range from 63.60% to variant grassing with *Lolium perenne* (V2) and 72.53% in black field variant (V2);
- Coefficient of relative fertility (CFR) varies between 0.57 to variant grassing with *Lolium perenne* (V2) and 0.79 in black field variant (V1);

Table 3

**The influence of the soil maintenance system on the percentage of the fertile shoot and fertility coefficient**

| Variant                         | Fertile shoots (%) |        |      | Coefficient of relative fertility (Cfr) |        |      |
|---------------------------------|--------------------|--------|------|---|--------|------|
|                                 | Val.               | Dif.   | Sem. | Val.                                    | Dif.   | Sem. |
| V1 – black field                | 72,53              | 3,50   | *    | 0,79                                    | 0,09   | *    |
| V2 – grassing <i>Lolium p.</i>  | 63,60              | -5,43  | oo   | 0,57                                    | -0,13  | oo   |
| V3 –grassing <i>Medicago s.</i> | 70,87              | 1,84   | -    | 0,73                                    | 0,03   | -    |
| Average exp.(Mt.)               | 69,03              | 0,00   | -    | 0,70                                    | 0,00   | -    |
| DL 5%                           |                    | 2,5681 |      |   | 0,0849 |      |
| DL 1%                           |                    | 3,8888 |      |   | 0,1285 |      |
| DL 0,1%                         |                    | 6,2472 |      |   | 0,2605 |      |

For the two indicators - percentage of fertile shoots and coefficient of relative fertility, compared with control variant (Mt) represented by average experience, black field variant (V1) showed a significant positive difference (3.50 and 0.09), and the variant grassing with *Lolium perenne* (V2) showed a distinct negative difference significant (-5.43 respectively -0.13).

Variant of grassing with *Medicago sativa* (V3) did not show significant differences from the average experience that control variant (Mt). The data obtained on the influence of the soil maintenance system on characteristics of grape production, summarized in table 4, is observed:

- Average number of grapes per vine varies from 15.37 to variant grassing with *Lolium perenne* (V2) and 22.51 to field black variant (V1);
- The average weight of the grape varies between 101.33 g of variant grassing with *Lolium perenne* (V2) and 161.67 g to black field variant (V1);
- Weight of 100 grains varies between 124.33 g of variant grassing with *Lolium perenne* (V2) and 159.33 g to black field variant (V1).

Table 4

The influence of the soil maintenance system on grape production characteristics

| Variant                             | Grapes              |       |    |                    |        |     |                          |        |    |
|-------------------------------------|---------------------|-------|----|--------------------|--------|-----|--------------------------|--------|----|
|                                     | Average number/vine |       |    | Average weight (g) |        |     | Weight of 100 grains (g) |        |    |
|                                     | Val.                | Dif.  | S. | Val.               | Dif.   | S.  | Val.                     | Dif.   | S. |
| V1 – black field                    | 22,51               | 3,48  | *  | 161,67             | 24,00  | **  | 159,33                   | 18,00  | ** |
| V2 – grassing <i>Lolium perenne</i> | 15,37               | -3,66 | o  | 101,33             | -36,34 | ooo | 124,33                   | -17,00 | oo |
| V3 –grassing <i>Medicago sativa</i> | 19,33               | 0,30  | -  | 150,33             | 12,66  | -   | 141,00                   | -0,33  | -  |
| Average exp.(Mt.)                   | 19,03               | 0,00  | -  | 137,67             | 0,00   | -   | 141,33                   | 0,00   | -  |
| DL 5%                               | 2,9995              |       |    | 14,7717            |        |     | 7,8544                   |        |    |
| DL 1%                               | 4,5421              |       |    | 22,3685            |        |     | 11,8939                  |        |    |
| DL 0,1%                             | 7,2967              |       |    | 35,9343            |        |     | 19,1071                  |        |    |

The interpretation of statistical and mathematical data in table 4, compared with control variant (experimental average), is observed:

- Variant grassing with *Lolium perenne* (V2) has significant differences in the negative, for the number of grapes per vine (-3.66), distinct differences significant negative for weight of 100 grains (-17.00), and differences very semmificative in a negative way for the average weight of the grape (-36.34);
- Variant black field (V1) showed positively significant differences for the number of grapes per vine (3.48) and positively distinct significant differences for mean weight of grapes (24.00) and weight of 100 grains (18 , 00);
- Variant grassing with *Medicago sativa* (V3) not shown significant differences for the parameters analyzed.

The data obtained on the influence of the soil maintenance system on grape production, summarized in table 5, it is noted that:

- Average yield on the vine is between 1.59 kg/vine for the variant grassing with *Lolium perenne* (V2) and 3.57 kg/vine for the black field variant (V1);
- Average production value per hectare ranges from 8.84 t / ha for variant grassing with *Lolium perenne* (V2) and 19.84 t / ha to field black variant (V1).

Table 5

**Influence of the soil maintenance system on grape production**

| Variant                         | Grape production/vine (Kg) |        |    | Production evaluated (t/ha) |        |    |
|---------------------------------|----------------------------|--------|----|-----------------------------|--------|----|
|                                 | Val.                       | Dif.   | S. | Val.                        | Dif.   | S. |
| V1 – black field                | 3,57                       | 0,90   | ** | 19,84                       | 5,00   | ** |
| V2 – grassing <i>Lolium p.</i>  | 1,59                       | -1,08  | oo | 8,84                        | -6,00  | oo |
| V3 –grassing <i>Medicago s.</i> | 2,85                       | 0,18   | -  | 15,83                       | 0,99   | -  |
| Average exp.(Mt.)               | 2,67                       | 0,00   | -  | 14,84                       | 0,00   | -  |
| DL 5%                           |                            | 0,4839 |    |                             | 2,6789 |    |
| DL 1%                           |                            | 0,7328 |    |                             | 4,0567 |    |
| DL 0,1%                         |                            | 1,1772 |    |                             | 6,5169 |    |

The statistical and mathematical interpretation of data recorded in Table 5, compared with control variant represented by experimental average, observed:

- Variant grassing with *Lolium perenne* (V2) show distinct differences significant negative for average production per vine (-1.08 kg) and estimated production per hectare (-6.00 t / ha);
- Variant black field (V1) show distinct differences significant positive for average production/vine (0.90 kg) and estimated production per hectare (5.00 t/ha).

The influence of the soil maintenance system on productivity indices (IPR - relative productivity index and IPA - absolute productivity index) and accumulated sugar content in grapes is presented in table 6.

Table 6

**The influence of the soil maintenance system on productivity index and sugar content of grapes**

| Variant                         | Productivity index |         |    |        |         |    | Sugar content of grapes (g/l) |         |     |
|---------------------------------|--------------------|---------|----|--------|---------|----|-------------------------------|---------|-----|
|                                 | Ipr                |         |    | Ipa    |         |    | Val.                          | Dif.    | S.  |
|                                 | Val.               | Dif.    | S. | Val.   | Dif.    | S. |                               |         |     |
| V1 – black field                | 134,87             | 31,90   | ** | 247,17 | 37,50   | ** | 196,03                        | -22,67  | ooo |
| V2 – grassing <i>Lolium p.</i>  | 63,73              | -39,24  | oo | 151,67 | -58,00  | oo | 242,53                        | 23,83   | *** |
| V3 –grassing <i>Medicago s.</i> | 118,73             | 15,76   | -  | 231,07 | 21,40   | -  | 217,53                        | -1,17   | -   |
| Average exp.(Mt.)               | 102,97             | 0,00    | -  | 209,67 | 0,00    | -  | 218,70                        | 0,00    | -   |
| DL 5%                           |                    | 16,3252 |    |        | 24,1005 |    |                               | 7,8771  |     |
| DL 1%                           |                    | 24,7210 |    |        | 36,4950 |    |                               | 11,9282 |     |
| DL 0,1%                         |                    | 39,7135 |    |        | 58,6280 |    |                               | 19,1623 |     |

Analyzing the data obtained it is found that:

- Value of the relative productivity index (IPR), ranges from 63.7 to variant on grassing with *Lolium perenne* (V2) and 134.87 to field black version (V1);
- Value of the absolute productivity index (IPA), ranges from 151.7 to variant grassing with *Lolium perenne* (V2) and 247.17 to field black version (V1);
- The sugar content of grapes ranges from 196.03 g / l to the variant field black (V1) and 242.53 g / l to the variant grassing with *Lolium perenne* (V2).

Analyzing the data obtained compared to the average experience (Mt) pointed out that:

- Variant black field (V1) show distinct differences significant positive for value of the relative productivity index (31.90) and value the absolute productivity index (37.50);
- Variant grassing with *Lolium perenne* (V2) show distinct differences significant negative for the both productivity indices.

The results obtained for the sugar content of grapes, to the variant black field (V1) show very significant differences in the negative (-22.67 g / l) than the average experience (Mt), while the variant grassing with *Lolium perenne* (V2) recorded very significant differences positive (23.50 g / l). Analysis of variance on the acidity of must (g / l), showed no statistical significance between the three soil maintenance systems.

The soil maintenance system variant grassing with *Medicago sativa* (V3) not significant differences from the variant control (Mt) or experimental average.

## CONCLUSIONS

1. The soil maintenance system strongly influence characteristics the agro biological and productive of the variety;
2. Agro biological characteristics of the variety (fertile shoots, coefficients of fertility) and quantitative characteristics of grape production are adversely affected if the practice of variant grassing with *Lolium perenne* (V2);
3. A characteristic quality of grape production (sugar content) is positively influenced if applied the variant grassing with *Lolium perenne* (V2);
4. Variant grassing with *Medicago sativa* (V3), statistically similar experience average (Mt), provides an optimal balance between vegetative growth and quantitative and qualitative characteristics of grape production.

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# VARIATION OF PHOTOSYNTHETIC PIGMENTS CONTENT UNDER THE INFLUENCE OF TREATMENTS WITH BIOSTIMULATING SUBSTANCES AT SOME TABLE GRAPE VARIETIES GROWN IN IASSY VINEYARD AREA

## VARIAȚIA CONȚINUTULUI ÎN PIGMENȚI FOTOSINTETICI SUB INFLUENȚA TRATAMENTELOR CU SUBSTANȚE BIOSTIMULATOARE LA UNELE SOIURI PENTRU STRUGURI DE MASĂ CULTIVATE ÎN AREALUL PODGORIEI IAȘI

*COTOVANU FILIMON Roxana*<sup>1</sup>, *ROTARU Liliana*<sup>1</sup>,  
*CĂULEȚ Raluca*<sup>1</sup>, *FILIMON V. R.*<sup>1</sup>,  
e-mail: roxanacotovanu@yahoo.com

**Abstract.** *This study aims to determine the chlorophyll (a and b) and carotenoid content, using spectrophotometric methods, from leaves of Chasselas doré and Gelu table grape varieties (Vitis vinifera L.), grown in Iasi vineyard, treated with two organic fertilization products, Cropmax and Kelpak, in different doses. Physiological role of assimilating pigments is complex, being involved in oxidation-reduction and photosynthesis processes, fructification and protection against ultraviolet radiation. Following the study were observed statistically significant differences between experimental variants, which confirms the suitability of using these products in sustainable viticulture.*

**Key words:** chlorophyll, carotenoids, *Vitis vinifera* L., organic foliar fertilization.

**Rezumat.** *Studiul are ca scop determinarea spectrofotometrică a conținutului în clorofile (a și b) și carotenoizi din frunzele soiurilor de viță de vie (Vitis vinifera L.) Chasselas doré și Gelu, cultivate în podgoria Iași, tratate cu două produse ecologice de fertilizare a viței de vie, Kelpak și Cropmax, în diferite doze. Rolul fiziologic al pigmentilor asimilatori este complex, fiind implicați în procesele de oxido-reducere, în procesele de fotosinteză, fructificare și de protecție față de radiațiile ultraviolete. În urma observațiilor efectuate au fost identificate diferențe statistic semnificative între variantele experimentale, fapt ce confirmă preabilitatea folosirii acestor produse în viticultura sustenabilă.*

**Cuvinte cheie:** clorofilă, carotenoizi, *Vitis vinifera* L., fertilizare foliară organică.

### INTRODUCTION

Photosynthetic pigments are represented by green chlorophyll pigments (chlorophylls a and b) and yellow carotenoid pigments (carotenes and xanthophylls), being essential compounds in light energy conversion (Toma and, Jităreanu, 2003).

The quantity of light radiation absorbed by the leaf depends mainly on the amount of photosynthetic pigments that they contain (Steele et al., 2008). *Vitis*

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

*vinifera L.* leaves contain high levels of photosynthetic pigments, their value being dependent on the stage of leaf maturation and the phenophase of vegetation in which the plant is found, the percentage of chlorophyll in leaves increasing, during phenophases, until ripening. Usually, in vines, chlorophyllian and carotenoid pigments content was reported to be in the range of 1.05 mg/g fresh weight (f.w.) and 1.58 mg/g f.w., respectively from 0.33 to 0.65 mg/g f.w. (Burzo et al., 2005, Acatrinei and Andor, 2006).

Ratio between chlorophyll a and b (a/b), at *Vitis vinifera L.*, is maximum at the beginning of the growing season, reaching up to 3/1 and decreases during the grapes ripening, while the ratio chlorophyll/carotenoids can register values till 4/1 (Toma and Jităreanu, 2003, Beceanu, 2011).

Plant growth and development is controlled, in addition to genetic factors, by endogenous substances that are part of the plant hormone group (Davies, 2004). Phytohormones are a class of organic substances that in low concentrations, influences physiological processes of growth, differentiation and development of plants with effect on biosynthesis of assimilating pigment and the relationship between them.

## MATERIAL AND METHODS

The study was conducted at the table grape varieties *Gelu*, new romanian creation obtained at Research and Development Station for Viticulture and Vinification Iassy (RDSVV) by free fecundation of the local variety Coarnă neagră, whose hybrid seeds were irradiated with X rays; and *Chasselas Doré*, old cosmopolitan variety, with medium maturation and mixed use, cultivated in Iassy vineyard area, Ampelographic Collection of Faculty of Horticulture, belonging to the University of Agricultural Sciences and Veterinary Medicine (UASVM) "Ion Ionescu de la Brad" Iassy.

Physiological analysis of assimilating pigments were made in berry growth phenophase, at 14 days after foliar treatments with biostimulating substances *Kelpak* in doses of 50 mL (V<sub>1</sub>K), 100 mL (V<sub>2</sub>K), 150 mL (V<sub>3</sub>K) and *Cropmax*, at 10 mL (V<sub>1</sub>C), 15 mL (V<sub>2</sub>C), 20 mL (V<sub>3</sub>C). For analyzes were collected leaves from the middle third of the shoots, which contain the highest amount of chlorophyllian pigments, compared with leaves from upper and lower third of the shoots (Burzo et al., 2005).

Research aimed to emphasize the influence of mentioned treatments on chlorophyllian and carotenoid pigment biosynthesis in leaves of varieties selected for study. Extraction of pigments was achieved with 99% purity acetone, in triplicate.

Chlorophylls and carotenoids were quantitatively determined spectrophotometrically by measuring absorbance at three wavelengths,  $\lambda$  662 nm, 645 nm and 470 nm, 1 cm optical path, using a UV-vis spectrophotometer T 70 PG Instruments and the calculation of results was performed according to formulas proposed by Lichtenhaler H., 1987, the data being reported in mg pigment/g leaf, with three decimals, to detect small differences that appear at some variants.

Results are average of three determinations having calculated the standard deviation, and for the statistical analysis of data was used application Data Analysis, from Microsoft® Excel software.



## RESULTS AND DISCUSSIONS

Chlorophylla content, for the two studied varieties, differed according to the biostimulating substance used and the applied doses. Chlorophyll content value registered was between 1.363 mg/g f.w. (V<sub>1</sub>C) and 2.257 mg/g f.w. (V<sub>1</sub>K) at the Gelu variety, and between 1.441 mg/g f.w. (V<sub>1</sub>C) and 2.351 mg/g f.w. (V<sub>1</sub>K) at the Chasselas Doré variety (fig. 1).

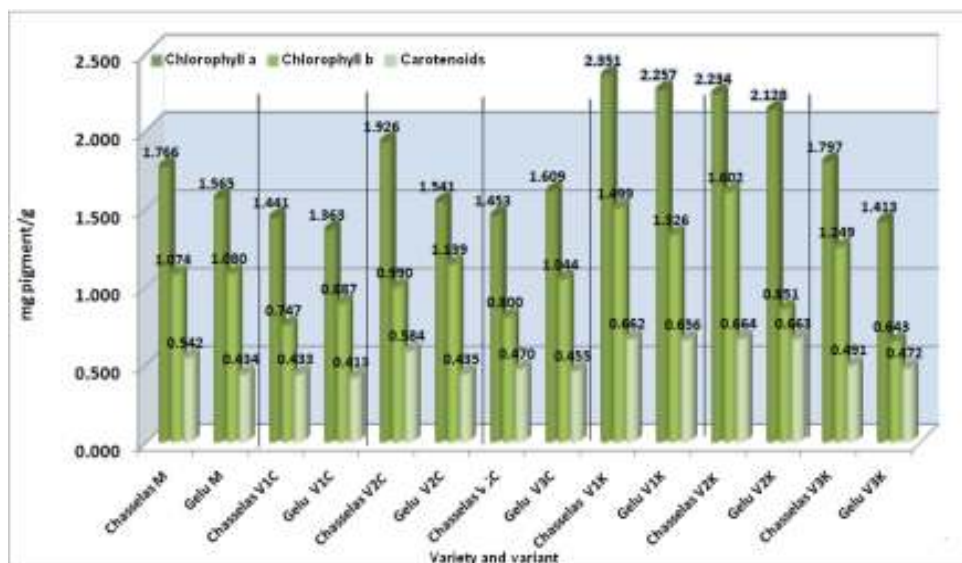


Fig. 1 - The amount of chlorophyll a, chlorophyll b and carotenoids (mg/g f.w.)

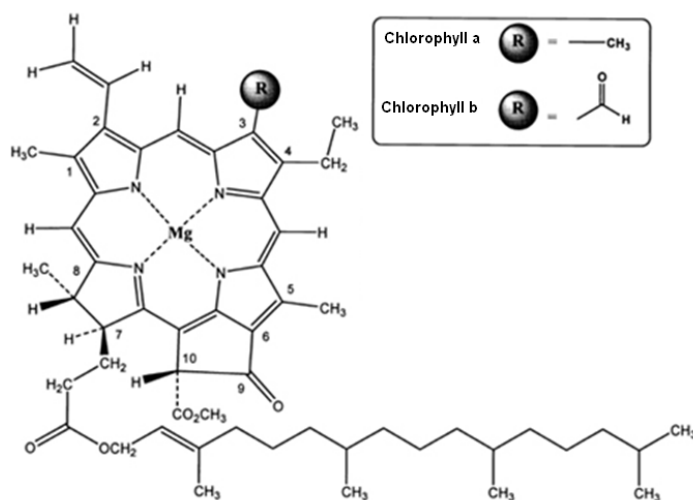


Fig. 2 - Chemical structure of chlorophyll a and b (adaptation after Streit et al., 2005)

Can be noted that low doses of Kelpak product applied to the first samples have positively influenced the content of chlorophyll a, chlorophyll b (fig. 2) and carotenoids compared with control sample, leading to an increase in both varieties in the pigment content. In contrast, low doses Cropmax led to low levels of chlorophyll a, compared to control version. The results of measurements were considered high compared with values found in the literature, presented introductory, fact correlated with the biostimulating treatments application.

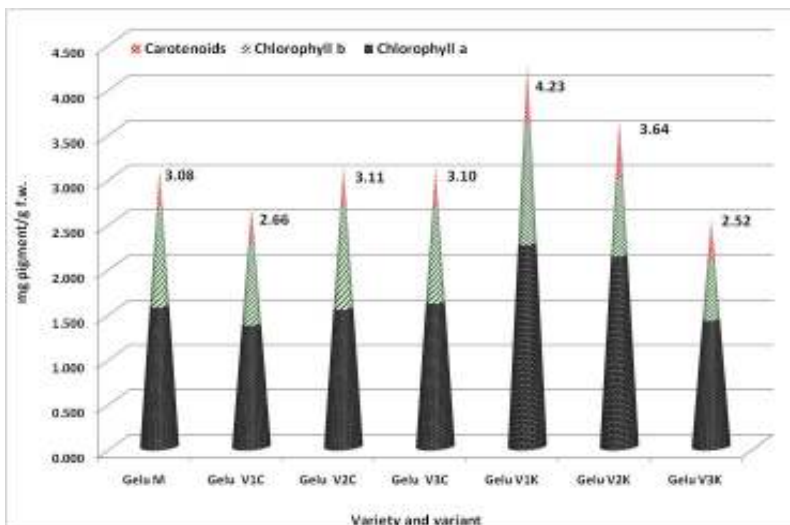


Fig. 3 - The total amount of photosynthetic pigments registered at Gelu variety

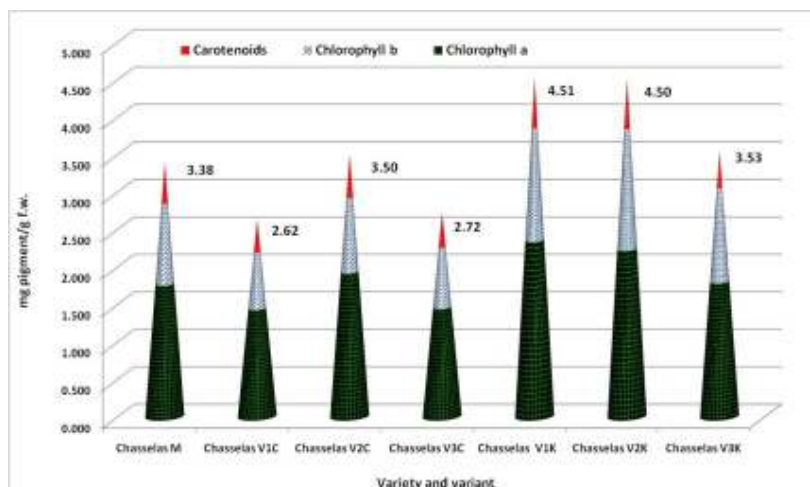


Fig. 4 - The total amount of photosynthetic pigments registered at Chasselas Doré variety

At the local variety Gelu, chlorophyll a and b content was the lowest in the variant treated with 150 mL Kelpak (V<sub>3</sub>K), 0.643 mg/g f.w., at the cosmopolitan

variety Chasselas Doré minimum value was identified at the variant treated with 10 mL Cropmax (V<sub>1</sub>C) 0.747 mg/g f.w.

Carotenoids content, at Gelu variety, ranged from 0.413 mg/g f.w. at V<sub>1</sub>C and 0.663 mg/g f.w. V<sub>2</sub>K version, and at the variety Chasselas Doré was found to be ranged from 0.433 mg/g f.w. (V<sub>1</sub>C) and 0.664 mg/g f.w. (V<sub>2</sub>K).

The total amount of photosynthetic pigments record a minimum of 2.52 mg/g f.w. and a maximum of 4.23 mg/g f.w. Gelu variety (fig. 3), and at Chasselas Doré variety ranged between 2.62 mg/g f.w and 4.51 mg/g f.w. (fig. 4).

Because of increasing values of chlorophyll *a* and chlorophyll *b*, caused by the treatments applied with the stimulant substances and also of the sampling moment, the ratio chlorophyll *a*/ chlorophyll *b* was less than 3/1, normal value given by the literature, and the chlorophylls/carotenoids ratio was much higher than the reference value, 4/1 (Table 1).

Table 1

The content of photosynthetic pigments in leaves at the variety Chasselas Doré

| Variety<br>Version | Total chlorophylls<br>(mg/g f.w.) | Chlorophyll a/<br>Chlorophyll b | Chlorophylls/<br>Carotenoids | Total photosynthetic<br>pigments<br>(mg/g f.w.) |
|--------------------|-----------------------------------|---------------------------------|------------------------------|---|
| Chasselas M        | 2.841 ± 0.11                      | 1.644 ± 0.02                    | 5.239 ± 0.01                 | 3.383± 0.13                                     |
| Chasselas V1C      | 2.187 ± 0.89                      | 1.659 ± 0.70                    | 4.266 ± 1.78                 | 2.621± 0.75                                     |
| Chasselas V2C      | 2.917 ± 0.14                      | <b>1.948± 0.07</b>              | 4.995 ± 0.10                 | 3.500± 0.16                                     |
| Chasselas V3C      | 2.253 ± 0.22                      | 1.835 ± 0.18                    | 4.791 ± 0.10                 | 2.723± 0.26                                     |
| Chasselas V1K      | <b>3.850± 0.76</b>                | 1.557 ± 0.11                    | 5.867 ± 0.31                 | <b>4.512± 0.93</b>                              |
| Chasselas V2K      | 3.836 ± 0.36                      | 1.403 ± 0.14                    | 5.787 ± 0.39                 | 4.500± 0.41                                     |
| Chasselas V3K      | 3.046 ± 0.63                      | 1.456 ± 0.14                    | <b>6.206± 0.36</b>           | 3.536±0.73                                      |
| Gelu M             | 2.646 ± 0.23                      | 1.455 ± 0.07                    | 6.087 ± 0.14                 | 3.080± 0.26                                     |
| Gelu V1C           | 2.250 ± 0.23                      | 1.540 ± 0.07                    | 5.453 ± 0.30                 | 2.663± 0.27                                     |
| Gelu V2C           | 2.680 ± 0.12                      | 1.359 ± 0.12                    | <b>6.180± 0.43</b>           | 3.115± 0.25                                     |
| Gelu V3C           | <b>3.816± 0.03</b>                | 1.695 ± 0.04                    | 5.872 ± 0.12                 | 3.108± 0.04                                     |
| Gelu V1K           | 3.584 ± 0.22                      | 1.703 ± 0.06                    | 5.466 ± 0.14                 | <b>4.239± 0.26</b>                              |
| Gelu V2K           | 2.979 ± 0.03                      | <b>2.518± 0.30</b>              | 4.494 ± 0.13                 | 3.642± 0.03                                     |
| Gelu V3K           | 2.056 ± 0.21                      | 2.200 ± 0.37                    | 4.401 ± 0.39                 | 2.529± 0.30                                     |

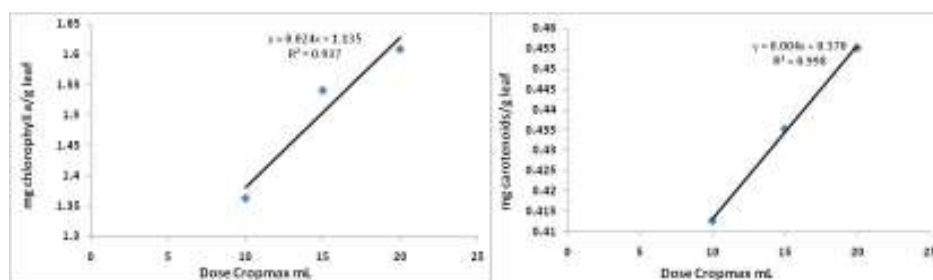


Fig. 5 - Correlation between chlorophyll a and carotenoid content, and applied dose at Gelu variety

Following statistical hypotheses by performing ANOVA test, *p-value* was less than 0.05 alpha (*p-value* calculated  $<p=0,05$ ), and F factor calculated was

higher than F critical ( $F_{calc} > F_{crit}$ ) concluded that differences statistically observed between the variants are caused by the treatments applied not by the experimental errors.

Only at Gelu variety were found positive correlations between the Cropmax doses applied and chlorophylls ( $R^2=0.937$ ) and carotenoids content ( $R^2=0.998$ ), indicating that at higher concentrations of the applied doses appears an increasing of chlorophylls and carotenoids content (fig. 5).

## CONCLUSIONS

1. The highest influence on the content of chlorophyll pigments had Kelpak product. The biggest amounts of assimilating pigments were recorded in both varieties at Kelpak with 50 mL and 100 mL concentration of solution. These increased amounts of assimilating pigments may be related to the hormonal substances applied and in a small measure to the microelements found in product composition.

2. Treatments applied had no significant influence on carotenoid content, the values oscillating around control sample value, unrelated with the concentration used.

3. Influence of biostimulating treatment at Chasselas Doré and Gelu varieties led to a reduction of the chlorophyll *a*/chlorophyll *b* ratio, and also to an increased value of chlorophylls/carotenoids ratio.

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# MARA, NEW VARIETY OF VINE FOR TABLE GRAPES, WITH INCREASED GENETIC RESISTANCE, CREATED AT S.C.D.V.V. IASI

MARA, SOI NOU DE VIȚĂ DE VIE DE STRUGURI PENTRU MASĂ, CU  
REZISTENȚĂ GENETICĂ SPORITĂ, CREAT LA S.C.D.V.V. IASI

*DAMIAN Doina*<sup>1</sup>, *CALISTRU Gh.*<sup>1</sup>, *NECHITA Ancuța*<sup>1</sup>, *SAVIN C.*<sup>1</sup>  
e-mail: doinadamian@yahoo.com

**Abstract.** Scientific research in the field of improving vines, with the permanent role of innovating and diversifying the assortments of vine, by creating new genotypes that are qualitatively and productively valuable, with a better resistance to diseases and stress factors, has an open way for the expansion and completion of the assortment of table grapes from the country's vineyards. As an answer to this, at SCDVV Iasi, through works of directed sexual hybridization between the interspecific hybrid Seyve-Villard 12303 and Ozana, has been obtained and homologated in 2011 the assortment called Mara. The new creation is characterized by medium-sized grapes (230 g), medium to large grains (3.7 g), with crisp core and black-cyan coloured skin. The average grape production is about 4.95 kg / vine, respectively 18.75 tons / ha calculated production, of which 90% is the commodity production. It has a good biological resistance to the mildew and powdery mildew and a middle one to the grape's gray rot. The assortment matures the grapes in their IV-V age.

**Key words:** sexual crossing, resistant varieties, statistical methods.

**Rezumat.** Cercetarea științifică din domeniul ameliorării viței de vie, cu rol permanent de înnoire și diversificare a sortimentelor viticole, prin crearea de noi genotipuri mai valoroase calitativ și productiv, cu rezistență mai bună la boli și factorii de stres, are cale deschisă pentru lărgirea și completarea sortimentului de struguri pentru masă din podgoriile țării. Pentru a răspunde la acest deziderat, la SCDVV Iași, prin lucrări de hibridare sexuată dirijată între hibridul interspecific Seyve-Villard 12303 și Ozana s-a obținut și omologat în anul 2011 soiul Mara. Noua creație se caracterizează prin struguri de mărime medie (230 g), boabe mijlocii spre mari (3,7 g), cu miezul crocant și epiderma colorată în negru-azuriu. Producția medie de struguri este de 4,95 kg/butuc, respectiv 18,75 tone/ha producție calculată, din care peste 90% reprezintă producția marfă. Prezintă rezistență biologică bună la mană și făinare și una mijlocie la putregaiul cenușiu al strugurelui. Soiul își maturează strugurii în epoca IV-V.

**Cuvinte cheie:** hibridare sexuată, soiuri rezistente, analiza statistică.

## INTRODUCTION

The varietal range of the varieties for tables grapes admitted for the reproduction in the recreation plantations from the population's households is rather limited and it needs to be supplemented and diversified with new genotypes.

The pronounced heterozygous character of the grape vine, the involvement

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<sup>1</sup> Research and Development Station for Viticulture and Vinifications Iasi, Romania

of the genes which control the resistance, the long period between seminal generations, as well as the appearance of determining factors which favor the action of plants sensitizing (rainy years for diseases resistance and cold winters for frost resistance), extend the research period. For definite conclusions, it was imposed the control of resistance's durability in time, in order to maintain this property. The research made in the last four decades in our country, have lead to the procurement of certain grape vine genotypes with increased resistance to diseases (Moldovan et al., 1987 and 1994, Oprea et al., 1994 and 2007, Calistru and Damian, 1999, Culcea et al., 1994 and 2004).

The new grape vine varieties with biological resistance to cryptogamic diseases and to frost, also have production and quality properties which are superior to the ones from Seyve – Villard class, but especially to the hybrid direct producers, these being created only for the recreation plantations from the non-winegrowing areas being destined either for fresh consumption or for obtaining different non-alcoholic drinks.

Following this direction of creation of new resistant genotypes, a new variety of table grapes, called Mara, has been approved at Research and Development Station for Viticulture and Wine-making Iași.

## MATERIAL AND METHODS

The research activity devoted to this purpose has started at SCDVV (Research and Development Station for Viticulture and Wine-making) Iași, after the 1980s. After a preliminary sustained study of the germplasm sources with resistance to diseases and frost, there was performed a wide range of controlled sexual hybridizations using as genitors vinifera varieties with superior properties of production, quality, precocity in grape ripening and inter-specific hybrids from Seyve – Villard class, hybrid direct producers and sometimes even varieties of vine rootstocks. From these hybrid combinations resulted Mara variety, which derives from the intercrossing of varieties S.V. 12303 with Ozana, which was approved in December 2011 (figure 1).



Fig. 1 – Mara Variety

By covering all the stages of the improvement scheme specific to the creation of new varieties with increased biological resistance, respectively the field of hybrids and the one for contest, the new variety has been tested and studied for the inspection of the properties of resistance to the main cryptogamic diseases (blight, mildew and grey mould of grapes), to frost and for the inspection of the production and quality properties. The experimental plantations have not been protected during the winter and during the vegetation period there have not been applied anti-cryptogamic treatments, with the exception of very rainy years, when there were required 1-3 interventions. During 2009–2011, which represented the final stage of the improvement scheme through controlled sexual hybridization, the agro-biological and technological studies have been carried out in compliance with ISTIS (The State Institute for Variety Testing and Registration) requirements. The description of distinctness characteristics was performed according to the descriptors adopted by UPOV (Union for the Protection of New Varieties of Plants). During the pre-approval stage, it was carried out the serological test (ELISA) of the presence of the main viruses, and the results were negative. For comparison it was used Milcov variety which is similar as direction of production, growth vigor and epidermis' color.

## RESULTS AND DISCUSSIONS

During the study period, the weather conditions in Iași vineyard, Copou wine-growing centre, where are located experimental fields, it was signalled the presence of cold winters, with absolute minimum temperatures which were under the freeze limit for the grape vine, respectively -27°C in the air and -35 °C at the ground surface ( 26.01.2010), with springs warmer than the standard temperature, but more poorer in precipitations, with the exception of the year 2010, very warm summers, with absolute maximum temperatures which frequently rose over 30°C in the air, and the average temperatures in July, August and September were over the standard, being very favorable to the completion of the main physiological and bio-chemical processes which influence its production and quality. The precipitations during the vegetation period were poorer in the year 2009 (214 mm) and in the other years they were close to the normal values, but they were irregularly distributed, the largest quantities being registered in June during the blooming period, with negative influence on the processes of fertilization and graining of the berries of grapes.

On the background of this weather conditions, Mara variety has started its vegetation through disbudding between 25<sup>th</sup> of April 6<sup>th</sup> of May, without significant differences in comparison with the witness, as well as the flowering phenophase located between 5<sup>th</sup>–8<sup>th</sup> of June. Grapes' ripping was realized between 30<sup>th</sup> of July 12<sup>th</sup> of August, and the grapes reached commercial maturity during the period 23<sup>rd</sup> of September 5<sup>th</sup> of October, with approximately three weeks later that the witness variety. Its extension offers the possibility of fresh consumption of grapes for a longer period (table 1).

Table 1

| Phenological spectrum |                 |              |                 |                     |             |
|-----------------------|-----------------|--------------|-----------------|---------------------|-------------|
| Variety               | Disbudding      | Flowering    | Grapes ripping  | Commercial maturity | Fall leaves |
| Mara                  | 25.04 - 06.05.  | 05 - 08. 06. | 30. 07 - 12.08. | 23.09-05.10.        | 25.10.      |
| Milcov (mt)           | 23.04 - 04. 05. | 03 - 10. 06. | 26.07 - 08.08.  | 21.08-15.09.        | 25.10.      |

Regarding the coverage of the phenological spectrum, the new Mara variety integrated in the ecosystem conditions, completing its vegetation period after 182 days.

Fertility and productivity of the new variety, appreciated through the percentage of fertile vine shoots, the absolute and relative fertility coefficients and the absolute and relative productivity indices (table 2) show its superiority for all the analyzed elements compared to the witness. The new variety show a high fertility potential, 87,6% of the vine shoots from a vine stalk being fertile, the absolute and relative fertility coefficients having values higher than 1, respectively 1,63 and 1,42, superior to Milcov variety (1,47 and 1,01). One can state significant differences between the values of the productivity indices, these ones being higher in the case of Mara variety when compared to the witness.

Table 2

| <b>Fertility and productivity elements of the Mara variety</b> |                  |                        |          |                      |          |
|--|------------------|------------------------|----------|----------------------|----------|
| Variety  | Fertile shoots % | Fertility coefficients |          | Productivity indices |          |
|  |                  | absolute               | relative | absolute             | relative |
| Mara   | 87,6             | 1,63                   | 1,42     | 389                  | 339      |
| Milcov (mt)  | 65,4             | 1,47                   | 1,01     | 278                  | 191      |

The studied technological properties complete the elements of acquaintance with the new variety, under the aspect of the opportunity of its homologation and cultivation in recreation plantations, in population's households within the incorporated area or in the non-winegrowing areas (table 3).

Table 3

| <b>The technological properties of the Mara variety</b> |                          |                     |        |           |                          |                            |            |  |                       |
|---|--------------------------|---------------------|--------|-----------|--------------------------|----------------------------|------------|--|-----------------------|
| Variety   | Grapes production:       |                     |        |           | Average weight / grape g | Average weight 100 berry g | Sugars g/L | Total acidity g/L H <sub>2</sub> SO <sub>4</sub> | Maturati on of grapes |
|   | effective kg/ vine stalk | calculated t/ha     | high % | increa se |                          |                            |            |  |                       |
| Mara  | 4,95                     | 18,75 <sup>xx</sup> | 91     | -         | 239                      | 370                        | 180        | 4,9  | IV - V                |
| Milcov (mt)   | 3,88                     | 14,69               | 80     | 27        | 189                      | 280                        | 138        | 3,0  | III - IV              |

DL 5% = 0.368; DL 1% = 0.609; DL 0,1% = 1,14.

Grape size realized in the case of this new variety, appreciated for its average weight is of 239 g/grape compared to 189 g/grape in the case of the witness. The berry is middle-sized, weighting 3,7 g/berry, also superior to the witness variety (2,8 g/berry), with black/blue, uniform epidermis and crunchy pulp. The effective production of grapes for each vine stalk and calculated per hectare reveals the increased biological potential of the new variety, this one achieving a multi-annual average production of 4,95 kg/vine stalk, respectively of 18,75 t/ha, ensuring a production increase with 27% in comparison to the witness, assured significantly and distinctly from a statistical point of view.

The commercial and consumption values are reflected through the high proportion of production to be delivered, which represent 91% of the total production. Quality-related elements show an increased potential of accumulation



of sugar (180 g/L) and total acidity in must (4,9 g/L H<sub>2</sub>SO<sub>4</sub>), which are more reduced in the case of Milcov variety, in the conditions of the winegrowing ecosystem of Copou.

The values of the technological indices resulted from the physical-mechanical analysis of one kilogram of grapes complete the qualitative properties of the new Mara variety (table 4).

Table 4

**Physico-mechanical composition of 1 kg grapes and technological indices of Mara variety compared with control**

| Elements determined                   | Mara | Milcov (mt) |
|---------------------------------------|------|-------------|
| <b>1 kg grapes:</b>                   |      |             |
| no. berry normally developed, healthy | 262  | 380         |
| berry, g                              | 980  | 972         |
| bunch, g                              | 20   | 28          |
| must, g                               | 640  | 630         |
| volume of must, cm <sup>3</sup>       | 585  | 600         |
| no. berry/100 g bunch                 | 27   | 39          |
| <b>100 berry:</b>                     |      |             |
| average weight, g                     | 389  | 280         |
| volume, cm <sup>3</sup>               | 350  | 250         |
| number of seeds                       | 237  | 243         |
| seeds weight, g                       | 12   | 14          |
| skin weight, g                        | 23   | 22          |
| core weight, g                        | 354  | 244         |
| <b>Technological indices:</b>         |      |             |
| berry index                           | 27   | 39          |
| structure of the grape index          | 49   | 35          |
| composition of berry index            | 10,1 | 6,78        |

Checking the resistance properties of the variety to low temperatures and to the attack of the main cryptogamic diseases, one can state that they have been influenced by the evolution of the climate conditions in the ecosystem (table 5).

Table 5

**Resistance to frost and fungal diseases main (notes OIV scale)**

| Variety | % Viable buds | Blight |        | Mildew |        | Grey mould of grapes |
|---------|---------------|--------|--------|--------|--------|----------------------|
|         |               | leaf   | grapes | leaf   | grapes |                      |
| Mara    | 39 – 95       | 9      | 9      | 9      | 9      | 7 – 8                |
| Milcov  | 27 – 93       | 7      | 8      | 7      | 7      | 6                    |

If vine stalks are not protected during winter, in the presence of some absolute minimum temperatures of – 27 °C in the air and – 35 °C at soil surface, Mara variety has been affected by frost in a quite high proportion, the viability of the winter grape buds was of 39% only, being similar to *vinifera* varieties. The biological resistance has been good to the main cryptogamic diseases, when applying a reduced number of treatments during the period of vegetation (1-3) in the years with abundant precipitations, being appreciated with 9 for blight and mildew on leafs and grapes and with 7-8 for grey mould of grapes in the OIV (International Organisation of Vine and

Wine) scale, values which are superior to the witness, Milcov variety, in the conditions of applying 6-7 phytosanitary treatments.

## CONCLUSIONS

1. The new created variety, called Mara, is an original and valuable creation that enriches the source of germoplasm, completes and diversifies the varietal conveyor of the table grapes, with increased resistance to diseases.

2. Presents a high potential of fertility (87,6% fertile vine shoots), reflected in the high and permanent productions, of 18,75 t/ha, of which 91% represents the production to be delivered.

3. Grapes are middle-sized, with large berries, black/bluish coloured, crunchy pulp and pleasant taste, semi-compact, which reach maturity at the 4<sup>th</sup> – 5<sup>th</sup> ages.

4. This new creation is recommended in case of reproduction for recreation plantations, population's households or in areas outside the winegrowing ones.

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# IMMATURE EMBRYO RESCUE OBTAINED BY INTRASPECIFIC HYBRIDIZATION PERFORMED ON THE GRAPEVINE

## RECUPERAREA EMBRIONILOR IMATURI OBTINUȚI PRIN HIBRIDĂRILE INTRASPECIFICE EFECTUATE LA VIȚA DE VIE

*DONICI Alina*<sup>1</sup>, *ȚÂRDEA C.*<sup>2</sup>  
e-mail: donicialina@yahoo.com

**Abstract.** *In contrast to conventional breeding methods, biotechnological approaches in recovery in ovules embryos rescue opening new perspectives in grapevine varieties. Works were performed the optimization of recovery paths for immature embryos from 4 genotypes hybrid vines in the collection of Research Station for Development Viticulture and Oenology Bujoru. Culture medium and genotype that participated in hybridization has strongly influenced the viability of eggs inoculated. Use of liquid culture medium under the bridges of filter paper showed a higher rate of egg viability. Supplementation of medium with 100 mg ascorbic acid and citric acid 100 mg resulted in greater potential results favorable. Un viable eggs was obtained from hybrid combinations Madeleine Angevine x Afuz Ali si Apiren alb x Kişmiş alb.*

**Key words:** grapevine, hybridization, seedless varieties, in vitro culture, embryos

**Rezumat.** *Spre deosebire de metodele conventionale de reproducere, aplicarea biotehnologiei in recuperarea embrionilor in ovulo a deschis noi perspective in obtinerea soiurilor apirene de viță de vie. S-au efectuat lucrări privind optimizarea căilor de recuperare a embrionilor imaturi de la 4 genotipuri hibride de viță de vie din colecția Stațiunii de Cercetare Dezvoltare pentru Viticultura si Vinificatie Bujoru. Mediul de cultură și genotipurile care au participat la hibridare, au influențat semnificativ viabilitatea ovulelor inoculate. Utilizarea mediilor de cultură lichide prevăzute cu punți din hârtie de filtru a evidențiat o rată a viabilității ovulelor mai mare. Suplimentarea mediilor de cultură cu acid ascorbic 100 mg și acid citric 100 mg a condus la rezultate favorabile. Un potențial mai mare de ovule viabile s-a obținut la combinațiile hibride Madeleine Angevine x Afuz Ali si Apiren alb x Kişmiş alb*  
**Cuvinte cheie:** vița de vie, hibridare, soiuri apirene, cultura in vitro, embrioni

### INTRODUCTION

The study seedless varieties existing in Romania has shown that they, in addition to quality, they have some shortcomings under the aspect adapting them to specific vineyards preserve ecosystems. This situation requires the need to obtain new seedless varieties which to adapt to environmental conditions

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<sup>1</sup> Research and Development Station for Viticulture and Winemaking Bujoru, Romania

<sup>2</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

characteristic vineyard climate in Romania, to ensure production quality, used either that grapes of table varieties or as a raw material for industrialization.

In ovulo embryo rescue thus provides a solution not only for obtaining intraspecific and interspecific crosses, but also allows using seedless vines as female parents (Cain et al. 1983; Emershad and Ramming 1984). Results of the previous works pointed out that not only the composition of the medium culture but also the genotype affects the success of the culture (Bouquet and Davis 1989).

Problem in creating seedless varieties genitor is the choice that must be to obtain the form large grain, more productive, with periods of maturation, to transmit to progeny seedless, to a lesser degree as high as possible (Cachiță, 1987). Recovery and in vitro culture of the zygotic embryos at a stage early stage of development, is the only way to get the hybrids from crosses in which one or both parent varieties are seedless (Bharathy et al., 2005). Embryo culture propagation is used for obtaining and selection.

## MATERIAL AND METHODS

In the method conventional have been carried out four hybrid combinations, using the genitor seedless varieties and varieties of flowers normal hermaphrodite. The objective pursued in experiences carried out has been developing culture media favorable seed to maintain the viability and excised-sample test results by default save immature embryos. For this purpose have been tested several types of nutrient media. In vitro growth processes are directed in particular to the hormone use in the environment. Nutrient media are distinguished by the content of macro and trace elements, but also on the report auxine / cytokinine thus, in dependence of the intended purpose they have been used following media:

1. Immature embryos to save environments have been used:

• M1 - macroelemente Nitsch & Nitsch and micronutrients after MS ( Murashige-Skoog , 1962) supplemented, 6 g/l agar, 30 g/l sucrose, 100 mg ascorbic acid and 100 mg citric acid; M2 - micro, macroelemente after MS ( Murashige-Skoog , 1962), and liquid. The medium was supplemented with: 10 mg/l THAT (indolilacetic acid) 0,05 mg-biotin, 100 mg ascorbic acid and 100 mg citric acid; M3 - micro, macroelemente after MS ( Murashige-Skoog , 1962). The medium was supplemented with: 10 mg/l (indolilacetic acid), 0,05 mg-biotin.

2. For maintaining viable seed has used the medium MS (Murashige-Skoog, 1962). supplemented with: 20 mg/l that is why (indolilacetic acid), 3 mg/l BAP, 0, 05 mg-biotin, 6 g/l agar, 30 g/l sucrose, 100 mg ascorbic acid and 100 mg citric acid. Environments described differs from both after the factor a hormonal and after pH value, which varies within the range 5.8 -7.0. Explants inoculation has been carried out under aseptic conditions into the speaker or in laminar air flow sterile.

Material has been taken from the SCDVV Bujoru collection ampelographic where they carried out the work of hybridization. Grapes were harvested at 69 and 86 days after pollination. After seed inoculation on the medium culture, the vessels have been kept under controlled conditions of the medium 25-27 °C, ensuring a photoperiod 16 hours light and 8 hours dark. Choosing the optimal temperature, the optimization of other factors externally induced, which contributes to the completion framework for the conduct of the multiplication process, growth and differentiation, has been taking account of the circumstances to which it is adapted to ecological vines in natural circumstances of cultivation. The bird seed in early stage on nutrient media

fresh is mandatory to maintain the viability. This procedure has been carried out at regular intervals to 5 weeks. Duration of a subculture has been 30 days.

## RESULTS AND DISCUSSIONS

As a result intraspecific hybridization has been obtained in all four grape hybrid combinations. Kernels were harvested at 2 different stages namely: 69 days to 86 days after pollination. Most have had an average weight of between 0, 35 -0, 47 g to those harvested at 69 days after pollination (table 1). The difference in weight was not significant. The smallest weight is obtained to the combination Seedless white x Kismis white. The ratio of grain/ova signifies the number of ova recovered from grain usable. The report heavily/ovum has been greater than to the combination Gelu x Kismis black 1.44 , while the combination Seedless white x Kismis white has had the lowest report 1.27 user's manual (table 1). Variation in seed grain can be amended by begets pattern and/or endogens hormones levels. Another aspect would be that the number of seeds/heavily is a character intrinsic genotype.

Table 1

The genitors influence on grain characteristics

| Combination hybrid            | Days after pollination | No. of the berries used | Weight of berries (g) | No. of seed excised | Report berries/seed | Seed size mm | Average weight of the seed mg |
|-------------------------------|------------------------|-------------------------|-----------------------|---------------------|---------------------|--------------|-------------------------------|
| Madeleine Angevine x Afuz Ali | 69                     | 300                     | 0,37                  | 420                 | 1,40                | 0,5          | 11,20                         |
|                               | 86                     | 445                     | 2,47                  | 623                 | 1,40                | 1,0          | 14,63                         |
| Azur x Kismis black           | 69                     | 150                     | 0.44                  | 208                 | 1,39                | 0.4          | 9,61                          |
|                               | 86                     | 149                     | 2,30                  | 193                 | 1,39                | 0,9          | 10,76                         |
| Seedless white x Kismis white | 69                     | 120                     | 0,35                  | 152                 | 1,27                | 0,3          | 8,14                          |
|                               | 86                     | 129                     | 1,55                  | 154                 | 1,27                | 0,8          | 9,75                          |
| Gelu x Kismis black           | 69                     | 200                     | 0,47                  | 288                 | 1,44                | 0,5          | 9,81                          |
|                               | 86                     | 203                     | 2,38                  | 284                 | 1,40                | 1,0          | 11,27                         |

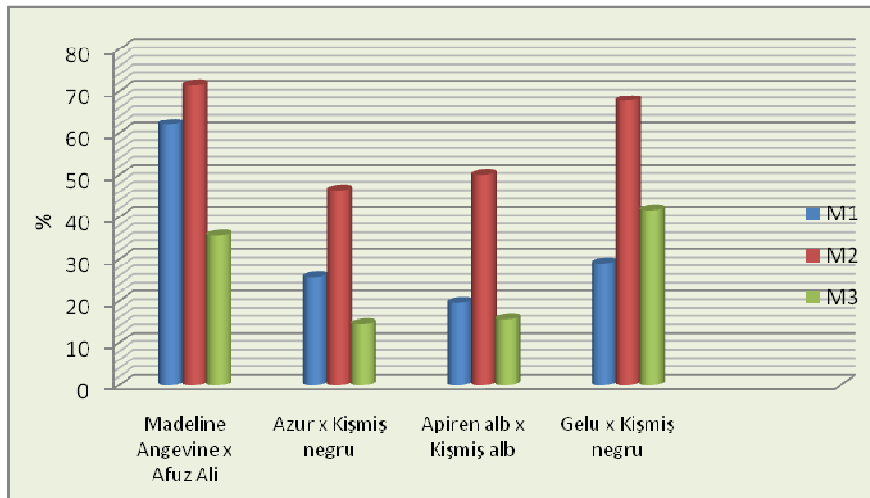
The variation in weight of the seed has been observed in all 4 combinations. The greatest weight was recorded to the combination Madeleine Angevine x Afuz Ali with 11.20 mg. The variation in weight of the seeds is given by genotypic parental characteristics, compatibility and the nutritional factories involved in the process of hybridization.

Berries harvest at 86 days after pollination brought us a plus as regards weight of grain which varied from 1.55 to Seedless white x Kismis white, and 2.47 to Madeleine Angevine x Afuz Ali. It should be noted that the ratio of

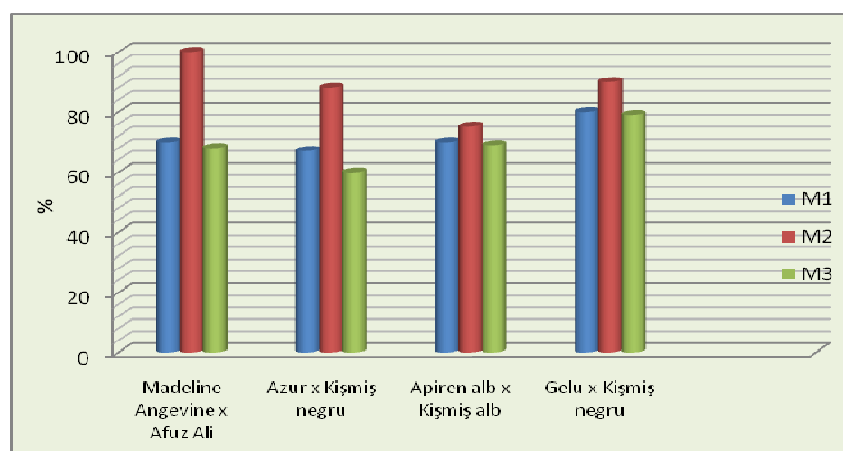
grain/ova has not changed in relation to stage of 69 days after pollination. Low ratio of ova despite a greater amount of weight of grain can be attributed to the effect of consagvinisation strenuousness, loss and exhaustion of the nutrient of the tissues in the developing berries. The variation in weight of the ovum has been observed in all four combinations. The greatest weight was recorded to the combination Madeleine Angevine x Afuz Ali with 14,63 g, and the lowest it has been registered with Seedless white x Kismis white respectively white 9.75 g.

Recorded results in experiments to save the embryos reflect the important role that clarity composition of the culture medium it owns in the assembly factors which makes the continued viability of seeds, conclusive proof in this respect be the effect different combinations of the same hormones in environments with basic composition sensitive different salts.

In figures 1-2 are highlighted the results of the summary on the influence of the culture medium of the percentage of viable seed after pollinisation able to germinal seeds. Seed viability rate has been associated with the type of culture medium and with the size/age inoculated seed. Significant results have been reported in case inoculation in culture medium M2- liquid medium. The supplement of the culture medium, 10 mg/l indolilacetic acid, 0, 05 mg-biotin, 100 mg ascorbic acid and 100 mg citric acid led to favorable results. The lowest rate of viability has been recorded in the case of the culture medium M3 to all hybrid combinations. In the case of M3, that there is no citric acid and the role-ascorbic antioxidant is reflected on viability low during the period of the seed inoculation. By comparing seed growing in their two stages, it has been demonstrated that the seed excised-sample test results at 86 days after pollination survived in a percentage of more than. Differences in nutrition, growth regulators and the age seeds lead to differences in survivability and germination of embryos.



**Fig. 1** - Influence of the culture medium of viability seeds harvested at 69 days after pollination



**Fig. 2** - Influence of the culture medium of viability seeds harvested at 86 days after pollination

Increase in embryos has been obtained directly on solid medium. It was not noticed callus development. In each seed has been obtained from single seedlings; what zygotic indicating the origin of these. Of a total of 2322 immature seed grown have been obtained 189 embryos viable (8 %), of which only 38 have generated vitro plants (table 2). The rate of viability of the embryos and the generation vitro plants was slightly greater than the 1.6 - 3, 7 % of seeds harvested at 86 days after pollination. Significant differences between the number embryos that have developed of the four crossovers, it shows that an important factor is genitors who participate in crossing. In the case combination Kismis white x Seedless white, the transfer vitro plants are problematic due to their considerable size small. As to the other three hybrid combinations that have a parent with seeds, vitro plants have had a good development of cotyledons.

*Table 2*

**Number of viable embryos obtained by growing seeds**

| Combination hybrid            | Days after pollination | No. of seeds cultivated | Viable embryos |     | Vitro plants regenerate from embryos |      |
|-------------------------------|------------------------|-------------------------|----------------|-----|--------------------------------------|------|
|                               |                        |                         | No.            | %   | No.                                  | %    |
| Madeleine Angevine x Afuz Ali | 69                     | 420                     | 32             | 7,6 | 7                                    | 21,9 |
|                               | 86                     | 623                     | 58             | 9,3 | 15                                   | 25,9 |
| Azur x Kismis black           | 69                     | 208                     | 18             | 8,7 | 3                                    | 16,7 |
|                               | 86                     | 193                     | 15             | 7,8 | 2                                    | 13,3 |
| Seedless white x Kismis white | 69                     | 152                     | 8              | 5,3 | 0                                    | 0    |
|                               | 86                     | 154                     | 13             | 8,4 | 1                                    | 7,7  |
| Gelu x Kismis black           | 69                     | 288                     | 20             | 6,9 | 4                                    | 20   |
|                               | 86                     | 284                     | 25             | 8,8 | 6                                    | 24   |

## CONCLUSIONS

1. To avoid necrosis seed it is very important addition in the environment for cultivation of 100 mg/l, abscisic acid and citric acid, substances with role antioxidant;
2. Use of the culture medium liquid provided with miss bridges of filter paper to highlight the rate of seed viability greater.
3. The culture medium and genotypes which participate in hybridize, the period of harvest of grain were influenced by embryo germination inoculate significantly.

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# RESEARCHES REGARDING *IN VITRO* REGENERATION CAPACITY OF THE GRAPEVINE VARIETY

## CERCETĂRI PRIVIND CAPACITATEA DE REGENERARE *IN VITRO* A UNOR SOIURI DE VIȚĂ DE VIE

**DONICI Alina**<sup>1</sup>

e-mail: donicialina@yahoo.com

**Abstract.** *Research has been conducted to determine the capacity in vitro multiplication of seedless grapevine varieties, defining the basic conditions for initiating, maintaining, proliferation regeneration and rooting explants. Biological material investigated was represented by two genotypes of grapes, Otilia and Călina, and biological material to initiation in vitro cultures was the apexes and meristems. A higher potential for regeneration was obtained from variety Călina. Viable plants were obtained by cultivating Murashige - Skoog medium (1962) supplemented with AIA and GA3.*

**Key words:** grapevine, in vitro regeneration, regeneration potential , explant, culture medium

**Rezumat.** *Cercetările au fost realizate în scopul determinării capacității de multiplicare in vitro a unor soiuri apirene de viță de vie , definerii condițiilor de bază pentru inițierea, menținerea – proliferarea, regenerarea, și înrădăcinarea explantelor. Materialul biologic investigat a fost reprezentat de două genotipuri de vița de vie, Otilia și Calina, iar materialul biologic pentru inițierea culturilor in vitro a fost reprezentat de apexuri și meristeme. Un potențial mai mare de regenerare s-a obținut la soiul Calina. Plantele viabile au fost obținute prin cultivarea pe mediu agarizat Murashige – Skoog (1962) suplimentat cu AIA și GA3.*

**Cuvinte cheie:** viță de vie, regenerare in vitro, potential de regenerare, explant, mediu de cultură

### INTRODUCTION

Most of the studies carried out for a century in the field in a plant vitro culture have been used to clarify several aspects of regeneration, growth, organogenesis, embryogenesis systemic and , of tissue structures or cell types, depending on the composition of the culture medium and to the conditions of eco physiologic containers of culture and of the room for growth. In vitro cultures were found in a very short period of time much practical application in improving numerous species of crop, by default to vines, on multiplication genotypes valuable, propagation of plants-free, conservation of genetic resources and more.

Healthy plants obtained by regeneration in vitro, grown in the field, as a rule they're canned vigurocity and productive capacity. The method of multiplication on aseptic environments propagation by growing in vitro of

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<sup>1</sup> Research and Development Station for Viticulture and Winemaking Bujoru, Romania

fragments derived from apices intense regenerative and meristems taken from the tip shoots, leads to the primordia caulinary in 20 -50 days of inoculation (Vişoiu, et al., 2000). Axillary buds are under dominance apical meristem technique is used: that once deleted increase meristems underarms, from shoots apices.

Induction processes of regeneration in vitro culture and vegetative multiplication depend to a great measuring tool of knowledge of mechanisms of operation of caulinary meristems. The higher the level of organization of inocul is most simple, the more nutrient needs and the culture conditions are more demanding, and nutrient substrate must be more complex and contain a wide range of organic substances. In this context, it considers a good knowledge of the factors which sequentially, or during the entire culture, may influence triggering organogenesis or carrying out processes. Most of the times, the reactivity of different explants, has been linked with the potential regenerative native genotypes studied (Vişoiu et al., 2006).

## MATERIAL AND METHODS

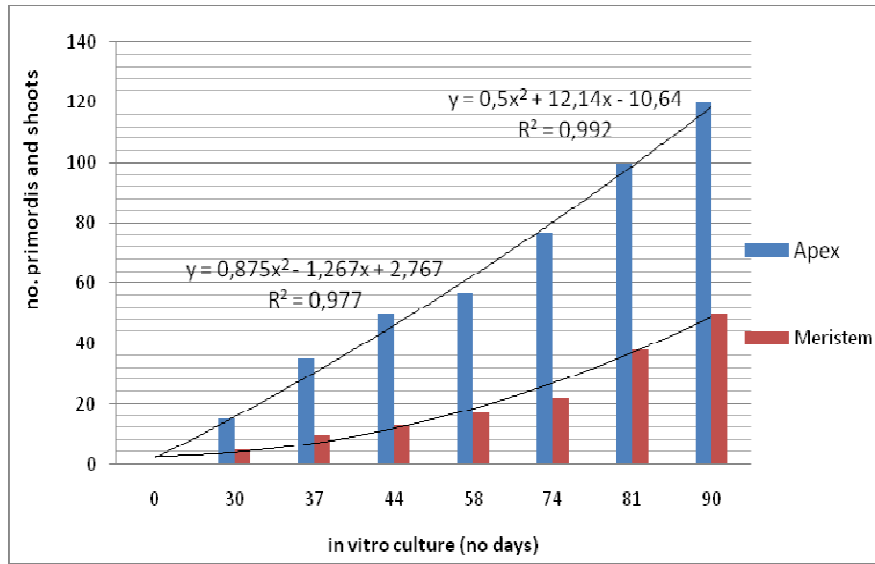
Biological material, as represented by apex and shoots axillary, has been taken from varieties *Vitis vinifera*. In the varieties of plant material and them Călina was harvested from the collection of ampelographic collection a SCDVV Bujoru. Sampling explants has been carried out in shoots harvested during the growing season. Explants used for initiating cultures in vitro have been disinfected with calcium hypochlorite ( $\text{CaCl}_2\text{O}_2$  -6 %), in sterile conditions in fume cupboard with laminar, for five minutes to small apices and meristems. Initiating and multiplication Călina variety and they have been carried out on the culture medium Murashige and Skoog (M&S, 1962), supplemented with 1 mg/l benzilaminopurine (BAP) and 0,5 mg/l  $\beta$ - indolacetic acid (IAA). As a source of carbon was used sucrose (20 g/l), and for solidification culture medium use has been made of agar-agar as a function of the amount of 6 g/l. Rooting shoots it has been on the Murashige and Skoog (M&S, 1962) to one-half, supplemented with 2 mg/l IAA, in the presence of 10 g/l sucrose; pH of the media was adjusted to 5.7 -5.8, before autoclaving. Sterilization vessels and of the culture medium was made by autoclave at  $120^\circ\text{C}$  (1 atm.), for 20 minutes. The inoculation operations and transfer to the medium fresh have been carried out in the spaces sterile. Culture medium inoculated was maintained under controlled conditions, the temperature  $25 \pm 10^\circ\text{C}$ ; for and lighting within the limits of 16 hours light. A periodic bird to small shots and bird fragment of callus on nutrient media fresh is mandatory to maintain the viability.

## RESULTS AND DISCUSSIONS

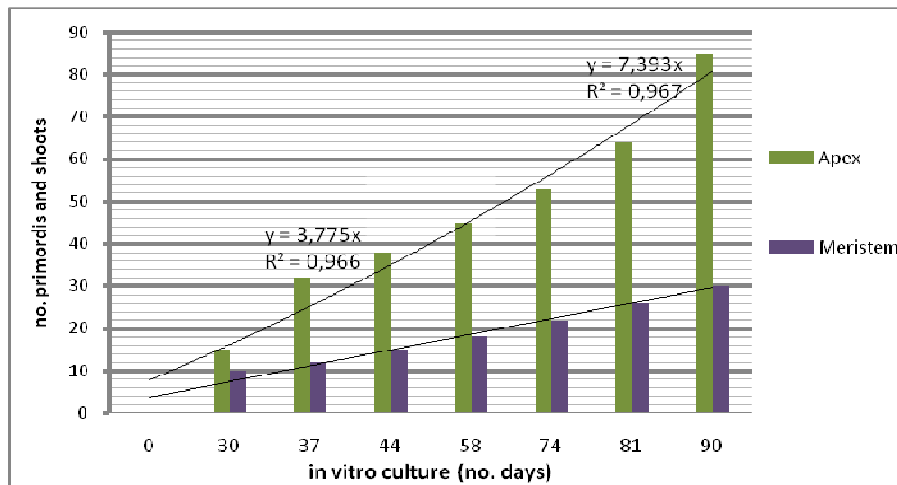
The first days of inoculation, have been carried out observations of processes of growth and differentiation of explants inoculated. After the first week of the cultivation at the explants have been reported their first signs of reaction.

It has pursued regenerative capacity in-vitro cultivar depending on the components donor explants, their nature and culture medium used. This aspect has been pursued on a culture medium Murashige and Skoog (M&S, 1962), supplemented with 1 mg/l benzilaminopurine (BAP) and 0,5 mg/l  $\beta$  indolacetic acid (IAA) which has shown that explants originating in apices have been intensely .

Regenerative potential of the varieties has been carried out after 90 days of culture in vitro, at which time explants reaction has become positive, for the purposes of training resulting from play against as a result of stimulation at the level existing vegetative explants. A significant increase in the plants was carried out after a period of 140 days. The best results under the aspect regeneration, of the number of small plant and rysogenesis has been carried out at the explants harvested from apices at both varieties. It should be noted that the values of the indices multiplication was assessed as being in a report in proportion to the number ignorance isn't linked and can be fully justified in this experiment the influence and the nature explants genotyping. (fig. 1-2).



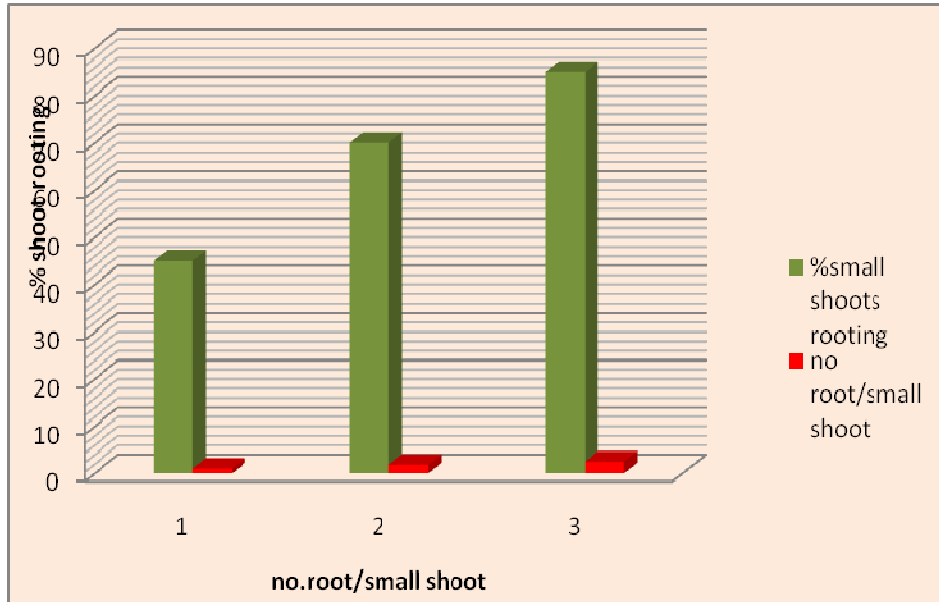
**Fig. 1** - The regenerative potential of Călina grapevine variety



**Fig. 2** - The regenerative potential of Otilia grapevine variety

For the function of rizogen small shoots detach regularly subculture, have been made comments during the sequence of taking root all over. Duration of the process of rysogenesis has been 50 days. So, based on the culture medium specific (1/2 M&S + 1 mg/l AIA), it is noted that small shoots coming from apices of 0.9 -3 cm have presented primordis root in proportion of progressed 30-45 %, after 15-20 days.

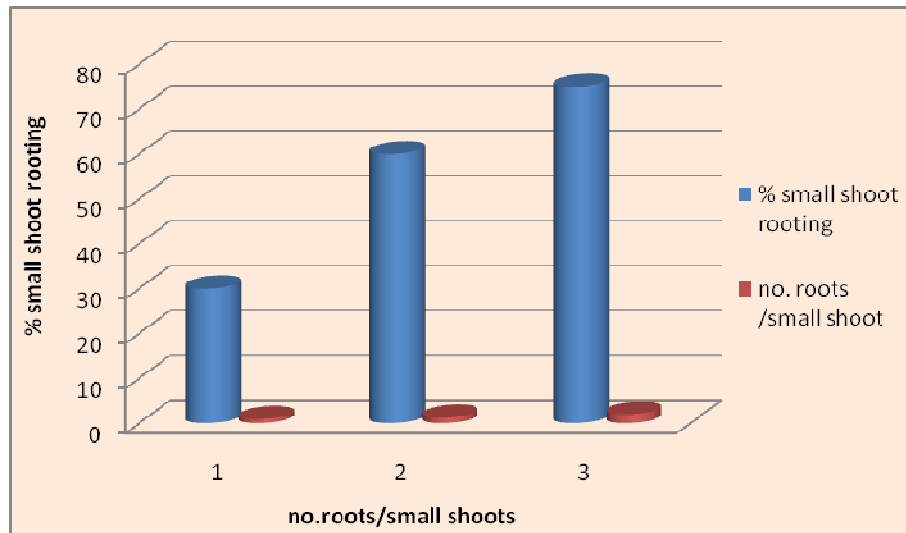
After 25- 27 days primordis root have developed in the 2- 2.5 cm to 60-70% of small shoot inoculate. Observations and tests carried out on biological material is inoculated on average rooting showed that the process of rysogenesis was completed after about 50 days when 75-85% of small shoots formed part of a well-developed system roots (fig. 3-4) , and shoots have been raised up to 4-7 cm.



**Fig 3 - Morphogenesis of Călina grapevine variety**

In the case coming from meristems foliar, small shoots had a production low, have shown primordis root in a rate of 10-25 %, after 15-20 days. After 25- 27 days the root primordis have developed in radices of 1.5 - 1.8 cm to 35-45% of small shoots inoculating, and after 50 days only 50% of shoot formed part of a well-developed system roots.

Another aspect that was noted was that a part of cultures have had an indirect regeneration through the formation of his gag. The callus diameter had sizes between 0.4 -1.8 cm, for a consistency slightly soft, green in color. Its capacity to regenerate the calus has been insignificant



**Fig. 4 - Morphogenesis of Otilia grapevine variety**

## CONCLUSION

1. Results of the research on the expression capacity of the two types of explants, varieties and they Călina, depend on the origin of explants;

2. A significant increase in the rate of multiplication has been registered after approximately 140 days of cultivation in vitro;

3. Type of meristem influenced significantly the plant neoformation rooted. Developments in the best have had the apical meristem;

4. The process of rysogenesis to biological material is inoculated on average nursery has been completed after approximately 50 days when 80-85% of small shoot formed part of a well-developed system roots (roots main ramifications secondary and tertiary), and shoots were height until 4-7 cm;

5. The difference of potentials of organogenesis of the two types of explants can be attributed to cellular metabolism of apices more active.

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# THE INFLUENCE OF TRELLISING SYSTEM ON PHENOLIC COMPOSITION OF GRAPES AND WINES FROM GREEK VARIETY XINOMAVRO

## INFLUENȚA SISTEMULUI DE CONDUCERE A VIȚELOR ASUPRA CONȚINUTULUI DE COMPUȘI FENOLICI ÎN STRUGURII ȘI VINUL SOIULUI GRECESC XINOMAVRO

**KYRALEOU Maria<sup>1</sup>, KALLITHRAKA Stamatina<sup>1</sup>, PROXENIA N.<sup>1</sup>,  
KOUNDOURAS S.<sup>2</sup>, KOTSERIDIS Y.<sup>1</sup>**  
e-mail: mkyraleou@yahoo.gr

**Abstract:** *The effects of three trellising systems Guyot, Royat and Lyre on phenolic composition in grapes and wines from Vitis vinifera var. Xinomavro have been studied. Total phenolic and anthocyanin content, tannin content, anthocyanin extractability (AE%) and antioxidant activity were measured in berries. Individual monomeric anthocyanins of skin extracts and wines were determined by high performance liquid chromatography. The results showed that the grapes derived from the Lyre system were richer in anthocyanins and total phenols in comparison with the grapes derived from the other two training systems studied. Moreover, it was observed, that grapes from the Lyre system were characterized by more anthocyanins. Significant differences were also observed among wine anthocyanin content. However, the training system did not affect significantly wine total phenolic and antioxidant activity.*

**Key words:** var. Xinomavro, trellising systems, total polyphenolic content, anthocyanins, antioxidant activity

**Rezumat:** *Articolul studiază efectele a trei sisteme de conducere Guyot, Royat și Lyre asupra compoziției fenolice a strugurilor și vinurilor din Vitis vinifera var. Xinomavro. S-a analizat conținutul fenolic total și conținutul de antociani, dar și extractibilitatea antocianilor (AE%), și capacitatea lor antioxidantă, în struguri. Antocianii monomerici din piețile strugurilor și din vinuri au fost analizați prin cromatografie lichidă. Rezultatele demonstrează că strugurii din viile conduse tip Lyre au o concentrație de antociani și compuși fenolici totali mai mare decât strugurii butucilor celorlalte sisteme de conducere. S-a observat, în plus, că strugurii obținuți prin utilizarea sistemului Lyre au o cantitate mai mare de antociani. Diferențe semnificative au fost observate și la conținutul de antociani din vinuri, totuși, sistemul de conducere nu a afectat în mod semnificativ conținutul total de antociani și activitatea antioxidantă a vinurilor.*

**Cuvinte cheie :** var. Xinomavro, sisteme de conducere, conținut total de polifenoli, antociani, activitate antioxidantă

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<sup>1</sup> Department of Food Science & Technology, Agricultural University of Athens, Greece

<sup>2</sup> Laboratory of Viticulture, School of Agriculture, Aristotle University of Thessaloniki, Greece

## INTRODUCTION

Grape phenolic compounds besides their antioxidant properties are very important constituents of wines since they contribute to color, astringency and bitterness (Ribereau-Gayon et al., 1999, Río Segade et al., 2008, Río Segade et al., 2009), oxidation reactions, interactions with proteins and ageing behavior of wines (Hertog et al., 1997).

Several strategies for increasing the content of phenolic compounds in wine have been made during the process of wine making and grape growing. Modification on the vine trellising system is one of the techniques applied to vines for this purpose. Trellising system is the arranging of the vine in space. It could affect the proportion of leaves to fruits, fruit zone microclimate, grape maturity and as a consequence the phenolic and anthocyanin composition of grapes (Ribereau-Gayon et al., 1999, Río Segade et al., 2008, Río Segade et al., 2009, Smart, R. and Robinson, M., 1991, Downey et al. 2004, González-Neves et al. 2004, Mota et al. 2011, Orlandini et al. 2008).

Xinomavro is the main red grape variety cultivated in North Greece. Grapes and wines produced from this variety are characterized by high acidity, phenolic richness and high ageing potential. Fresh wines usually contain “hard” tannins and have a unique aromatic character consisting of fruity and vegetal aromas. The cultivation of Xinomavro is difficult and is a challenge for the winemakers. To increase the tannin and anthocyanin concentration of Xinomavro wines, local grape growers are applying viticultural practices that, according to literature, are considered beneficial such as modifications in training systems. However, there is a lack of information concerning the relationship between practical implications and wine quality due to the uniqueness of this grape variety and its limited cultivation.

In this study, three different training systems Guyot, Lyre and Royat were applied in the vineyard and the quality of the produced grapes and wines was determined based on two quality parameters; anthocyanin content and tannin composition.

## MATERIAL AND METHODS

### ***Experimental conditions***

The experimental vineyard was situated in North Greece, in the region of Naoussa and was planted with *Vitis vinifera* L. cv. Xinomavro. Samples were collected from three different trellising systems Guyot, Lyre and Royat, at maturity in September 2010 in triplicates for each treatment and were kept frozen until analysis. Three red wine vinifications took place, one for each treatment.

### ***Analyses of grapes***

#### ***Phenolic compounds in whole berries***

50 berries from each treatment were homogenized using Ultra Turrax T25 at 24.000 rpm for 1min.

Total phenols and anthocyanin content were measured according to Iland et al. (2004) method: 1 g of the homogenate (in triplicate) was transferred into a centrifuge tube. An amount of 10 mL 50% v/v aqueous ethanol, pH 2, was then added and mixed for 1 hour. After centrifugation at 3500 rpm for 10 minutes, 0.5 mL of the



supernatant was added to 10 mL 1M HCl and mixed thoroughly. After 3 hours, absorbances at 520 nm and 280 nm were recorded.

Potential anthocyanins, extractable anthocyanins and phenolic composition of skins (dpell) and seeds (dTpep) were determined according to *Glorie's* method (1, 11) with some modifications. An amount of 20g of the homogenate was macerated for 4 hours in two different buffers. The pH of the first buffer was 1 and of the second 3.6. After 4 h, the macerated samples were centrifuged (4000 rpm, 10 min) and were used for the measurement of the anthocyanin and total phenolic content.

#### *Phenolic content and antioxidant activity in seed and skins extracts*

Seeds and skins of 150 berries were manually removed from grapes, lyophilised for 2 days and stored at -20 °C. The frozen seeds and skins were finally ground to obtain powder.

Extractions of seeds were carried out according to the well established methods (Chira et al. 2009, Lorrain et al. 2011). A 3 g portion of the obtained powder was extracted using 25mL of acetone/ water (80:20, v/v) for 3 h and 25 mL of methanol/water (60:40, v/v) for 2.5 h. After centrifuge, the supernatants were combined and evaporated under reduced pressure at 30 °C to remove organic solvents; the residue was then dissolved in water and lyophilised to obtain a crude tannin extract. Crude extracts were resolubilised in a model solution (12% ethanol; 5g/L tartaric acid; pH 3.5 adjusted with 1N NaOH) for the determination of total phenol content by Folin Ciocalteu test and antioxidant activity.

Anthocyanins were extracted with acidified methanol (0.1% HCl 12 N) from 1 g of dried skin powder at three successive times (for 4, 18 and 24 h). After centrifuge, the supernatants were combined and analysed for total anthocyanins (Ribereau-Gayon et al., 1999) and antioxidant activity (Brand-Williams et al., 1994). In addition, HPLC analysis was carried out according to Kallithraka et al. (2005) and Kallithraka et al. (2006) for the determination of monomeric anthocyanins. Identification was based on comparing retention times and UV spectra of the peaks detected with those of original compounds. Quantification was performed by establishing calibration curves for each compound determined, using the standards. Results were expressed as mg malvidin per dry skin weight. All analyses were performed in triplicate.

#### **Analyses of wines**

In wines several classical analysis (%vol, hue, color intensity, total polyphenols - OD280, pH, total acidity) were carried out immediately after bottling. In addition, total phenolic content (Folin–Ciocalteu test) (Waterman and Mole 1994), total anthocyanin content, ionization index, total tannins (Ribereau-Gayon et al., 1999), antioxidant activity (Brand-Williams et al., 1994) and monomeric anthocyanins by HPLC (Kallithraka et al., 2005, Kallithraka et al., 2006) were determined after 3 and 12 months of bottling. All analyses were performed in triplicate.

#### **Statistics**

Data were subjected to one-way analysis of variance (ANOVA), of Statistica V.7 Software (Statsoft InC., Tulsa, OK). Comparison of mean values was performed using Tukey's HSD test in case that samples were significantly different ( $p < 0.05$ ).

## **RESULTS AND DISCUSSION**

### ***Grapes***

As reported in table 1, the grapes of the lyre trellising system contained significantly higher values of total and extractable anthocyanins. In addition, their phenolic content was significantly higher than the corresponding contents of the Guyot and Royat trellising systems. Phenolics accumulated in grape skin are an

important determinant of wine quality. Although, there were no statistical differences regarding skin and seed tannin content, it was observed that the grapes from Guyot system contained less seed tannins in contrast with the grapes of the Lyre system which contained higher concentration of skin tannins.

Table 1

Analysis of grapes

| Specification                       | Royat                    | Lyre                     | Guyot                    |
|-------------------------------------|--------------------------|--------------------------|--------------------------|
| mg anthocyanins/berry               | 0,647±0,032 <sup>b</sup> | 0,883±0,016 <sup>a</sup> | 0,626±0,032 <sup>b</sup> |
| total anthocyanin mg/L juice        | 217,99±5,83 <sup>b</sup> | 252,75±4,85 <sup>a</sup> | 201,01±4,14 <sup>b</sup> |
| extractable anthocyanins mg/L juice | 123,69±6,38 <sup>b</sup> | 143,70±4,05 <sup>a</sup> | 121,85±6,52 <sup>b</sup> |
| anthocyanin extractability (AE%)    | 43,30±1,97 <sup>a</sup>  | 43,09±2,00 <sup>a</sup>  | 39,46±1,97 <sup>a</sup>  |
| dpell (skin tannins)                | 4,94±0,25 <sup>a</sup>   | 5,74±0,16 <sup>a</sup>   | 4,87±0,26 <sup>a</sup>   |
| dTpep (seed tanins)                 | 23,72±0,41 <sup>a</sup>  | 27,69±1,40 <sup>a</sup>  | 26,01±0,96 <sup>a</sup>  |
| total phenolics (au)/berry          | 1,714±0,045 <sup>b</sup> | 2,170±0,054 <sup>a</sup> | 1,510±0,045 <sup>c</sup> |

Significant differences were found regarding total anthocyanin content and antioxidant activity of skin anthocyanin extracts (figure 1). The highest anthocyanin contents, was observed in grapes originated from the lyre system. However, these grapes showed the lowest antioxidant activity value.

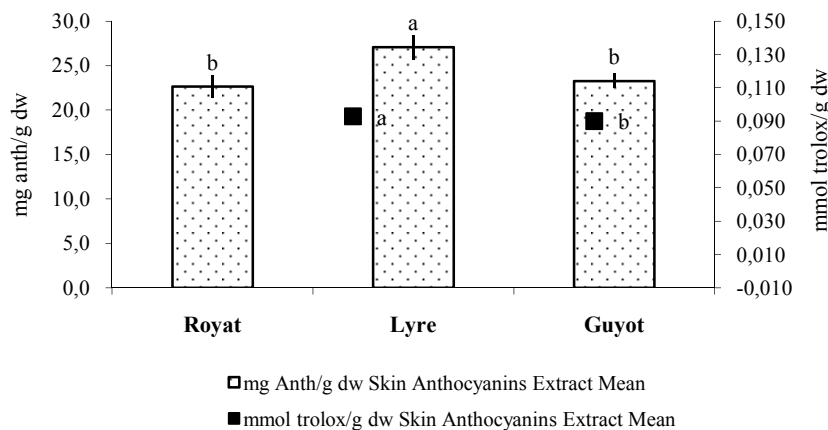


Fig. 1 - Total anthocyanin content and antioxidant activity of skin anthocyanin extracts

Figure 2 shows the individual anthocyanin content of the skin extracts obtained from the three trellising systems. There were not observed significant differences in the profiles of anthocyanins among the three different systems. These results are in contrast with those reported by other authors, who found that

the architecture of the plant and the pruning method may modify the anthocyanin profile (González-Neves et.al., 2004). However, the skin extracts obtained from the grapes of the lyre system contained the highest concentration of malvidin-3-glucoside, which is the main anthocyanin in grapes and wines.

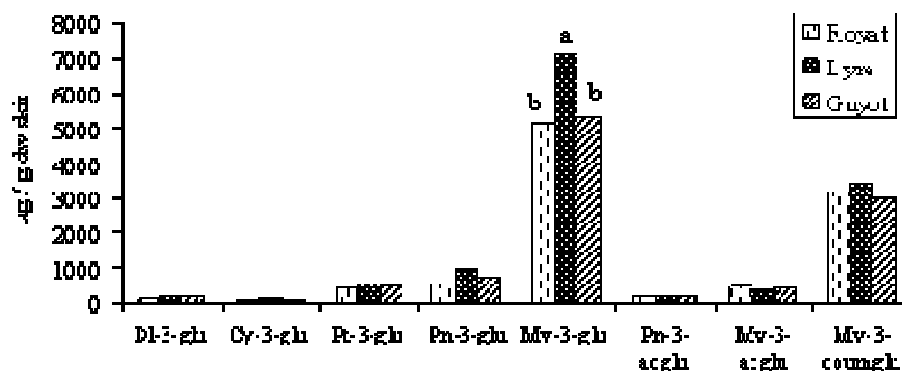


Fig. 2 - Anthocyanin profile of grape skin extracts obtained from the three trellising systems.

Trellising systems (Royat, Lyre, Guyot) did not significantly affected phenolic composition and antioxidant activity of skin and seed extracts (table 2). However, it could be observed that seed extracts were richer in phenolic compounds and showed higher antioxidant activity than the skin extracts.

Table 2

Total phenols and antioxidant activity of skin and seed extracts

| Specification                               | Organ | Royat                    | Lyre                     | Guyot                    |
|---|-------|--------------------------|--------------------------|--------------------------|
| total phenols<br>mg gallic<br>acid/g dw     | seeds | 121,46±1,02 <sup>a</sup> | 130,05±1,04 <sup>a</sup> | 122,86±4,62 <sup>a</sup> |
|   | skins | 69,75±2,59 <sup>a</sup>  | 77,50±1,29 <sup>a</sup>  | 66,70±4,03 <sup>a</sup>  |
| antioxidant<br>activity<br>mmol trolox/g dw | seeds | 0,185±0,002 <sup>a</sup> | 0,234±0,003 <sup>a</sup> | 0,213±0,008 <sup>a</sup> |
|   | skins | 0,118±0,003 <sup>a</sup> | 0,134±0,005 <sup>a</sup> | 0,118±0,005 <sup>a</sup> |

### Wines

Table 3 shows the results obtained by the classical analyses of the wines immediately after bottling. No statistical differences were observed regarding alcohol content; color hue and phenolic content (OD280). As far as lyre system is concerned, wines were characterized by higher color intensity and lower total acidity. This parameter is of high importance since wines from Xinomavro variety are often characterized by high acidity values.

Table 3

## Analytical parameters of wines

|       | %vol              | Intensity         | Hue               | OD 280          | pH                | total acidity (g/L) |
|-------|-------------------|-------------------|-------------------|-----------------|-------------------|---------------------|
| Royat | 15,6 <sup>a</sup> | 4,26 <sup>b</sup> | 7 <sup>a</sup>    | 45 <sup>a</sup> | 3,31 <sup>b</sup> | 7 <sup>a</sup>      |
| Lyre  | 15,5 <sup>a</sup> | 4,77 <sup>a</sup> | 0,68 <sup>a</sup> | 44 <sup>a</sup> | 3,41 <sup>a</sup> | 6,4 <sup>b</sup>    |
| Guyot | 15,5 <sup>a</sup> | 4,02 <sup>b</sup> | 0,68 <sup>a</sup> | 40 <sup>a</sup> | 3,3 <sup>b</sup>  | 6,8 <sup>a</sup>    |

Table 4 shows the results obtained after analyzing the wines during ageing. In all cases studied, the concentration of anthocyanins was decreased, however the higher values were observed in wines originated from the Lyre system. Furthermore, Guyot system resulted in wines with higher ionization indexes and lower absorbance's at 280 nm. As far as the other analytical parameters are concerned, trellising system did not affect the tannin content and antioxidant activity of the wines studied.

Table 4

## Analytical parameters of the wins after 3 and 12 months of bottling

|                        | ionization index%   | total anthocyanins mg/L | intensity         | hue               | OD 280             | pH                | total tannins (g/L) | antioxidant activity mMtrollox | total phenolics g gallic acid/L |
|------------------------|---------------------|-------------------------|-------------------|-------------------|--------------------|-------------------|---------------------|--------------------------------|---------------------------------|
| <i>after 3 months</i>  |                     |                         |                   |                   |                    |                   |                     |                                |                                 |
| Royat                  | 71,28 <sup>b</sup>  | 46,52 <sup>b</sup>      | 4,11 <sup>b</sup> | 0,71 <sup>a</sup> | 45,54 <sup>a</sup> | 3,42 <sup>b</sup> | 2,37 <sup>a</sup>   | 1,56 <sup>a</sup>              | 2,21 <sup>a</sup>               |
| Lyre                   | 66,00 <sup>c</sup>  | 51,13 <sup>a</sup>      | 4,28 <sup>a</sup> | 0,74 <sup>a</sup> | 43,08 <sup>a</sup> | 3,56 <sup>a</sup> | 2,42 <sup>a</sup>   | 1,59 <sup>a</sup>              | 2,38 <sup>a</sup>               |
| Guyot                  | 87,70 <sup>a</sup>  | 42,54 <sup>b</sup>      | 3,91 <sup>c</sup> | 0,72 <sup>a</sup> | 38,29 <sup>b</sup> | 3,41 <sup>b</sup> | 2,04 <sup>a</sup>   | 1,62 <sup>a</sup>              | 1,82 <sup>b</sup>               |
| <i>after 12 months</i> |                     |                         |                   |                   |                    |                   |                     |                                |                                 |
| Royat                  | 96,43 <sup>b</sup>  | 26,30 <sup>b</sup>      | 4,23 <sup>a</sup> | 0,89 <sup>a</sup> | 42,60 <sup>a</sup> | 3,51 <sup>b</sup> | 1,92 <sup>a</sup>   | 1,44 <sup>a</sup>              | 2,40 <sup>a</sup>               |
| Lyre                   | 82,50 <sup>c</sup>  | 32,75 <sup>a</sup>      | 4,01 <sup>b</sup> | 0,88 <sup>a</sup> | 41,58 <sup>a</sup> | 3,66 <sup>a</sup> | 2,06 <sup>a</sup>   | 1,39 <sup>a</sup>              | 2,72 <sup>a</sup>               |
| Guyot                  | 108,86 <sup>a</sup> | 28,44 <sup>b</sup>      | 3,49 <sup>c</sup> | 0,85 <sup>a</sup> | 35,94 <sup>b</sup> | 3,56 <sup>b</sup> | 1,81 <sup>a</sup>   | 1,46 <sup>a</sup>              | 1,65 <sup>b</sup>               |

The results obtained regarding malvidin-3-*O*-glucoside content of the wines were similar to those obtained for the skin extracts (figure 3). Malvidin-3-*O*-glucoside content of wines originated from Lyre system was significantly higher than the corresponding contents of the wines originated from the other two systems. In addition, trellising system did not affect the profiles of anthocyanins determined by HPLC.

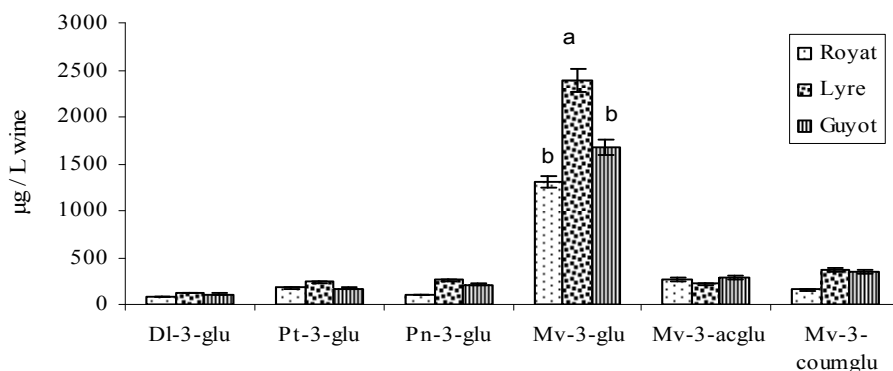


Fig. 3 - Anthocyanin profile of wines obtained from the three trellising systems.

## CONCLUSIONS

In conclusion, trellising system may affect grape anthocyanin concentration and total phenolic content. Grapes from Lyre system contained more anthocyanins and malvidin-3-*O*-glucoside, while berries from Guyot system were the poorest in total phenolics. Total tannin content, antioxidant activity and individual anthocyanin content of grapes originated from the three training systems did not differ significantly.

Regarding wines, the trellising system had a significant effect on anthocyanin concentration, color intensity, total acidity, pH and total phenolic content. Higher anthocyanin concentration, color intensity, pH values and lower total acidity were observed in wines from Lyre system, while Guyot system resulted in wines with lower total phenolic content. Finally, trellising system did not affect alcoholic content, color hue, total tannin concentration, antioxidant activity and anthocyanin profiles of the wines studied.

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# THE EVOLUTION OF THE MAIN METEOROLOGICAL PARAMETERS (YEAR 2011) IN THE EASTERN PART OF WINE REGION MOLDOVA HILLS

## EVOLUȚIA PRINCIPALILOR PARAMETRII METEOROLOGICI (ANUL 2011) ÎN PARTEA DE EST A REGIUNII VITICOLE A DEALURILOR MOLDOVEI

ROTARU Liliana<sup>1</sup>, AMĂRIUCĂI M.<sup>2</sup>, COLIBABA Cintia<sup>3</sup>, MACHIDON O.<sup>2</sup>  
e-mail: lirotaru@uaiasi.ro

**Abstract.** *Vine is a plant with a large plasticity and it adapts easily to different areas of culture, while the influence of climate and weather trends in a given time have a decisive role on the quality of the obtained products. The article aimed at studying the spatial distribution of meteorological parameters in the area of the eastern part of the Moldova Hills wine region during 2011. The results obtained show that, except in periods of heat, in the summer, when thermal stress exceeded the critical threshold of air and soil and an increase in droughts soil appeared, particularly in the southern part of Moldova, the year 2011 was favourable to vine culture.*

**Key words:** vine, agro-meteorology, climate, wine region

**Rezumat.** *Vița de vie este o plantă cu o largă plasticitate ecologică, adaptându-se cu ușurință în diverse areale de cultură, iar influența factorilor climatici cât și evoluția lor meteorologică într-un anumit interval de timp au un rol hotărâtor asupra calității produselor obținute. În lucrare a fost urmărită distribuția spațială a parametrilor meteorologici în arealul părții de est a regiunii viticole a Dealurilor Moldovei pe parcursul anului 2011. Rezultatele obținute au evidențiat faptul că, cu excepția unor perioade de caniculă din sezonul cald al anului când s-a depășit pragul critic al stresului termic din aer și sol și a avut loc o accentuare a fenomenului de secetă pedologică, în mod deosebit în sudul Moldovei, anul 2011 a fost favorabil culturii viței de vie.*

**Cuvinte cheie:** viță de vie, agrometeorologie, climă, regiune viticolă

### INTRODUCTION

The Romanian climate characteristics are given by the country's geographical position and air circulation in the earthly atmosphere. Because of its geographical position, mainly because of the tropical air masses blending with polar air masses, the Romanian climate is directly influenced by the great baric systems from Europe.

The viticultural plantations are situated, almost entirely, on the eastern sub-Carpathian hills, as well as on other hilltops and slopes, mostly along the rivers

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup> University "Al. I. Cuza" of Iasi, Romania

<sup>3</sup> Technical University "Gheorghe Asachi" of Iasi, Romania

that cross the Moldavian Plateau.

The relief insures very favourable conditions to vine culture, as it has many microareas with specific ecoclimates. In its whole, the ecoclimate, with strong east-European influences, is characterised through heliothermic resources with high values, the hydric resources being of course lower. The predominant climate is that of silvo-steppe, with continental accents especially in the north-eastern part, with very warm and dry summers, harsh winters that usually endanger the unprotected vines in culture. The eco-climatic variations are very wide. The soils that are used for vine culture are found in a large segment: grey forest soils, cernoschernozem soils in different evolution stages, podsoils, chalky rendsiness, sands and sandy soils (Mustea, 2003).

The viticultural area has as main aim the obtaining of wines from a very large palette, while in the southern part, the culture conditions allow the obtaining of table grapes with economical value (Rotaru and Colibaba, 2011).

## **MATERIAL AND METHODS**

The agro-meteorological peculiarities of 2011 and their influence on the grape vine were analysed over critic characteristic time intervals on the vegetation state and productive potential. Meteorological parameters from the main meteorological station from the east side of the region (Cotnari, Iași and Dealurile Bujorului vineyards) were registered. The indices that were analysed are: the annual and monthly average temperature, the absolute monthly minimum and maximum temperature, annual and monthly average precipitations, number of frosty nights and days in winter, depth of the snow layer, humidity reserve accessible to the plants during April - July and August-September (grape maturation period).

## **RESULTS AND DISCUSSIONS**

From a thermal point of view, the year 2011 was warmer than normal, the average temperature varying from 8,6<sup>0</sup>C at Darabani to 10,5<sup>0</sup>C at Galați (fig. 1). Minimal temperatures were registered between -12,8<sup>0</sup>C at Cotnari and -20,4<sup>0</sup>C at Negrești in February and March. The maximal temperature was registered in August, between 32,2<sup>0</sup>C at Darabani and 36,0<sup>0</sup>C at Galați (tab.1).

Analysing the precipitations quantity (fig. 2) it was registered that, as a whole, Moldova region, in 2011, had a 168,8 l/m<sup>2</sup> deficit compared to the multiannual average. The highest values were registered in the north of the region, the maximum being attained at Darabani where the deficit was of 238,4 l/m<sup>2</sup>. At the opposed pole is the southern part of the analysed region, the minimum being registered at Bârlad with a deficit of 70,0 l/m<sup>2</sup>.

Regarding the monthly evolution of precipitations during 2011, it can be registered that, except the months of April and June, all these areas were having a deficit in water in all of the other months (fig. 3). Especially interesting is the case of the month of November, when the whole region registered a deficit of 98% compared to the normal situation. A characteristic of the precipitations of 2011 was their relative homogeneity in space and time during April and July, fact that lead to the formation of a good water reserve accessible to the vines.



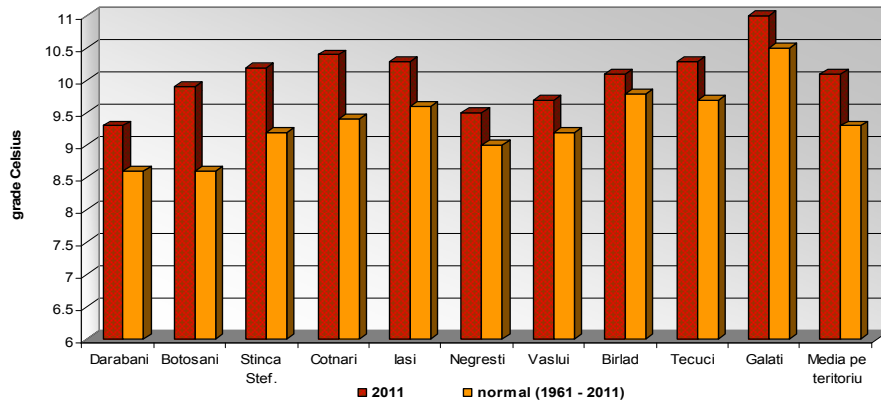


Fig. 1 – Average temperature in 2011 at meteorological stations from Moldova area (east of Siret river)

Table 1

Air temperature - °C (average, absolute minimum and maximum)

| Station    | Yearly average | Multiannual average (1961-2011) | Absolute minimum temperature (2011) | Absolute maximum temperature (2011) |
|------------|----------------|---------------------------------|-------------------------------------|-------------------------------------|
| Darabani   | 9,3            | 8,6                             | -17.8/5.01                          | 32,2/19.07                          |
| Botoșani   | 9,9            | 8,6                             | -20.0/5.01                          | 33,8/20.07                          |
| Stânca Șt. | 10,2           | 9,2                             | -17.0/5.01                          | 34,0/19.07                          |
| Cotnari    | 10,4           | 9,4                             | -12.8/16.02                         | 33,6/20.07                          |
| Iași       | 10,3           | 9,6                             | -16.6/5.01                          | 35,5/20.07                          |
| Negrești   | 9,5            | 9,0                             | -20.4/3.03                          | 34,7/20.07                          |
| Vaslui     | 9,7            | 9,2                             | -18.5/5.01                          | 34,5/19.07                          |
| Bârlad     | 10,1           | 9,8                             | -18.2/26.01                         | 34,3/19.07                          |
| Tecuci     | 10,3           | 9,7                             | -19.1/31.01                         | 34,3/9.07                           |
| Galati     | 11,0           | 10,5                            | -16.2/31.01                         | 36,0/9.07                           |
| Average    | 10,1           | 9,3                             | -20.4/3.03                          | 36,0/9.07                           |

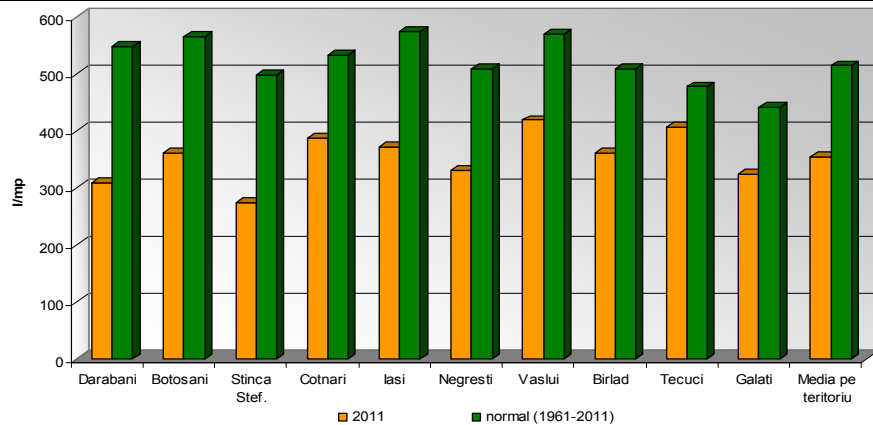
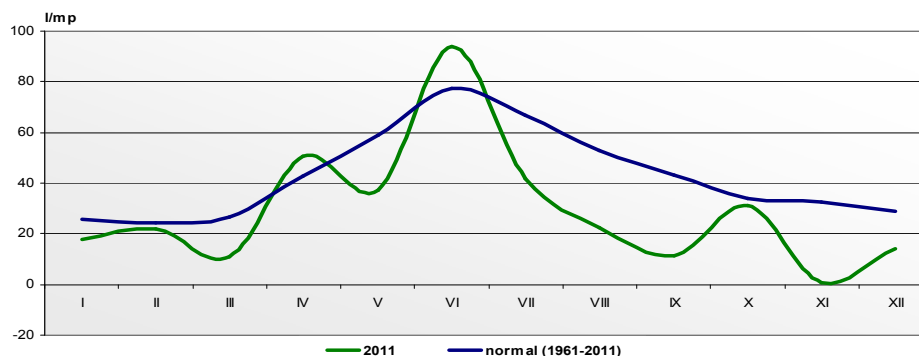


Fig. 2 – Average precipitations of 2011 in meteorological stations of Moldova (east of Siret river)



**Fig. 3** – Monthly regimen of 2011 compared to normal values of atmospheric precipitations in Moldova (east of Siret river)

2011 started with a gentle winter, characterised by a higher than usual thermal regime. In the last decade of January, the weather becomes cold, even frosty at nights and mornings, all over the agricultural soils of Moldova a layer of snow with a depth of 3 - 10 cm (northern half) being registered. In the 15 frosty nights and 38 winter days that were registered in average for the whole of Moldova (tab. 2), on viticultural areas without protective snow layer or a superficial one (under 10 cm), with extremely low minimal air temperatures, situated below the critical threshold for plants, partial damages were registered. The number of days with temperatures  $\leq -10^{\circ}\text{C}$  was higher in January (between 3 days at Cotnari and 11 days at Bârlad). Starting with the last decade of January, during the whole month of February and the first half of March, the soil was frozen at depths of 5-15 cm in the majority of the territory. Locally, the frost reached a depth of 20 cm in February. In the beginning of April, a level of average and good vegetation state of the vineyard was registered.

Table 2

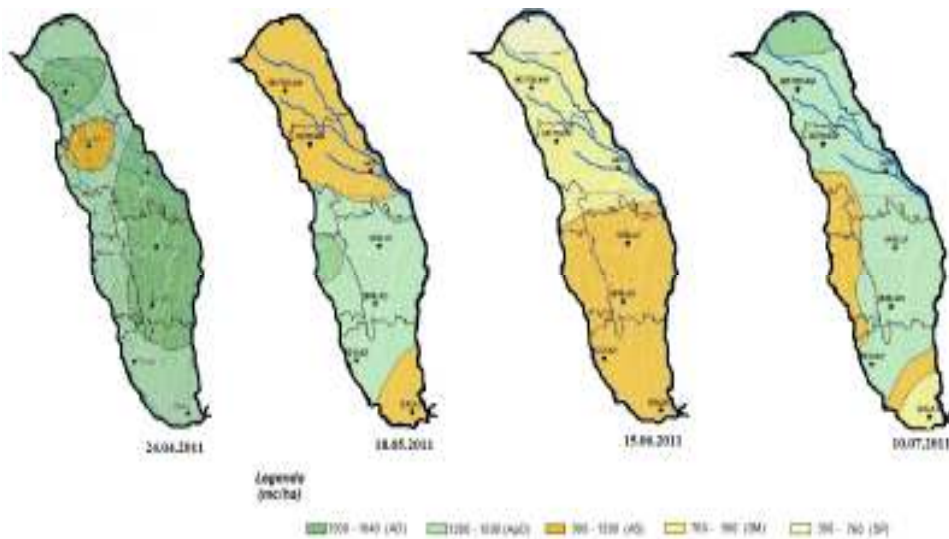
**Number of frosty nights, winter days and snow layer in 2011**

| Station    | Frosty nights (min, $\leq 10^{\circ}\text{C}$ ) (monthly sum) |    |     |      | Winter days (max, $\leq 0^{\circ}\text{C}$ ) (monthly sum) |    |     |     | Snow layer (cm) - monthly average- |    |     |     |         |
|------------|---|----|-----|------|--|----|-----|-----|------------------------------------|----|-----|-----|---------|
|            | I   | II | III | Suma | I  | II | III | Sum | I                                  | II | III | XII | Average |
| Darabani   | 6   | 4  | 2   | 12   | 16   | 17 | 5   | 38  | 6                                  | 5  | 3   | 1   | 3.8     |
| Botoşani   | 8   | 5  | 4   | 17   | 11   | 15 | 3   | 29  | 6                                  | 5  | 3   | 0   | 3.5     |
| Stânca Şt. | 9   | 3  | 5   | 17   | 16   | 17 | 5   | 38  | 2                                  | 3  | 2   | 0   | 1.8     |
| Cotnari    | 3   | 3  | 0   | 6    | 15   | 16 | 5   | 36  | 2                                  | 7  | 6   | 0   | 3.8     |
| Iaşi       | 8   | 3  | 2   | 13   | 14   | 16 | 1   | 31  | 6                                  | 4  | 1   | 0   | 2.8     |
| Negreşti   | 10  | 5  | 4   | 19   | 15   | 14 | 4   | 33  | 3                                  | 5  | 4   | 0   | 3.0     |
| Vaslui     | 10  | 5  | 3   | 18   | 16   | 15 | 2   | 33  | 5                                  | 6  | 3   | 0   | 3.5     |
| Bârlad     | 11  | 5  | 4   | 20   | 17   | 16 | 3   | 36  | 6                                  | 15 | 2   | 0   | 5.8     |
| Tecuci     | 9   | 7  | 2   | 18   | 15   | 15 | 2   | 32  | 5                                  | 6  | 2   | 0   | 3.3     |
| Galaţi     | 8   | 2  | 0   | 10   | 16   | 15 | 1   | 32  | 11                                 | 7  | 0   | 0   | 4.5     |

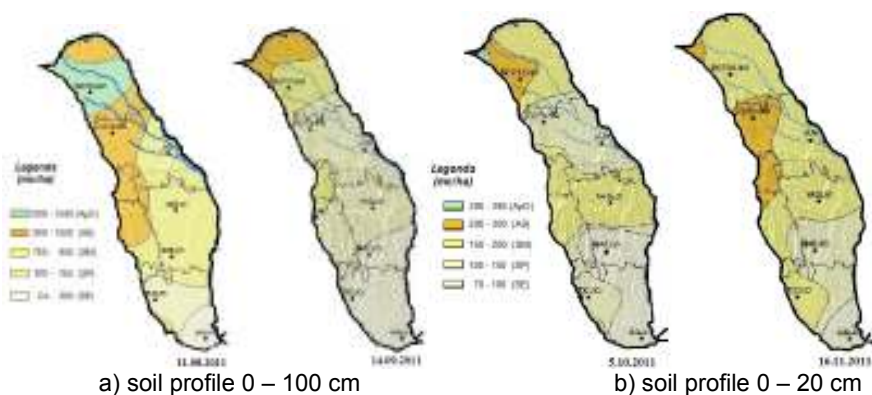
Regarding the accessible humidity reserve in the soil at 0-100 cm, at the beginning of spring, it was satisfactory (AS), close to the optimal (ApO) and optimal (AO) all over Moldova (900-1600 m<sup>3</sup>/ha). During May-July, when the vine passes through its most important vegetation stages, and the water request is the highest, the accessible humidity reserve for plants presented the same characteristics (fig. 4), fact that was due to a relatively uniform time repartition of precipitation quantities. The exception was the month of June, when, because of reduced rains and high evaporation, in the northern part of Moldova, a moderate pedological drought (SM) and even strong pedological drought (SP) appeared.

The favourable air and soil hydrothermal regime from the months of August and September 2011 registered normal vegetation rhythms of corn plants on the lands of the north and north-west of the studied territory where the soil water reserve was satisfactory and close to the optimum. The uniformity and vigour of the plants was good and average, respectively average and weak at phenologically late cultures and on agricultural surfaces affected by pedological draught phenomena (fig. 5a.).

Autumn started with warm weather, but the precipitations were reduced. During this season, because of low quantity of precipitations, the deficit of humidity content led to the establishment of a pedological drought, with different degrees of intensity, respectively average, strong and excessive (fig. 5b.), affecting somewhat the grape maturation. Due to the lack of water in the soil, the grapes had the possibility to accumulate important quantities of sugars, so that the wines were of quality, although the harvest loss was quite big.



**Fig. 4** – Accessible humidity reserve of plants in 0-100 cm level on Moldova territory (eastern of Siret river) during April – July 2011



**Fig. 5** – Humidity reserve of Moldova (east of Siret river) during August-November 2011

The end of autumn (November) and beginning of winter were characterised by the maintaining of a high thermal degree and the registering of very low quantities of precipitations. An important fact is that the snow layer was absent in all of the area, except its extreme northern part where it was insignificant (tab. 1).

## CONCLUSIONS

1. Except some drought periods of the warm season when the critical threshold of the thermal stress from air and soil, and the pedological drought phenomena was accentuated in the south of Moldova or the precipitations interval, that had a temporary torrential character that were sometimes teamed up with hail and short time wind enhancements, in general, 2011 was a year when the vine had a good and average vegetation state.

2. Exception is made by the period at the end of the year, characterised by deficit precipitations almost all over the territory, the vine being affected especially in the south of Moldova, the values of the precipitation quantities indicating pedological drought, under the resistance limit of the vine (250 mm). Therefore, in these areas, in some years, vine irrigation is necessary even if the vine is know for its drought resistance

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# FETEASCA REGALĂ CL.1 IȘ - A VALUABLE CLONE FOR WHITE WINES OBTAINED AT S.C.D.V.V. IASI

## FETEASCĂ REGALĂ CL.1 IȘ – O CLONĂ VALOROASĂ PENTRU VINURI ALBE OBTINUTĂ LA S.C.D.V.V. IASI

SAVIN C.<sup>1</sup>, DAMIAN Doina<sup>1</sup>, MĂNTĂLUȚĂ Alina<sup>1</sup>  
e-mail: statiunea\_viticola\_iasi@yahoo.com

**Abstract.** *The clonal selection carried out between the vine assortments aims to improve the agro biological and technological characteristics of the local, domestic and valuable varieties, and of the foreign ones introduced in the culture, that make up the traditional assortments of the country's vineyards. By the clonal selection work applied to Feteasca Regala in 2011 resulted the homologation of a new clone, which is characterized by average yields of 4,78 kg/vine, respectively 18,1 tons/ha calculated production, 16 % higher than the population assortment. The potential accumulation of sugars in the must of 208 g/L provides an increase of about 22 % compared to the reference assortment. The wines resulted from processing the grapes is characterized by an alcoholic potential between 11,3 and 12,2 % vol, non reducing extract of 24,6 g/L and 7,9 g/L glycerol, with a typical specific of the Feteasca Regala assortment.*

**Key words:** genotypes indigenous, clone selection, clones

**Rezumat.** *Selecția clonală efectuată în cadrul soiurilor de viță de vie are ca scop îmbunătățirea însușirilor agrobiologice și tehnologice ale soiurilor locale, autohtone, valoroase, sau a celor străine introduse în cultură, ce alcătuiesc sortimentele tradiționale ale podgoriilor țării. Prin lucrări de selecție clonală aplicate în cadrul soiului Fetească Regală, în anul 2011 a fost omologată o nouă clonă, care se remarcă prin producții medii de 4,78 kg/butuc, respectiv 18,1 tone/ha producție calculată, cu 16% mai mari decât soiul populație. Potențialul de acumulare a zaharurilor în must de 208 g/L asigură un spor de cca 22% față de martor. Vinurile rezultate în urma procesării strugurilor se caracterizează printr-un potențial alcoolic cuprins între 11,3 și 12,2 % vol., extract nereducător de 24,6 g/L și 7,9 g/L glicerol, cu tipicitate specifică soiului Fetească regală.*

**Cuvinte cheie:** genotipuri autohtone, selecție clonală, clone

### INTRODUCTION

The autochthonous valuable varieties cultivated today are a permanent source of germplasm, which is very important for the improvement of the biological material cultivated currently. The achievement of some varieties and clones of vine with superior characteristics of production and quality implies a continuous activity of selection and improvement applied within the genotypes of traditional varieties of famous vineyards. Along time, they were submitted to inappropriate cultural measures, genetic erosion, produced because of irregularities occurred in the

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<sup>1</sup> Research and Development Station for Viticulture and Vinifications Iasi, Romania

replication of the genome and of the viral infections transmitted through negative multiplication. This is the case of the Fetească regală variety, recommended and authorized for multiplication in the vineyard of Iași, Copou viticultural center, but due to its vocation of adaptability to different climatic conditions, it is currently cultivated in most viticultural areas of the country. Manifesting great variability of characters, the soil was submitted to a rigorous cloning selection, which led to the achievement of 3 valuable clones by the Station of Research and Development for Viticulture and Winemaking of Blaj, the National Institute of Research and Development for Biotechnologies in Horticulture of Ștefănești and the vineyard in Iași, which was homologated in 2011. In Romania, 88 clones of vine were obtained, of which 77 for wine grapes and 11 for table grapes (Damian et al., 2003, 2008, Moldovan et al., 2001, Popa, 2010, Savin et al., 2001).

## MATERIAL AND METHOD

The cloning selection works of the Fetească regală variety were initiated 16 years before, in the plantations cultivated with this variety at the Station of Research and Development for Viticulture and Winemaking of Iași. Initially, from the mother plantation, 16 cloning elites were selected and studied for 3 consecutive years as regards the ampelographic characters, the phytosanitary state, the productive potential and especially the qualitative potential related to the accumulation of sugars, as the purpose was to obtain a quality clone.

The cloning elites that presented stability of the desired characteristics were multiplied by grafting, and the resulted cloned material was used to create the comparative field, with 28 stocks for each elite. In this stage of the cloning selection diagram, we selected 6 cloning elites for future perspective, which were multiplied, ensuring 100 vines of each, with which a contest (verification) field was created, organized in five repetitions for each elite, according to the experimental technique rules and the diagrams of improvement through cloning selection. In order to compare results, the controls used were the average of elites and the variety population. After another 7 years of study, of which 3 years of fructification in the contest plantation, we observed a clone elite, with code 1, which corresponded to the proposed purpose and was tested by the State Institute for the Soil Testing and Registration with the purpose of homologation and whose results are the object of this study (fig. 1).



**Fig. 1** - Fetească regală clona 1 Is

Between 2009 and 2011, which was the final stage of the official diagram of vine clone selection, adopted nationally and internationally, the research continued according to the rules of the State Institute for the Soil Testing and Registration. The characters of distinctiveness of the clone were described in comparison to the population according to the descriptors of the UPOV. Concomitantly we carried out phytosanitary and conservative selection works, serological testing of the presence of the main viruses. For an objective appreciation of the qualitative characteristics of the new clone, we proceeded to vinifying grapes and completely testing the wines obtained from the clone meant for homologation, in comparison to the control varieties.

## RESULTS AND DISCUSSIONS

The climatic conditions in the vineyard in Iași, Copou viticultural center, from the period of time when the clone of the Fetească regală variety was tested are characterized by the presence of cold winters with absolute minimum temperatures under the limit of frost of the vine, namely  $-27^{\circ}\text{C}$  in the air and  $-35^{\circ}\text{C}$  on the surface of the snow layer, which affected for the most part the winter buds. Springs were warmer but with less rain, while summers were very hot, with many days with absolute maximum temperatures over  $30^{\circ}\text{C}$ , and the daily average temperatures from the second half of the vegetation period were favourable to the processes of growth and maturation of grapes.

The beginning of the vegetation process represented the disbudding, between the 23<sup>rd</sup> and the 30<sup>th</sup> of April, and there are no differences between clones, the average of the elites and the population variety, as well as the blooming phenophase between the 4<sup>th</sup> and the 8<sup>th</sup> of June, the beginning of the ripping process between the 30<sup>th</sup> of July and the 8<sup>th</sup> of August, and the technological maturation of grapes in September (table 1). The analysis of the fulfillment of the phonological specter reveals that the new clone of Fetească regală is integrated in the variety characteristics, crossing the vegetation period during 174-178 days.

Table 1

Phenological spectrum

| Variety                    | Disbudding  | Flowering | Grapes ripping | Technological maturity | Fall leaves | During the vegetation, days |
|----------------------------|-------------|-----------|----------------|------------------------|-------------|-----------------------------|
| Fetească regală cl 1 ls    | 23. - 30.04 | 4 - 8. 06 | 30.07 - 6.08   | 15 - 25.09             | 15 - 25.10  | 174 - 178                   |
| Elites average             | 23. - 30.04 | 4 - 8. 06 | 30.07 - 6.08   | 15 - 25.09             | 15 - 25.10  | 174 - 178                   |
| Fetească regală-population | 23. - 30.04 | 4 - 8. 06 | 30.07 - 6.08   | 15 - 25.09             | 15 - 25.10  | 174 - 178                   |

The fertility and productivity of the Fetească regală clone, estimated due to the percentage of fertile sprouts, the coefficients of fertility and the productivity indexes bring out close values of the clone and the average of elites slightly higher than those of the population variety (table 2).

Table 2

**Fertility and productivity elements of the Fetească regală cl 1 Is**

| Clone / Witness              | Bud loss % | Fertile shoots % | Fertility coefficients |          | Productivity indices |          |
|------------------------------|------------|------------------|------------------------|----------|----------------------|----------|
|                              |            |                  | absolute               | relative | absolute             | relative |
| Fetească regală cl 1 Is      | 48         | 78               | 1,57                   | 1,43     | 220                  | 200      |
| Population variety (witness) | 46         | 72               | 1,48                   | 1,23     | 175                  | 145      |
| Elites average               | 47         | 76               | 1,56                   | 1,39     | 200                  | 178      |

The estimation of certain physiological characteristics revealed that the characteristics of resistance to frost of the clone resemble the ones of the population variety, the bud loss oscillating between 48 and 78%, at temperatures of -27o C in the air and -35o C on the soil. The behaviour upon attack of the cryptogamic diseases, with the application of phytosanitary treatments is specific to the Vinifera variety. The serological tests by the ELISA process confirmed that this clone is free of the main viruses in Romania. There were significant differences between the values of the average mass of a grape and of 100 berries, which are favourable to the clone, in comparison to the control varieties.

The grape production achieved per stock and calculated per hectare supports the characteristic of high productivity of the new creation, in comparison to the population variety. In the researched period of time, the clone Fetească regală-1 Is achieved average productions of 18.1 t/ha, ensuring a harvest increase of 16% in comparison to the population. The harvest increase is also supported by the statistic-mathematical calculus, being distinctly significant.

This clone was submitted to homologation due to its qualitative potential, even if from the production point of view, it is overcome by the average of the elites. The grape and the berry size and the average content of sugar of the unfermented wine (208 g/L) are in favour of the superiority of the grape harvest quality, overcoming the population variety by 22% and the average of the elites by 6%, being ensured statistically-mathematically distinctly significant. This fact reveals the clone potential for wine quality (table 3).

Table 3

**Quality and productivity elements of the Fetească regală cl 1 Is**

| Clone / Witness              | Grapes production:       |                      |                  | Average weight / grape g | Average weight 100 berry g | Sugar s g/L        | Total acidity g/L H <sub>2</sub> SO <sub>4</sub> | Yield in must % |
|------------------------------|--------------------------|----------------------|------------------|--------------------------|----------------------------|--------------------|--|-----------------|
|                              | effective kg/ vine stalk | calculated t/ha      | harvest increase |                          |                            |                    |  |                 |
| Fetească regală 1Is          | 4,78                     | 18,10 <sup>xxx</sup> | 116              | 140                      | 208                        | 208 <sup>xxx</sup> | 4,8  | 76              |
| Population variety (witness) | 4,13                     | 15,64                | 100              | 118                      | 162                        | 186                | 5,3  | 65              |
| Elites average               | 4,82                     | 18,25                | 117              | 128                      | 198                        | 196                | 5,1  | 74              |

DL towards population:

5% = 0.60;  
1% = 0.86;  
0,1% = 1,31.

5% = 5,48;  
1% = 9,06;  
0,1% = 16,96.



The technological characteristics of the clone are completed by the values of the technological indexes of the grapes resulted from the physical-mechanical analysis of 1 kg of grapes (table 4).

Table 4

**Physico-mechanical composition of 1 kg grapes and technological indices of Fetească regală 1Is clone, compared with control**

| Elements determined                   | Fetească regală 1Is clone | Population variety (witness) |
|---------------------------------------|---------------------------|------------------------------|
| <b>1 kg grapes:</b>                   |                           |                              |
| no. berry normally developed, healthy | 501                       | 580                          |
| berry, g                              | 974                       | 958                          |
| bunch, g                              | 28                        | 42                           |
| must, g                               | 790                       | 730                          |
| volume of must, cm <sup>3</sup>       | 764                       | 653                          |
| marc, g                               | 184                       | 228                          |
| <b>100 berry:</b>                     |                           |                              |
| average weight, g                     | 205                       | 162                          |
| volume, cm <sup>3</sup>               | 165                       | 140                          |
| number of seeds                       | 168                       | 175                          |
| seeds weight, g                       | 6,33                      | 6,8                          |
| skin weight, g                        | 17,76                     | 17,84                        |
| core weight, g                        | 156,82                    | 112,72                       |
| marc weight, g                        | 24,09                     | 24,64                        |
| <b>Technological indices:</b>         |                           |                              |
| structure of the grape index          | 34,78                     | 22,80                        |
| composition of berry index            | 6,51                      | 4,57                         |
| yield index                           | 4,29                      | 3,20                         |

The obtained wines have the characteristics specific to the variety, having an alcohol concentration of 11.3% vol., close to the control variety (11% vol.), are fructuous, stout, rich in non-reducing extract (24.6 g/L) and in glycerol (7.9 g/L), and can be included in the category of the wines of controlled origin (table 5).

Table 5

**Physico-chemical characteristics of wines from grapes vinification Feteasca regală 1Is clone compared with control**

| No. | Physical - chemical parameters | U.M.   | Fetească regală 1Is clone | Fetească regală, population variety |
|-----|--------------------------------|--|---------------------------|-------------------------------------|
| 1   | Alcohol                        | % vol.   | 11,3                      | 11,0                                |
| 2   | Total acidity                  | g/L C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> | 7,2                       | 6,0                                 |
| 3   | Volatile acidity               | g/L CH <sub>3</sub> COOH                         | 0,39                      | 0,45                                |
| 4   | Sugars                         | g/L  | 1,4                       | 0,45                                |
| 5   | Non-reducing extract           | g/L  | 24,6                      | 22,5                                |
| 6   | Total poliphenols              | g/L  | 0,31                      | 0,30                                |
| 7   | pH                             | unit. pH   | 2,98                      | 2,90                                |
| 8   | Glycerol                       | g/L  | 7,9                       | 6,9                                 |

## CONCLUSIONS

1. The cloning selection of the Fetească regală variety, meant for the production of superior white wines resulted in the homologation of the clone of Fetească regală vine cl. 1 IS and the certification by the State Institute for the Testing and Registration of Varieties (405/19.01.2012) and the recommendation for the cultivation in the areas favourable to the vine.

2. The production of grapes achieved per stock and calculated per hectare supports the characteristic of high productivity of the new creation in comparison to the population variety.

3. The obtained wines are high quality wines, fructuous stout wines rich in non-reducing extract, which are characteristics that include them in the categories of wines of controlled origin.

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# SEEDLESS VARIETIES IN REPUBLIC OF MOLDOVA: ASCERTAINMENT AND PERSPECTIVES

## SOIURI APIRENE ÎN REPUBLICA MOLDOVA: CONSTATĂRI ȘI PERSPECTIVE

SAVIN GH.<sup>1</sup>, CORNEA V.<sup>1</sup>, NOFIT Daniela<sup>1</sup>  
e-mail: ghsavin@yahoo.com

**Abstract.** Traditionally in Carpathian-Danubian-Pontic region, inclusively in Republic of Moldova, in grapevine assortment did not existed seedless varieties, they having geographical origin from *Proles orientalis* Negr. The researches, testing of some introduced seedless varieties denoted the absence of any prospect for its cultivation in industrial vineyards: they are seriously affected by critical winter temperatures and have a low crop capacity. In this context it was expressed an opinion that in our region is not possible the cultivation of seedless varieties. Following amelioration researches disproved the skepticism regarding the possibilities to create a seedless assortment, adapted to the specific of climate conditions. Stable harvest and advanced resistance of new created genotypes during more than 20 years cultivation without protection during the winters, inclusively of varieties already included in register, are a convincing argument. At the same time accumulated biological material represents a significant genotypic diversity: diverse degree of seedlessness, earliness, quality, productivity, diverse utilization, resistance to abiotic unfavorable factors, and the presence of these properties in diverse combinations at diverse genotypes allows the creation of newly competitive varieties.

**Key words:** grapevine, seedless varieties, resistance, biodiversity

**Rezumat.** Tradițional, în spațiul carpato-danubiano-pontic, inclusiv Republica Moldova, în sortimentul viticol n-au existat soiuri apirene, ele având origine din *Proles orientalis* Negr. Cercetările, testările unor soiuri apirene introduse au demonstrat lipsa de perspectivă a cultivării lor în plantații industriale: sunt grav afectate de temperaturile critice în timpul iernii și manifestă o productivitate scăzută. În acest context s-a vehiculat ipoteza că în regiunea noastră nu este posibilă cultivarea soiurilor apirene. Cercetările ulterioare de ameliorare au infirmat scepticismul privind posibilitatea creării unui sortiment apiren, adaptat specificului condițiilor climatice. Producția stabilă și rezistența avansată, manifestată pe parcursul a mai bine de 20 ani de genotipurile noi create, cultivate fără protejare pe parcursul iernilor, inclusiv de soiurile deja înscrise în registru, sunt un argument convingător în acest sens. Totodată, materialul biologic acumulat prezintă o diversitate genotipică semnificativă: grad diferit de apirenitate, timpurietate, calitate, productivitate, utilizare diversă, rezistență la factorii abiotici restrictivi, iar prezența acestor însușiri în diferite combinații la diferite genotipuri permite de a crea noi soiuri competitive.

**Cuvinte cheie:** viță de vie, soiuri apirene, rezistență, biodiversitate

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<sup>1</sup> Research and Practical Institute for Horticulture and Food Technologies, Republic of Moldova

## INTRODUCTION

In the context of dynamic market conditions as well as more frequent climatic challenge, amelioration of grapevine assortment, inclusive of seedless varieties with various utilization and advanced resistance to unfavorable factors of environment is an actual task. During the last 30 years in the Republic of Moldova were created many genotypes with different degree of seedlessness and diverse agro biological and technological characters regarding time of full maturity of berries, color and size of berry, size of the bunch, direction of use, resistance to restrictive a biotic factors. After preliminary evaluation in State Commission for Plant Testing, six new seedless varieties had been admitted for cultivation (2011). In order to diversify existing assortment other seedless varieties are evaluated and promoted. In the same time, the diversity of seedless grapevine resources from Institute's Genofond had been completed with new genotypes from other viticulture centers of the world. Appearance in the world viticulture of new seedless varieties, inclusive with very early time of berry maturity, large or very large berry, advanced resistance to winter conditions (<http://vinograd.info/sorta/>) determine new tasks and objectives in future amelioration of assortment adapted to the specific of climatic conditions of Carpathian – Danubian –Pontic region. Mobilization of these resources allows the accumulation of “critical mass” of genetic diversity for future efficiency of breeding process of new seedless varieties for industrial cultivation.

## MATERIAL AND METHODS

Is analyzed the segment of seedless grapevine genetic resources from the grapevine Genofond of Research and Practical Institute for Horticulture and Food Technologies (further the Institute), inclusive varieties Apiren alb, Apiren negru de Grozesti, Apiren roz, Romulus homologated in republic during the last six years as well as new varieties Apiren roz extratimpuriu and Apiren roz Basarabean with perspective for homologation. In the basis of analysis of diverse sources of literature was revealed and presented a segment of seedless grapevine assortment existed in other viticulture centers that represent interest for breeding.

Ampelographical descriptions and agrobiological estimations were effectuated according the OIV methodology (2009). Processing of experimental data with STATGRAPHICS Plus 5.1 software.

## RESULTS AND DISCUSSIONS

The diversity of grapevine genetic resources with diverse degree of seedlessness presented in Institute's Genofond include about 65 genotypes with various ecological – geographical and genetic origin: traditional seedless varieties Kishmish belyi, Kishmish chernyi, Sultanina, Perlette et al. and new forms and elites obtained during the years in the result of breeding programs (Savin, 2002). Also were introduced new genotypes (Tab. 1) with favorable characteristics for amelioration of seedless assortment according the criteria: early and medium time

of full berry maturity, large berry and bunch, some of them with relative or advanced resistance to wintering.

Table 1

**Diversity of some seedless genotypes from the Genofond of ISPHTA**

| Genotype                | Country of origin | Time of berry maturity | Berry color  | Berry weight, g |
|-------------------------|-------------------|------------------------|--------------|-----------------|
| Besemeannii ghibrid V-6 | Bulgaria          | Medium-late            | Green-yellow | 4,5             |
| Besemeannii pozdnii     | Uzbekistan        | Late                   | Blue black   | 2,0             |
| Besemeannii ranii       | Russia            | Early                  | Green-yellow | 3,0             |
| Călina                  | Romania           | Early                  | Rose         | 1,9             |
| Centennial seedless     | USA               | Early                  | Green-yellow | 2,8             |
| Flame seedless          | USA               | Early                  | Red          | 2,0             |
| Himrood                 | USA               | Very early             | Green-yellow | 1,5             |
| langhi Er               | Uzbekistan        | Early                  | Green-yellow | 1,6             |
| Interleikin             | USA               | Early                  | Green-yellow | 1,6             |
| Kis-mis Vatcana         | Uzbekistan        | Medium                 | Rose         | 2,6             |
| Mecita                  | Ukraine           | Early                  | Rose         | 2,1             |
| Perlon                  | USA               | Medium                 | Red-violet   | 3,0             |
| VIII-1-24               | R.Moldova         | Medium-late            | Red-violet   | 4,5             |
| XI-37-38                | R.Moldova         | Late                   | Red-violet   | 4,5             |

According the preliminary estimations seedless varieties originated from USA (Loose Perlette, Dawn seedless, Summer muscat et al.), admitted for testing in production conditions of Republic of Moldova (2010), it was established that although have high quality (large and attractive bunch, early maturity), most of them are very susceptible to wintering and diseases.

A valuable potential for breeding for advanced resistance to unfavorable conditions of environment, inclusively wintering, have recently homologated varieties Apiren alb, Apiren roz, Apiren negru de Grozesti and varieties evidentiati as perspective for implementation in production – Apiren roz extratimpuriu and Apiren roz Basarabean. Most of them have early or medium time of berry maturity and variety Apiren roz extratimpuriu can complement the list of varieties with very early maturity (Tabelul 2). The period bud burst – harvest vary for these varieties between 106-139 days, but indicated period may be different in function of direction of utilization of grapes. Thus, full maturity for consumption of fresh grapes is reached earliest by one week in comparison with the indicated mean value. For technological processing (for must, juice, homogenate, marinate, jam, raisins) and wine making the harvesting is effectuated on respective conditions of quality. The variety Apiren roz extratimpuriu, with the same time of full maturity like variety Perla de Csaba, can complete the existing assortment for this period, having at the same time colored berry and specific aroma. In comparison with other varieties of the same time of maturity included in Register, the grapes of Apiren roz extratimpuriu are less affected by cryptogam diseases, the grapes can be preserved on the bush for the long time (Figura 1) and in favorable for viticulture years the berries can accumulate already at the middle

of August 240-260 g/l of sugar. The estimations of the possibilities of technological processing of these varieties (Savin et al., 2005) confirmed its utility for this purpose.

Table 2

Phenology of seedless varieties (2006-2011, Genofond of RPIHFT)

| Genotype                 | Bud burst*  | Full bloom  | Berry ripening | Full maturity  | Days from bud burst to maturity |
|--------------------------|-------------|-------------|----------------|----------------|---------------------------------|
| Apiren alb               | 26.IV       | 09.VI       | 28.VII         | 01.IX          | 128                             |
|                          | 21.IV-02.V  | 08.VI-10.VI | 20.VII-06.VIII | 26.VIII-07.IX  |                                 |
| Apiren negru de Grozești | 24.IV       | 08.VI       | 29.VII         | 10.IX          | 139                             |
|                          | 22.IV-27.IV | 07.VI-10.VI | 25.VII-02.VIII | 06.IX-15.IX    |                                 |
| Apiren roz               | 26.IV       | 08.VI       | 28.VII         | 02.IX          | 129                             |
|                          | 25.IV-28.IV | 05.VI-12.VI | 22.VII-03.VIII | 25.VIII-10.IX  |                                 |
| Apiren roz Basarabean    | 23.IV       | 07.VI       | 24.VII         | 04.IX          | 134                             |
|                          | 20.IV-27.IV | 04.VI-10.VI | 21.VII-28.VII  | 28.VIII-11.IX  |                                 |
| Apiren roz extratimpuriu | 23.IV       | 08.VI       | 16.VII         | 07.VIII        | 106                             |
|                          | 21.IV-26.IV | 07.VI-09.VI | 13.VII-19.VII  | 30.VII-16.VIII |                                 |
| Romulus                  | 23.IV       | 09.VI       | 29.VII         | 29.VIII        | 128                             |
|                          | 20.IV-27.IV | 08.VI-10.VI | 25.VII-02.VIII | 22.VIII-05.IX  |                                 |

\* - is indicated mean value and the limits

The majority of these varieties have medium or large grapes, with medium weight and small or medium berries.



30.VII: Z= 183 g/l;  
t.a.= 9,15 g/l



12.IX: Z=240 g/l;  
t.a.=5,7 g/l

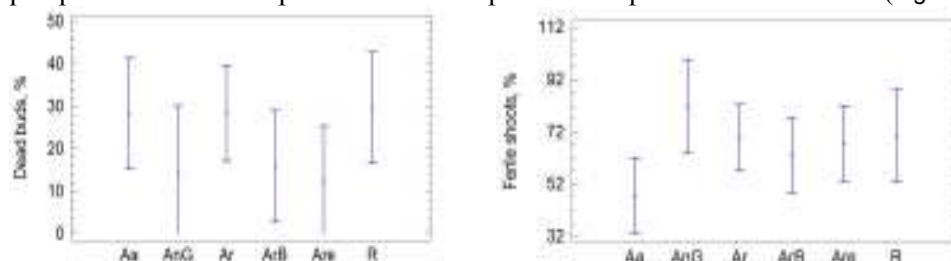


18.IX: Z=272 g/l;  
t.a.=4,5 g/l

\* Z= sugar content of must  
t.a.= total acidity of must (g/L tartaric acid)

Fig. 1 – The evolution of grapes of variety Apiren roz extratimpuriu

With very good resistance to wintering (according the percent of dead buds) is distinguished the homologated variety Apiren negru de Grozesti and new perspective varieties Apiren roz extratimpuriu and Apiren roz Basarabean (Fig. 2).



**Fig. 2** – Mean values and 95,0 percent LSD intervals for the percent of dead buds and percent of fertile shoots (2008-2011) (varieties in order according Table 2)

These creations represent a modest beginning, but they disprove the scepticism concerning the possibility to create an assortment adapted to the climatic conditions specific for Carpathian-Danubian-Pontic region, confirmed and by the practice of neighboring countries (2005). Information from diverse sources (e.g.: <http://vinograd.info/sorta/>, [http://www.anfic.com.au/table\\_grape.htm](http://www.anfic.com.au/table_grape.htm)) (Tab. 3) denotes the appearance, on international level of an avalanche of new seedless varieties, inclusive with the characteristics valuable for breeding purposes: early and medium time of berry maturity, various berry color, large bunch and berry, specific aroma, good resistance to frosts.

Table 3

**Diversity of seedless genotypes resistant to critical temperatures**

| Genotype               | Country of origin | Time of maturity | Berry color  | Berry weight, g | Bunch weight, g | Resistance to low temperatures |
|------------------------|-------------------|------------------|--------------|-----------------|-----------------|--------------------------------|
| Alexandrit             | Russia            | Early            | Green-yellow | 5-6             | 450-600         | -25°C                          |
| Arsenevskii            | Russia            | Medium-late      | Green-yellow | 9               | 1000-2000       | -25°C                          |
| Attica Seedless        | France            | Very early       | Blue black   | 4-5             | -               | -                              |
| Besemeannâi krasnâi    | Russia            | Early            | Red          | 5               | 400-500         | -20°C                          |
| Besemeannâi rozovâi    | Russia            | Medium           | Rose         | 5               | 200-500         | -22°C                          |
| Black Emerald Seedless | USA               | Early            | Blue black   | 3-5             | 500             | -23°C                          |
| Black Finger *         | Israel            | Early            | Blue black   | 12-14           | -               | -                              |
| Concord Seedless       | USA               | Late             | Blue black   | 2-3             | 230-300         | -29°C                          |
| Desert seedless        | RSA               | Early            | Blue black   | -               | -               | -                              |
| Canadice               | USA               | Medium-early     | Rose         | 2-3             | 250-450         | -29°C                          |

| Genotype           | Country of origin | Time of maturity | Berry color  | Berry weight, g | Bunch weight, g | Resistance to low temperatures |
|--------------------|-------------------|------------------|--------------|-----------------|-----------------|--------------------------------|
| Kişmiş Askaiskii   | Russia            | Early            | Rose         | 4-5             | 500-600         | -26°C                          |
| Kişmiş Zaporozskii | Ukraine           | Early            | Rose         | 2-2,5           | 600-900         | -25°C                          |
| Mystery            | Israel            | Early            | -            | 6               | -               | -                              |
| Polinka            | Russia            | Early            | Rose         | 2-3             | 400-500         | -26°C                          |
| Prime*             | Israel            | Early            | Green-yellow | 6               | -               | -                              |
| Venus              | USA               | Early            | Blue black   | 2-3             | -               | -26°C                          |

\* Berry weight after growth stimulation

Consequently, the diversity of seedless genetic resources allows initiation and realization of breeding programs for creation of new seedless varieties for industrial cultivation.

## CONCLUSIONS

1. Seedless genetic resources from Institute's genofond contain, in various combination, the favorable characteristics: annually ensured quality and productivity, competitive production; early maturity and resistance to stress factor of environment.

2. This diversity allows the creation of new competitive varieties for fresh consumption, for storage in refrigerator for 3-4 months, technological processing, wine, raisins.

3. The variety Apiren roz extratimpuriu is recommended for testing in State Commission for Plant Testing.

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# THE INFLUENCE OF ROOTSTOCK ON GRAPEVINE NUTRITIONAL DISORDERS INDUCED BY THE INCREASE OF SOIL CHLOROSANT POWER

## INFLUENȚA PORTALTOIULUI ASUPRA DEREGLĂRILOR NUTRIȚIONALE LA VIȚA DE VIE INDUSE DE CREȘTEREA PUTERII CLOROZANTE A SOLULUI

ȘERDINESCU A.<sup>1</sup>, PÎRCĂLABU Liliana<sup>1</sup>  
e-mail: serdinescuadrian@yahoo.com

**Abstract.** Under controlled conditions, using calcareous soils with  $\text{CaCO}_3$  contents between 30 and 70% were studied the nutritional disorders induced by the increase of soil chlorosant power on three vinifera varieties grafted on seven rootstocks having a different resistance to iron chlorosis. The leaf diagnosis showed an increase of P, K, Mn and B contents and a decrease of N, Ca, Mg and Fe contents in the leaves of vinifera varieties in accordance with the increase of soil chlorosant power. This aspect determined a modification of global nutrition and especially of the nutritional equilibrium and of the nutritional ratio between macro and micronutrients related with rootstocks characteristics. Iron immobilization at the mesophyll level of leaves was correlated with the ratio  $\text{P/Fe} > 12$ , the ratio  $\text{Fe/Mn}$  near one value and with high ratio between K/Mg, K/Fe and K/Ca which expressed a potassium excess in the chlorotic leaves inducing an increase of leaves ash alkalinity and a high pH at the apoplast level causing an iron precipitation at this level.

**Key words:** grapevine, nutritional disorders, iron chlorosis

**Rezumat.** În condiții controlate, utilizând soluri calcaroase cu conținuturi de  $\text{CaCO}_3$  total cuprinse între 30 și 70% au fost studiate dereglările nutriționale induse de creșterea puterii clorozante a solului la 3 soiuri vinifera altoite pe 7 portaltoi cu rezistență diferită la cloroza ferocalcică. Analizele de diagnoză foliară au evidențiat o creștere a conținuturilor de P, K, Mn și B și o scădere a celor de N, Ca, Mg și Fe în frunzele soiurilor vinifera pe măsura creșterii puterii clorozante a solului. Acest lucru a determinat o modificare a alimentației globale, dar mai ales a echilibrului nutritiv și a rapoartelor nutriționale dintre macro și microelemente, diferențiat în funcție de caracteristicile portaltoiului. Blocarea Fe la nivelul mezofilului frunzelor, care determină apariția simptomelor de cloroză a fost corelată cu raportul  $\text{P/Fe} > 12$ , raportul  $\text{Fe/Mn}$  apropiat de 1 și rapoartele ridicate K/Mg, K/Fe și K/Ca care evidențiază un exces de potasiu în frunzele clorozate, determinând o creștere a alcalinității cenușii frunzelor și implicit un pH ridicat la nivelul apoplastului, blocând prin precipitare Fe la acest nivel.

**Cuvinte cheie :** vița de vie, dereglări nutriționale, cloroza ferică

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<sup>1</sup> Research Institute for Viticulture and Enology, Valea Călugărească, Romania

## INTRODUCTION

The presence in excess of calcium carbonate in the calcareous soils determine a series of changes in soil chemistry which induce a disturbance of the main physiological and biochemical processes in plants, including mineral nutrition, finally leading to the appearance of iron chlorosis symptoms (Mengel and Geurtzen, 1986; Bavaresco et al., 1992; Fregoni and Bavaresco, 1997). In case of the vine these disorders are manifested differently according to the rootstock resistance to the chlorosant power of the soil (Pouget and Ottenwaelter, 1978). This study aims to identify these nutritional disorders and to establish their magnitude depending on the grafting partner in order to determine their impact on the manifestation of the iron chlorosis in vines.

## MATERIAL AND METHODS

The research works have been carried out in pots under controlled environmental conditions using calcareous soils with total  $\text{CaCO}_3$  content ranged between 30 to 70 %. It was mounted also a control treatment using a noncalcareous soil. Biological material was represented by grafted vines belonging to Afuz Ali, Feteasca regala and Cabernet Sauvignon varieties grafted on 7 rootstocks with different resistance to the chlorosant power of the soil, namely: K 5BB, Cr. 26, SO4-4, Precoce de Miniş, 41B, 59VI, Ru140 and Fercal. To determine the changes induced in the nutrition of vinifera/rootstock combinations have been carried out analysis concerning leaves content in macro and micronutrients and their ash alkalinity in June and August. It was also determined the chlorophyll content of the leaves and were noticed the appearance of iron chlorosis symptoms. Correlative links were established between soil carbonate content and the ash alkalinity of the leaves and their chlorophyll content, these data been interpreted in correlation with the appearance of iron chlorosis symptoms.

## RESULTS AND DISCUSSION

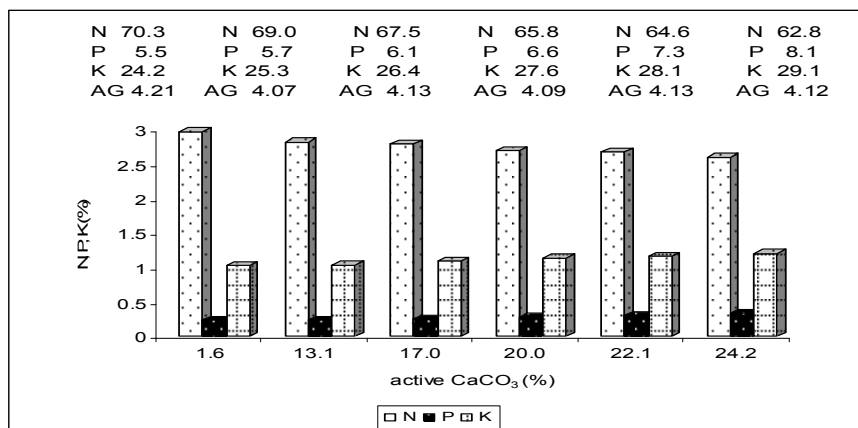
The experimental results obtained showed that the presence in excess of calcium carbonate in the calcareous soils affected by an obvious way the macro and micronutrient nutrition of vinifera/rootstock combinations used in the experiment (table 1).

Table 1

**The influence of the increase of soil chlorosant power on macro and micronutrient contents in vine leaves**

| <b>CaCO<sub>3</sub><br/>level</b> | <b>N<br/>%</b> | <b>P<br/>%</b> | <b>K<br/>%</b> | <b>Ca<br/>%</b> | <b>Mg<br/>%</b> | <b>Fe<br/>ppm</b> | <b>Mn<br/>ppm</b> | <b>B<br/>ppm</b> |
|-----------------------------------|----------------|----------------|----------------|-----------------|-----------------|-------------------|-------------------|------------------|
| Control                           | 2,96           | 0,23           | 1,02           | 3,04            | 0,25            | 240               | 113               | 36,5             |
| 30%                               | 2,81           | 0,23           | 1,03           | 2,86            | 0,25            | 238               | 143               | 39,0             |
| 40%                               | 2,79           | 0,25           | 1,09           | 2,85            | 0,23            | 228               | 147               | 40,7             |
| 50%                               | 2,69           | 0,27           | 1,13           | 2,77            | 0,22            | 217               | 152               | 41,2             |
| 60%                               | 2,67           | 0,30           | 1,16           | 2,74            | 0,22            | 201               | 150               | 40,8             |
| 70%                               | 2,59           | 0,33           | 1,20           | 2,66            | 0,20            | 199               | 154               | 39,8             |

Considering the average values for the 21 vinifera/rootstock combinations it was noticed a decrease of N content in leaves (from 2.96% to 2.59%) and an increase of P content (from 0.23% to 0.33%) as well as of K content (from 1.02% to 1.20%), which highlights a change in nutrient balance in sense of increasing the share of P and K in vine nutrition in accordance with the increase of soil chlorosant power. The overall nutrition showed only a slight decrease because the reduction of N content in leaves was compensated by a corresponding increase of P and K contents (fig. 1).



**Fig. 1** – The evolution of NPK contents and of the nutritional equilibrium in vine leaves according to the increase of soil chlorosant power expressed by its content in active CaCO<sub>3</sub> (%)(mean values for the 21 vinifera/rootstock combinations)

Ca and Mg showed a slight tendency of decrease, whereas in case of micronutrients has been registered a slight decrease of Fe content and an increase of Mn and B contents in the vine leaves while the soil chlorosant power was increasing. The modifications induced in the vine nutrition determined also an obvious modification of the nutritional ratios between macro and micronutrients (table 2).

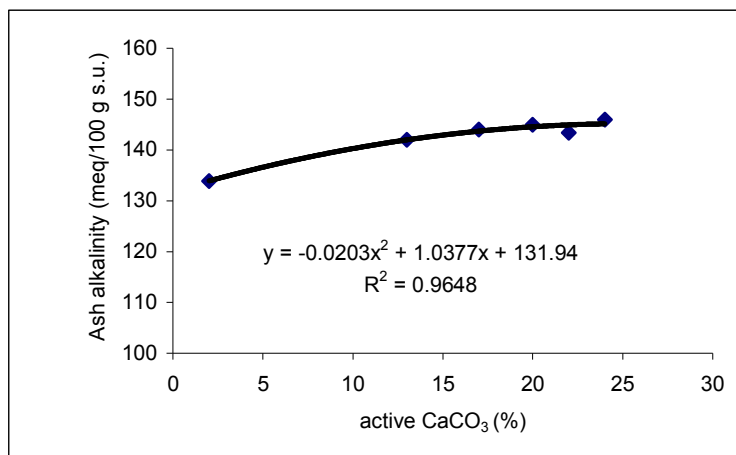
*Table 2*  
**The influence of the increase of soil chlorosant power on the main nutritional ratios in vine leaves (mean value for June and August)**

| CaCO <sub>3</sub> level | P/Fe | Ca/Fe | K/Fe | Mg/Fe | Fe/Mn | Ca/Mg | K/Mg | K/Ca |
|-------------------------|------|-------|------|-------|-------|-------|------|------|
| Control                 | 9,6  | 126,6 | 42,5 | 10,4  | 2,1   | 12,2  | 4,1  | 0,33 |
| 30%                     | 9,7  | 120,2 | 43,3 | 10,5  | 1,7   | 11,5  | 4,2  | 0,36 |
| 40%                     | 11,0 | 125,0 | 47,8 | 10,1  | 1,5   | 12,4  | 4,7  | 0,38 |
| 50%                     | 12,4 | 127,6 | 52,1 | 10,1  | 1,4   | 12,6  | 5,1  | 0,41 |
| 60%                     | 14,9 | 136,3 | 57,7 | 10,9  | 1,3   | 12,4  | 5,3  | 0,42 |
| 70%                     | 16,6 | 133,7 | 60,3 | 10,1  | 1,3   | 13,3  | 6,0  | 0,45 |

Thus, we can notice a clear increase of P/Fe and K/Fe ratios and a decrease of Fe/Mn ratio, which reaches almost the value of 1 in the chlorotic leaves. The

ratios Ca/Fe and Mg/Fe remained relatively constant with the increase of soil chlorosant power, because all the three nutrients showed a downward trend in the chlorotic leaves. It's important to mention also the increase of K/Mg and K/Ca ratios, this fact emphasizing a K surplus in the chlorotic leaves.

The modifications occurring in vine nutrition, mainly in case of the nutrition with alkaline nutrients, influenced the ash alkalinity of the leaves, inducing a constant increase of this one in correlation with the increase of soil chlorosant power (fig. 2).

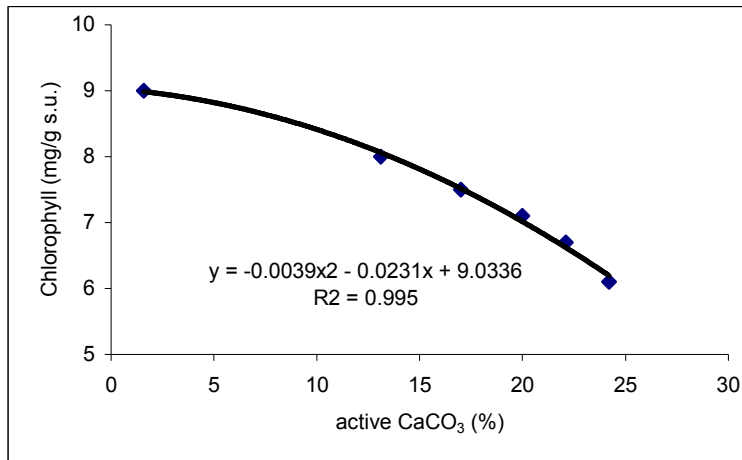


**Fig. 2** - Relationship between the soil chlorosant power expressed by its content in active CaCO<sub>3</sub> and leaf ash alkalinity (mean values for the 21 vinifera/rootstock combinations)

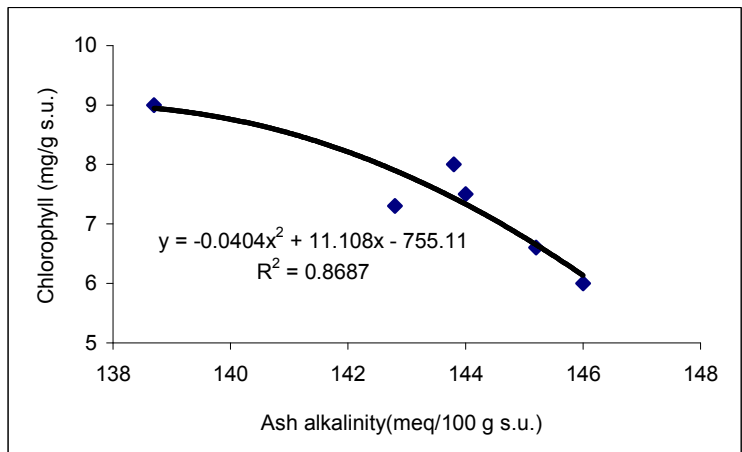
We believe that the changes occurring in the nutritional ratios in vine leaves and the increase of the proportion of alkaline nutrients, especially K, induced a high pH at the level of the appoplast and the Fe precipitation at this space this one becoming thus inactive for the synthesis of the chlorophyll, even if quantitatively it was within relatively normal limits in leaves. In this respect the experimental data revealed a close correlation between the increasing of soil chlorosant power, the ash alkalinity and the chlorophyll content in leaves (figures 3 and 4).

The inactivation of Fe at the mesophyll level, phenomenon that causes the appearance of the iron chlorosis symptoms, was also correlated with the P/Fe ratio > 12 and Fe/Mn ratio close to one.

The nutrition of the three vinifera varieties planted on calcareous soils was influenced in an obvious manner by the rootstock used in the grafting combinations, their resistance to the soil chlorosant power being different according to their genetic origin.



**Fig.3** - Relationship between the soil chlorosant power expressed by its content in active CaCO<sub>3</sub> (%) and the chlorophyll content in vine leaves (mean values for the 21 vinifera/rootstock combinations)



**Fig.4** - Relationship between the leaf ash alkalinity of the vine leaves and the chlorophyll content (mean values for the 21 vinifera/rootstock combinations)

The analytical data emphasize the fact that under the conditions of a very chlorosant soil (70 % total CaCO<sub>3</sub>) Fe concentration in the leaves of vinifera varieties and the value of the main nutritional ratios showing Fe nutrition (P/Fe, K/Fe and Fe/Mn) were highly influenced by the rootstock used in the grafting combination (table 3).

The biggest Fe concentration in the leaves and the balanced Fe/Mn, K/Fe and P/Fe ratios were registered in case of 140Ru 59V1, Fercal and SO4-4 rootstocks, which have a highly resistance to the chlorosant power of the soil and a reduced one in case of Precoce de Minis and 41B rootstocks.

Table 3

Chemical composition of the leaves of vinifera varieties and some nutritional ratios according to the rootstock used in the grafting combination and to the level of total CaCO<sub>3</sub> from soil ( mean values for the three vinifera varieties)

| Rootstock     | CaCO <sub>3</sub> level | N %  | P %  | K %  | Fe ppm | Mn ppm | P/Fe | Fe/Mn | K/Fe |
|---------------|-------------------------|------|------|------|--------|--------|------|-------|------|
| K5BB          | 1*                      | 3,24 | 0,25 | 1,03 | 211    | 117    | 11,8 | 1,80  | 48,8 |
|               | 2**                     | 2,66 | 0,36 | 1,20 | 196    | 165    | 18,4 | 1,19  | 61,2 |
| C-26          | 1                       | 2,94 | 0,23 | 1,02 | 215    | 80     | 10,7 | 2,68  | 47,4 |
|               | 2                       | 2,48 | 0,34 | 1,18 | 187    | 145    | 18,2 | 1,29  | 63,1 |
| SO4-4         | 1                       | 2,94 | 0,23 | 1,03 | 240    | 111    | 9,6  | 2,16  | 42,9 |
|               | 2                       | 2,65 | 0,33 | 1,20 | 219    | 137    | 15,0 | 1,60  | 54,8 |
| Precoce Minis | 1                       | 2,98 | 0,22 | 1,01 | 240    | 143    | 9,2  | 1,68  | 42,1 |
|               | 2                       | 2,49 | 0,34 | 1,23 | 170    | 171    | 19,9 | 0,99  | 72,4 |
| 41 B          | 1                       | 2,88 | 0,25 | 1,01 | 273    | 108    | 9,2  | 2,53  | 37,0 |
|               | 2                       | 2,56 | 0,36 | 1,19 | 180    | 187    | 20,0 | 0,96  | 66,1 |
| 140 Ru. 59 VI | 1                       | 2,90 | 0,21 | 1,05 | 229    | 100    | 9,2  | 2,29  | 45,8 |
|               | 2                       | 2,68 | 0,30 | 1,24 | 231    | 139    | 13,0 | 1,66  | 53,7 |
| Fercal        | 1                       | 2,79 | 0,24 | 1,03 | 284    | 137    | 8,4  | 2,07  | 36,3 |
|               | 2                       | 2,64 | 0,32 | 1,17 | 224    | 128    | 14,2 | 1,75  | 52,2 |

\* - control; \*\* - treatment with 70% total CaCO<sub>3</sub> in soil

## CONCLUSIONS

1. The modifications induced in the chemistry of the calcareous soils by the excess of calcium carbonate influenced in an obvious manner the mineral nutrition of vinifera/rootstock combinations

2. The leaf diagnosis showed in the chlorotic leaves high values for P, K, Mn and Fe contents and low values for N, Ca, Mg and Fe contents. This caused a change in nutrient balance and in nutritional ratios between macro and micronutrients.

3. The inactivation of Fe at the mesophyll level was correlated with the ratio P/Fe>12, the ratio Fe/MN close to one and the high values of the ratios K/Mg, K/Fe and K/Ca which have induced a high pH at the appoplast level

4. The mineral nutrition of vinifera varieties was strongly influenced by the rootstock used in the grafting combination

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# SELECTED AUTOCHTHONOUS YEAST STRAINS WITH INFLUENCE ON WINE QUALITY

## TULPINI DE DROJDII AUTOHTONE SELECȚIONATE CU IMPACT ASUPRA CALITĂȚII VINURILOR

**BRÎNDUȘE Elena<sup>1</sup>, TUDORACHE Aurelia<sup>1</sup>, FOTESCU Laura<sup>1</sup>**

e-mail: elabrinduse@yahoo.com

**Abstract.** The aim of this work was to study the influence of some autochthonous yeast strains on the wines quality. For this purpose grape must from the Cabernet Sauvignon grapes, during 2 harvest years (2009 - 2010), was used. The selected autochthonous yeast strains are part from the microbiota collection created at the Research Institute for Viticulture and Enology, Valea Calugărească, during 2007 – 2009 period. The strains were isolated from the vineyard, from the grapes surface and during different phases of alcoholic fermentation and were identified as belonging to *Saccharomyces* genus. The selection of yeast strains was performed after metabolic evaluation (period of latency, fermentation kinetics, features of fermented mash, tolerance to alcohol, fermentation temperature), and oenological evaluation (compositional profile, sensory profile). From a total of 30 wine yeast strains, three strains (SC 46, SC 62 and SC 23) have been kept for the production of wines with improved varietal characteristics. SC 58 strain is destined for the production of qualitative dried red wine. Because of alcohol resistance (up to 17.5% vol) the strain can be used, also, to restart the alcoholic fermentation.

**Key words:** yeast, *Saccharomyces*, fermentation, wine

**Rezumat.** Acest studiu a avut drept scop analiza influenței unor tulpini de drojdii autohtone asupra calității vinurilor. Experimentările au fost efectuate timp de 2 ani consecutiv (2009 -2010), utilizându-se mustul obținut din struguri aparținând soiului Cabernet Sauvignon. Tulpinile de drojdii selecționate provin din colecția de microorganisme înființată la Institutul de Cercetare-Dezvoltare pentru Viticultură și Vinificație, Valea Calugărească, în perioada 2007 – 2009. Tulpinile au fost izolate de pe struguri și în diferite faze ale fermentației alcoolice, și au fost identificate ca aparținând genului *Saccharomyces*. Selecția tulpinilor de drojdii a fost bazată pe evaluarea caracteristicilor metabolice (perioada de latență, cinetica de fermentare, particularitățile mustului fermentat, toleranța la alcool, temperatura de fermentare), și a celor oenologice (profil compozițional, profil senzorial). Din 30 de tulpini analizate, 3 tulpini (SC 46, SC 62 și SC 23) au fost selecționate pentru a fi utilizate în producerea vinurilor cu caractere varietale intensificate. Tulpina SC 58 este recomandată pentru obținerea vinurilor roșii de calitate. Datorită rezistenței la alcool (până la 17,5% vol) tulpina poate fi utilizată și pentru a reporni fermentația alcoolică.

**Cuvinte cheie:** drojdie, *Saccharomyces*, fermentație, vin

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<sup>1</sup> Research and Development Institute for Viticulture and Enology, Valea Călugărească, Romania

## INTRODUCTION

The use of selected *Saccharomyces* yeasts for alcoholic fermentation in winemaking is essential for the continuous improvement of the wines quality and for food safety assurance. In order to avoid the risks of the must spontaneous fermentations, active dried yeast cultures are used. The wine is made in an integrated system, with a better control of alcoholic fermentation under inputs reducing. The final product is of a better quality than the wine produced by traditional spontaneous fermentation (Fleet and Heard, 1993, Giudici and Zambonelli, 1992, Regodón et al., 1997, Ribéreau-Gayon P., 1985).

In the last two decades, an interest has grown in using new autochthonous or local selected yeasts to control must fermentations. These yeasts are better acclimated to the local environmental conditions and can enhance the typical sensory properties of the wines produced in a given region (Degre, 1993; Maqueda, 2011).

The aim of this work was to study the influence of some autochthonous yeast strains isolated from Dealu Mare vineyards, Valea Calugarească center to the wine quality.

## MATERIAL SI METHOD

The isolation and selection of yeasts were performed in the 2007-2009 period in the Dealu Mare vineyard, Valea Calugarească center and were identified as belonging to *Saccharomyces* genus. From a total of 30 wine yeast strains tested 2 consecutive years (2009 – 2010), four strains (SC 46, SC 62; SC 58 and SC 23) have been kept for the production of wines with improved varietal characteristics.

The selection of yeast strains was performed after metabolic evaluation (period of latency, fermentation kinetics, features of fermented mash, tolerance to alcohol, fermentation temperature), and oenological evaluation (compositional profile, sensory profile).

The physicochemical analyses were performed in order to establish the basic composition (alcoholic strength, total acidity, volatile acidity, reducing sugar, total dry extract), the polyphenolic composition (Folin-Ciocalteu index, anthocyanins,) and the chromatic characteristics (Color intensity, hue).

Conventional oenological parameters of wines were determined in accordance with official International Organization of Vine and Wine (OIV) practices (Recueil International des Methodes d'Analyses).

Organoleptic analysis of the wines was performed using the sensory descriptive method by a panel of winetasters trained for this activity.

The sensory descriptors were evaluated by points awarded on a scale from 1 to 5 and then recorded in a special descriptive evaluation sheet. On the basis of the obtained results, the composition and the sensory profiles for each wine were realized.

## RESULTS AND DISCUSSION

The origin of the selected yeast strains is included in table 1.



Table 1

**The origin of the selected yeast strains**

| Yeast strain | Origin of wine yeast strains  |
|--------------|---|
| SC 58        | must in the tumultuos fermentation stage, made from Cabernet Sauvignon grapes – classical plantation  |
| SC 46        | must at the end of fermentation, made from Cabernet Sauvignon grapes – classical plantation           |
| SC 62        | must in the tumultuos fermentation stage, made from Cabernet Sauvignon grapes – ecological plantation |
| SC 23        | must in the tumultuos fermentation stage, made from Cabernet Sauvignon grapes – classical plantation  |

**Metabolic characteristics**

The selected yeast strains are characterized by a short latency stage, a short to medium, medium or slow fermentation kinetics. The must is clear, with a deposit of creamy-white or yellowish-white, fine, sunk, with a good separation from the glass walls of fermentation tanks, in a low or moderate amount. The foam is fine, in a small amount. (tab. 2).

Table 2

**Characteristics of musts fermented by selected yeast strains**

| Yeast strain | Latency stage  | Tolerance to alcohol | Fermentation temperature | Fermentation kinetics | Characteristics of fermented must |   |                         |                       |
|--------------|----------------|----------------------|--------------------------|-----------------------|-----------------------------------|---|-------------------------|-----------------------|
|              |                |                      |                          |                       | Must aspect                       | Deposit   |                         | Production of foam    |
|              |                |                      |                          |                       |                                   | Aspect  | Quantitative assessment |                       |
| SC 58        | short (< 24 h) | up to 17.5% vol      | 10-30°                   | short/medium          | clear                             | creamy-white, fine, sunk, very good separation    | moderate amount         | fine, in a low amount |
| SC 46        | Short (< 24 h) | 15% vol              | 10-35°                   | slow                  | clear                             | creamy-white, fine, sunk, very good separation    | moderate amount         | fine, in a low amount |
| SC 62        | short (< 24 h) | 16% vol              | 15-30°                   | slow                  | clear                             | yellowish-white, fine, sunk, very good separation | moderate amount         | fine, in a low amount |
| SC 23        | short (< 24 h) | 15% vol              | 15-30°                   | medium                | clear                             | creamy-white, fine, sunk, very good separation    | moderate amount         | fine, in a low amount |

**1. Composition profile**

Cabernet Sauvignon wines are dry (residual sugar ranged from 1.65 g/l, ( SC 23) and 1.75 g/l, (SC 58), with an alcohol degree ranging between

11.7% vol. (SC 58) and 13.2 % vol. (SC 62). The volatile registered values under the limit allowed for red wines (tab. 3).

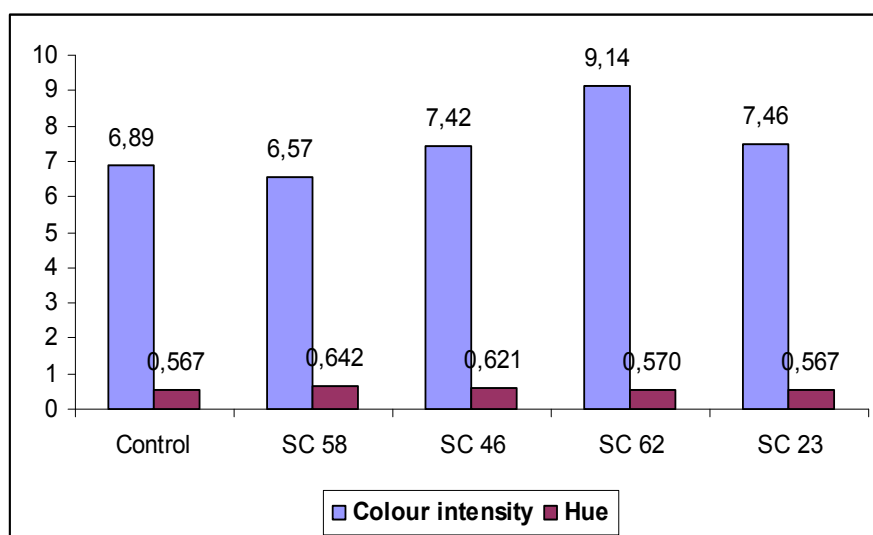
Table 3

**Physicochemical parameters of Cabernet Sauvignon young wines fermented with selected yeast strains**

| Yeast strain | Total acidity (g/l H <sub>2</sub> SO <sub>4</sub> ) | Volatile acidity (g/l CH <sub>3</sub> COOH) | Residual sugar (g/l) | Alcohol (% volum) | Total dry extract (g/l) |
|--------------|---|---|----------------------|-------------------|-------------------------|
| CS 0         | 7.05  | 0.43  | 1.70                 | 11.5              | 24.16                   |
| SC 58        | 6.45  | 0.25  | 1.75                 | 11.7              | 22.94                   |
| SC 46        | 6.00  | 0.27  | 1.68                 | 12.4              | 21.76                   |
| SC 62        | 6.38  | 0.27  | 1.70                 | 13.2              | 23.76                   |
| SC 23        | 6.00  | 0.32  | 1.65                 | 11.9              | 22.53                   |

### Chromatic characteristics

The use of yeast strains for Cabernet Sauvignon wines fermentation has resulted in a large variability in the color intensity of wines. Maximum value was observed with the SC 62 yeast strain which determined an increase in the color intensity with 32,65% compared with the control (fig. 1).



**Fig. 1** - Chromatic characteristics in case of the wine fermented with selected yeast strains

### Polyphenolic composition

Regarding the total polyphenols content, Cabernet Sauvignon wines registered values closed to the wine obtained in the presence of commercial

yeast used like control, with the exception of wine fermented with SC 58 strain, which showed significantly differences, in a negative way.

The yeast strains used in fermentations determined a high variability in terms of anthocyanin content, the differences being statistically assured. The maximum anthocyanin content was found in wine prepared from SC 62, and the lowest was recorded in wine made with the inoculation of SC 23 (data not shown).

### Sensory profile

Cabernet Sauvignon wines are lighter, with a medium colored intensity, equilibrated, with ordinary to strong alcoholic strength, slightly astringent with round tannins.

They are characterized by a fine and complex aroma with fruity (blackcurrant, blackberry, cherry), floral (violet) and vegetal notes.

The wine produced with the strain SC 23, clearly distinguished from the rest of the Cabernet Sauvignon wine samples.

Lighter wine, showed a highest red colored intensity, with a fine aroma, complex and strong in intensity.

The wine is equilibrated, with strong alcoholic strength, round, with ordinary, round tannins, slightly astringent. The equilibrium is ample, with long aromatic persistence (fig. 2).

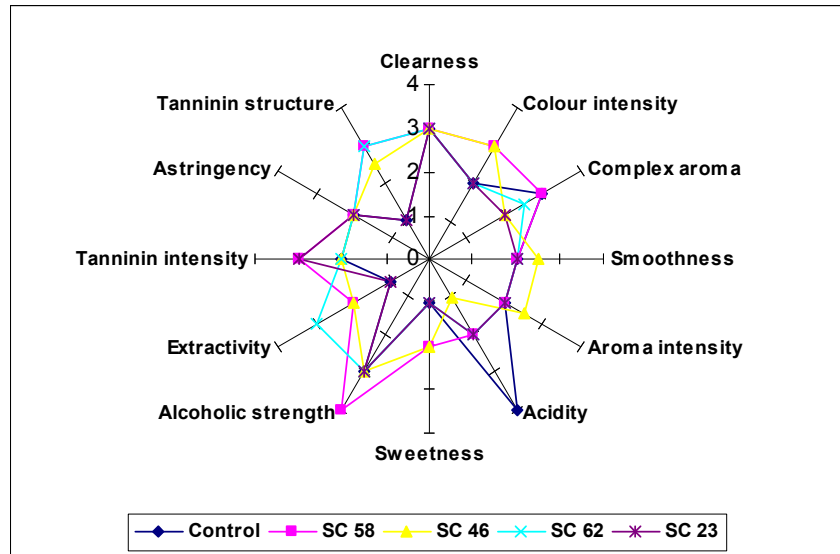
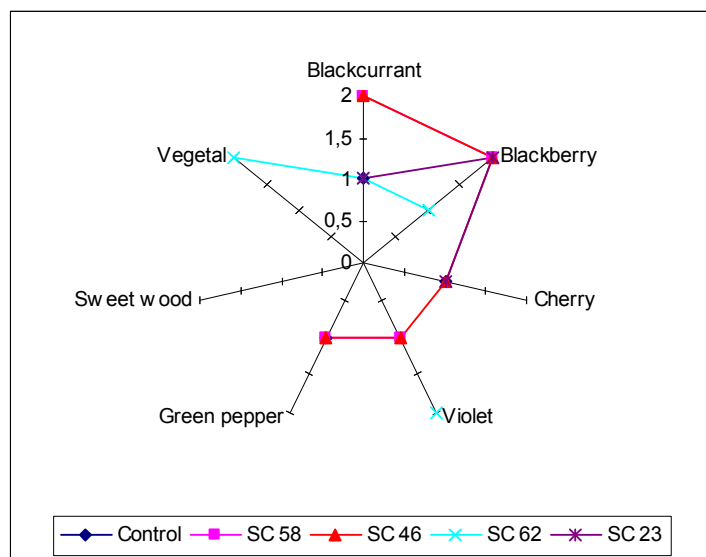


Fig. 2 - Sensory profile of Cabernet Sauvignon wines

Dominant spicy, slightly fruity (blackberry, blackcurrant, cherry) and floral (violet) notes were identified (fig. 3).



**Fig. 3 - Flavour specificity of Cabernet Sauvignon wines**

## CONCLUSIONS

1. The selected yeast strains contribute positively to the wine's sensory characteristics.

2. From a total of 4 autochthonous yeast strains selected, three strains (*SC 46*, *SC 62* and *SC 23*) have been kept for the production of wines with improved varietal characteristics. *SC 58* strain is recommended for the production of qualitative dried red wine.

3. Because of alcohol resistance (up to 17.5% vol) *SC 58* strain can be used, also, to restart the alcoholic fermentation.

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# STUDY OF PHYSICAL-CHEMICAL PARAMETERS OF ROMANIAN WHITE GRAPE VARIETIES AND THE EFFECT ON WINE QUALITY

## STUDIUL UNOR PARAMETRI FIZICO-CHIMICI LA SOIURI DE STRUGURI ALBI, ROMÂNEȘTI ȘI EFECTUL ASUPRA CALITĂȚII VINURILOR

BUBURUZANU C.<sup>1</sup>, COTEA V.V.<sup>1</sup>, NICULAUA M.<sup>2</sup>, MORARU I.<sup>1</sup>  
e-mail: cristianbuburuzanu@yahoo.com

**Abstract.** *This study is based on Romanian white grape varieties used in wine-making in Iasi vineyard. The analyses done on 20 Romanian grape varieties for wine production, during 2008 and 2009 have compared the photosynthesis parameters (chlorophyll a, chlorophyll b and carotenoids), total acidity and sugar content in grapes, as to establish specific correlations in the studied varieties.*

**Key words:** Romanian white grapes, chlorophyll, carotenoids, wine quality

**Rezumat.** *La baza acestui studiu stau soiuri albe românești de struguri pentru vin din podgoria Iași. În urma analizelor efectuate pe un număr de 20 de soiuri românești de struguri pentru vin din recoltele anilor 2008 și 2009 s-au comparat parametrii de la fotosinteză (clorofila a și b și carotenoizi), aciditatea totală și conținutul în glucide din struguri, dar și extractul nereducător din vin, în scopul realizării unor corelații specifice soiurilor luate în studiu.*

**Cuvinte cheie:** soiuri de struguri românești, clorofilă, carotenoizi, calitatea vinului

### INTRODUCTION

Copou vineyard in Iași has a very high potential for obtaining quality wines. The present article aims characterising from a physical–chemical point of view the white wines of local varieties, obtained without any addition of yeasts and enzymes, using the classical fermentation technology. At the same time, the raw matter that was used for the experiment (grapes and vine leaves) will be analysed.

The study of the physical-chemical parameters in the plant-grape-wine relationship from the Iași vineyard was done within the Pilot Station of the Horticultural Faculty, in the Oenological Laboratory of the University of Agricultural Sciences and Veterinary Medicine „Ion Ionescu de la Brad” – Iași, during 2007-2010.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup> Research Center for Enology, Romanian Academy, Branch Iasi

## MATERIAL AND METHOD

The base of this study is represented by white local grape varieties from the Copou-lași vineyard. After analysing Romanian grape varieties harvested from the Ampelographic collection of USAMV Iași in 2008 and 2009, a comparison of the photosynthetic parameters (chlorophyll *a*, *b* and carotenoids), total acidity and sugar content, non-reductive extract of wine was done in order to make specific correlations for each grape variety studied.

In order to reach the final objectives of the study, the following physical-chemical indices were analysed, according to speciality literature (Compendium of International Methods of Analysis of Wine and Musts, 2009; Cotea et al, 2009) and present standards (Colecție de standarde pentru industria vinului și băuturilor alcoolice, 1997; Târdea, 2007): chlorophyll *a* (Ca) (Arnon, 1949; Lichtenthaler and Buschmann, 2001), chlorophyll *b* (Cb) (Arnon, 1949; Lichtenthaler and Buschmann, 2001), carotenoids (Arnon, 1949; Lichtenthaler and Buschmann, 2001), reductive sugars (iodometric method STAS 6182/18-81), total acidity (titrimetric, in presence of bromothymol blue, STAS 6182/1-79) and non-reductive extract (EN)(densimetric, using the Tabarie formula, STAS 6182/9-80).

The comparative study was done on 3 grape variety classes, genetically related (Rotaru, 2009) group I – Galbenă de Odobești, Zghiara de Huși and Cruciuliță, group II – Fetească albă, Grasă de Cotnari, Fetească regală, Furmint and Armaș, group III – Șarbă and Tămâioasă românească.

## RESULTS AND DISCUSSIONS

In order to have a complete image of the studied parameters and as to start from a sturdy place, table 1 presents the data obtained after laboratory analysis of reductive sugars at harvest and processing (g/L) and the total acidity (g/L tartaric acid) as well as the non-reductive extract (g/L) of wines obtained in the harvest years of 2008 and 2009 (tab. 1). It can be easily seen that in the 2 harvest years, the values of the reductive sugars and total grape acidity are different: in 2008 the values are higher than in 2009, even if 2008 was a year that did not produce quality wines, proof being the values of the non-reductive extract of the obtained wines, turning around thus the mentioned situation.

Table 1

White Romanian grape varieties from Copou-lași vineyard

| No. | Grape Variety   | Reductive sugars (g/L) |      | Total acidity (g/L) C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> |       | EN g/L |       |
|-----|-----------------|------------------------|------|--|-------|--------|-------|
|     |                 | 2008                   | 2009 | 2008   | 2009  | 2008   | 2009  |
| 1   | Armaș           | 192                    | 152  | 11,24  | 9,05  | 22,58  | 25,49 |
| 2   | Blasius         | 176                    | 161  | 10,46  | 7,84  | 19,92  | 24,16 |
| 3   | Băbească gri    | 169                    | 149  | 9,05   | 6,32  | 12,71  | 26,53 |
| 4   | Cioinic         | 169                    | 131  | 9,61   | 7,14  | 15,95  | 18,25 |
| 5   | Creata de Banat | 164                    | 144  | 11,92  | 12,14 | 9,67   | 12,48 |
| 6   | Cruciuliță      | 119                    | 129  | 11,04  | 8,3   | 14,28  | 15,72 |
| 7   | Fetească albă   | 206                    | 154  | 7,03   | 6,57  | 23,03  | 22,66 |
| 8   | Fetească regală | 211                    | 169  | 8,17   | 7,38  | 25,06  | 22,21 |
| 9   | Frâncușă        | 159                    | 146  | 12,07  | 10,3  | 21,58  | 19,22 |

|    |                      |     |     |       |       |       |       |
|----|----------------------|-----|-----|-------|-------|-------|-------|
| 10 | Furmint de Miniș     | 131 | 158 | 11,94 | 10,09 | 17,79 | 23,83 |
| 11 | Galbenă de Odobești  | 188 | 155 | 8,08  | 7,21  | 16,57 | 19,39 |
| 12 | Gordan               | 118 | 107 | 11,83 | 11,09 | 17,89 | 7,41  |
| 13 | Gordin               | 138 | 139 | 11,59 | 9,36  | 16,86 | 16,36 |
| 14 | Grasă de Cotnari     | 189 | 172 | 8,38  | 7,22  | 13,52 | 23,83 |
| 15 | Miorița              | 148 | 138 | 7,34  | 6,71  | 13,98 | 20,68 |
| 16 | Mustoasă de Maderat  | 137 | 118 | 13,37 | 11,74 | 18,76 | 11,79 |
| 17 | Șarbă                | 167 | 149 | 9,79  | 8,73  | 18,72 | 21,32 |
| 18 | Selena               | 165 | 154 | 9,12  | 7,39  | 18,78 | 15,23 |
| 19 | Tămâioasă românească | 214 | 168 | 9,04  | 8,96  | 25,77 | 26,91 |
| 20 | Zghihară de Huși     | 189 | 165 | 13,02 | 11,73 | 25,82 | 24,09 |

The obtained results of analysing the white local grape varieties and their wines permitted them to be classified in three groups that are genetically related (Rotaru, 2009) as such: group I – Galbenă de Odobești, Zghihară de Huși and Cruciuliță, group II – Fetească albă, Grasă de Cotnari, Fetească regală, Furmint, Armaș, group III – Șarbă and Tămâioasă românească.

In the case of the Cruciuliță, Galbenă de Odobești and Zghihară varieties, genetically related grape sorts (from the Galbenă de Odobești variety Zghihară de Huși and Cruciuliță were obtained), the Zghihară variety shows a variation of the chlorophylls ratio, from 1,59 to 2,46, according to the harvest year, while at the other two varieties there is no evident variation (tab. 2). Therefore, we can safely say that the influence of the vegetative year can be observed along the entire plant-grape-wine relation, according to table 1, with higher values in 2009 compared to 2008 regarding wine quality.

Table 2

**Correlations of chlorophyll a, chlorophyll b and carotenoids as well as Ca/Cb ratio for group I of white Romanian grapes from Copou-Iasi vineyard**

| Grape variety       | Ca (mg/L) | Cb (mg/L) | carotenoids (mg/L) | Ca/Cb |
|---------------------|-----------|-----------|--------------------|-------|
| Harvest of 2008     |           |           |                    |       |
| Cruciuliță          | 8,65      | 4,23      | 2,89               | 2,05  |
| Galbenă De Odobești | 10,68     | 5,64      | 3,52               | 1,89  |
| Zghihară            | 11,88     | 4,83      | 3,48               | 2,46  |
| Harvest of 2009     |           |           |                    |       |
| Cruciuliță          | 1,73      | 0,95      | 0,56               | 1,82  |
| Galbenă De Odobești | 3,21      | 1,56      | 1,06               | 2,05  |
| Zghihară            | 3,09      | 1,94      | 1,40               | 1,59  |

The second group of genetically related varieties, according to the values for the chlorophylls ratio, sugars quantity (from most of the varieties, DOC wines can be produced) and the non-reductive extract (over the 19 g/L limit for qualitative wines) (table 3) are superior to the before mentioned group, fact already confirmed by the degree of exploitation of these varieties for producing quality wines.

As in the case of the latter group, the values of chlorophyll a, chlorophyll b and carotenoids have higher values in 2008 than 2009.

Table 3

**Correlations of chlorophyll a, chlorophyll b and carotenoids as well as Ca/Cb ratio for group II of white Romanian grapes from Copou-Iași vineyard**

| Grape variety    | Ca (mg/L) | Cb (mg/L) | carotenoids (mg/L) | Ca/Cb |
|------------------|-----------|-----------|--------------------|-------|
| Harvest of 2008  |           |           |                    |       |
| Armaș            | 8,97      | 3,99      | 2,74               | 2,25  |
| Fetească Albă    | 7,36      | 3,08      | 2,31               | 2,39  |
| Fetească Regală  | 8,75      | 3,45      | 2,46               | 2,54  |
| Furmint de Miniș | 15,60     | 6,93      | 5,20               | 2,25  |
| Grasă de Cotnari | 12,06     | 4,81      | 3,52               | 2,51  |
| Harvest of 2009  |           |           |                    |       |
| Armaș            | 2,95      | 0,98      | 0,51               | 3,03  |
| Fetească Albă    | 2,65      | 1,00      | 1,16               | 2,65  |
| Fetească Regală  | 2,40      | 1,26      | 0,90               | 1,91  |
| Furmint de Miniș | 3,54      | 1,37      | 1,37               | 2,58  |
| Grasă de Cotnari | 7,40      | 4,41      | 1,57               | 1,68  |

From the aromatic grape varieties, Tămâioasă românească and Șarbă (Șarba is a grape variety obtained by specialists at the Viticultural Research Station at Odobești through hybridising Tămâioasă românească with Riesling), have higher values for Ca, Cb and carotenoids compared to the other studied varieties. The quality of the grapes is limited by harvesting them before their technological maturity, but even so, the obtained wines are of superior quality (tab. 4).

Table 4

**Correlations of chlorophyll a, chlorophyll b and carotenoids as well as Ca/Cb ratio for group III of white Romanian grapes from Copou-Iași vineyard**

| Grape variety        | Ca (mg/L) | Cb (mg/L) | carotenoids (mg/L) | Ca/Cb |
|----------------------|-----------|-----------|--------------------|-------|
| Harvest of 2008      |           |           |                    |       |
| ȘARBĂ                | 20,03     | 8,52      | 5,92               | 2,35  |
| TĂMÂIOASĂ ROMÂNEASCĂ | 10,04     | 4,18      | 2,79               | 2,40  |
| Harvest of 2009      |           |           |                    |       |
| ȘARBĂ                | 6,21      | 3,19      | 1,83               | 1,95  |
| TĂMÂIOASĂ ROMÂNEASCĂ | 4,72      | 1,91      | 2,95               | 2,47  |



Fig. 1 represents the quantitative values of Ca, Cb and carotenoids of the studied white local grape varieties from the Ampelographic collection of UȘAMV Iași. The arrows mark the limits for the chlorophyll a quantity determined in the three genetically related groups. We can thus safely say that the majority of the varieties can be registered in to the first genetic group from a chlorophyll a potential (Băbească gri, Cioinic, Creț de Banat, Miorița, Mustoasa de Măderat and Selena), or in the second group (Blasius and Gordin), the exception being the variety Gordan where the Ca quantity surpasses these intervals in 2008 and 2009.

If the mode in which the Ca variety varies, the same classification is obtained, underlining Gordan variety again that can be registered due to its chlorophyll synthesis potential in to the third genetic group (as well as the aromatic varieties).

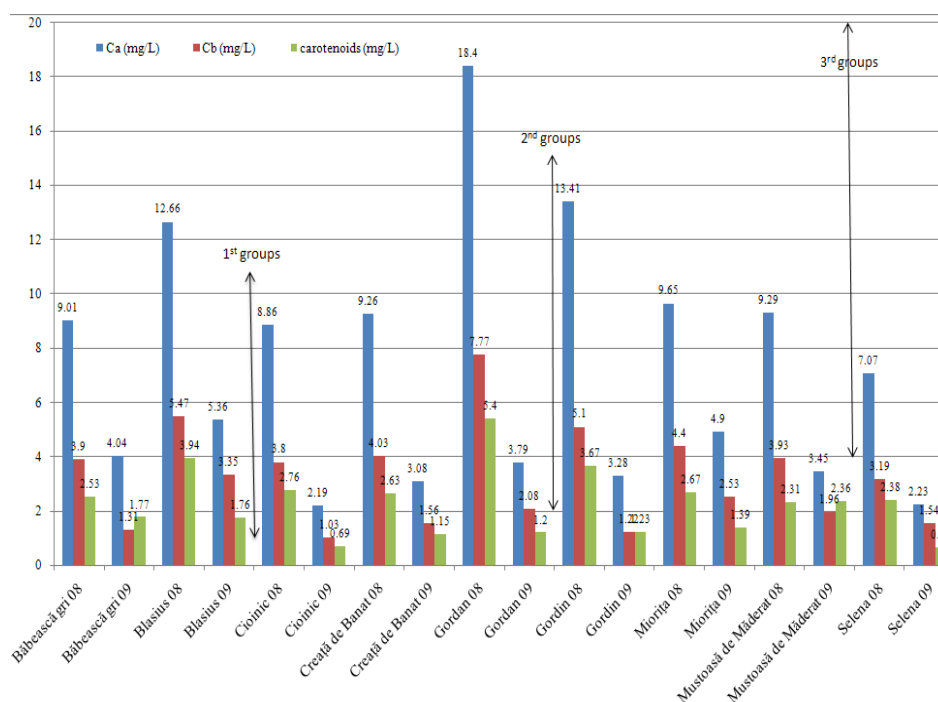


Fig. 1 - Correlations of chlorophyll a, chlorophyll b and carotenoids for white local grape varieties in Copou-Iași vineyard for harvest years 2008 and 2009

## CONCLUSIONS

1. The presented studies have confirmed the existence of a relation plant-fruit-final product (wine) by evaluating some parameters as the chlorophyll a and chlorophyll b quantity, sugar content in the grapes and non-reductive extract of wine.

2. There is an inverse proportional dependency between the chlorophyll quantity, the carotenoids and the quality of the obtained wine, proven by the analyses of Gordan variety.

3. The local grape varieties can be differentiated according to the chlorophyll quantity produced in a year (annual influence) in order to predict grape quality.

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# TECHNOLOGICAL AND OENOLOGICAL VALUE OF GRAPE VARIETIES AND WINES FROM COTNARI VINEYARD IN 2011

## VALOAREA TEHNOLOGICA SI OENOLOGICA A SOIURILOR DIN PODGORIA COTNARI IN CONDITIILE ANULUI 2011

*COLIBABA Cintia*<sup>1</sup>, *COTEA V. V.*<sup>2</sup>, *ROTARU Liliana*<sup>2</sup>, *NECHITA B.*<sup>2</sup>,  
*NICULAU M.*<sup>3</sup>, *ZAMFIR C.*<sup>2</sup>, *LACUREANU F.G.*<sup>2</sup>, *TUDOSE-SANDU-VILLE St.*<sup>2</sup>  
e-mail: cintia\_colibaba@yahoo.co.uk

**Abstract.** *Cotnari vineyard is one of the benchmarks of Romanian viticulture and wine-making. The four grape varieties of the vineyard, Tămâioasă românească, Fetească albă, Grasă de Cotnari and Frâncușă are undried resources of worldwide famous wines. This study wants to present from an ampelographic (technological) and oenological point of view the grapes and wines of the vineyard.*

**Key words:** Cotnari, analytical connection between grapes-must-wine

**Rezumat.** *Podgoria Cotnari reprezinta unul din stindardele viticulturii si oenologiei romanesti, fiind astfel permanent in vizorul cercetarilor din domeniu. Cele patru soiuri etalon ale podgoriei, si anume Tămâioasă românească, Fetească albă, Grasă de Cotnari and Frâncușă sunt surse inepuizabile de vinuri cu renume mondial. Studiul de fata doreste sa prezinte atat din punct de vedere ampelografic - tehnologic dar si din punct de vedere oenologic vinurile si strugurii podgoriei.*

**Cuvinte cheie:** Cotnari, conexiune analitica strugure-must-vin

## INTRODUCTION

Cotnari vineyard is situated in the north-east of Romania and is part of the renowned European vineyards found at the most northern limit of vine culture for economical purpose (45–50° north latitude): Tokaj - Hungary, Rheingau - Germany, Champagne - France, vineyards with world-wide recognized wines (Cotea, 1985).

From the oldest times, the Cotnari assortment is constituted of local grape varieties. The vines are the same with those cultivated in the time of Stephen the Great and are in the following proportion: Grasă de Cotnari 35%, Fetească albă 35 %, Frâncușă 20% and Tămâioasă românească (Busuioacă de Moldova) 10%. Cotnari is the only Romanian vineyard that has not changed the vine assortment after the phylloxera attack (Cotea et al., 2009).

The main aim of this article is the study of the analytical connection between grapes, must and wine, from a interdisciplinary (ampelography and oenology) point of view.

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<sup>1</sup> “Gheorghe Asachi” Technical University of Iași, Romania

<sup>2</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

<sup>3</sup> Oenology Research Center – Iasi Branch of the Romanian Academy, Romania

## MATERIAL AND METHOD

Frâncușă, Fetească albă, Grasă de Cotnari and Tămâioasă românească grapes were harvested from Cotnari vineyard in September 2011. Grapes were harvested from 45 vines chosen at random from the production parcels, with an area of 1 ha and a vine density of 3200 vinetrunks, so that the plants have variety-specific vegetative growths and production capacity. The harvest was programmed on the 25<sup>th</sup> of September, the average date for harvesting in Cotnari vineyard.

Before processing, some grape characteristics were determined: grape quantity (kg), 100 grape berries mass (g), marc quantity (L), stem mass (kg), grape marc quantity (kg), must quantity (L) and wine quantity of (L) for each grape variety.

The grapes' processing was done according to the technology of obtaining dry white wines. A series of preliminary tests for the must were registered: sugars quantity (g/L), total acidity (g/L tartaric acid). Selected yeasts (20 g/100 kg) were added to the must that were then settled in stainless steel tanks in order to finish their alcoholic fermentation. The obtained wines were conditioned and then bottled.

The following general physical-chemical analyses were determined: alcoholic concentration, reductive sugars, relative density, total acidity, volatile acidity, non-reductive extract, total dry extract. All the analyses were based on national and international standards as well as in the specialty literature (Compendium of International Methods of Analysis of Wines and Musts, 2011).

## RESULTS AND DISCUSSIONS

Table 1 present the technological characteristics of the raw matter from the analysed grape varieties. The lowest production is registered at the Frâncușă variety, of 82 kg, while Tămâioasă românească had the highest quantity of harvested grapes, of 199,5 kg.

The mass of 100 grape berries is mainly determined by the ampelographic characteristics of the variety and less by the applied agro-technical measures. The highest values are registered at Grasă de Cotnari, with 375,77 g, with berries of average to big dimensions. At the other end is Fetească albă variety, with small berries, the mass of 100 berries 225,01 g.

The grape structure index is within limits: for Tămâioasă românească, the ratio between berries' weight and stems weight is 33,25, for Grasă de Cotnari, 42,5, close to that of table grapes due to the berries' dimensions. Fetească albă has a structure index of 21,3, while Frâncușă has 27,3. Stem mass represents 4,69% of the total quantity of Fetească albă grapes, 3,65% of the total quantity of Francusa grapes, 2,35% of the total quantity of Grasă de Cotnari and 3% of the total quantity of Tămâioasă grapes.

After pressing, the grape marc represents 34,48% of the total quantity of crushed grapes of Fetească albă, 30,37 % of the total quantity of crushed grapes of Frâncușă, 36,14% of the total quantity of crushed grapes of Grasă de Cotnari and 34,62 of the total quantity of crushed grapes of Tămâioasă românească. The ratio between the obtained wine and the total quantity of harvested grapes is 56 L Fetească albă (52,58% ), 47 L Frâncușă (57,31% ), 43 L Grasă de Cotnari (50,58% ), 116 L Tămâioasă românească (58,14% ).

Table 1

**Technological characteristics of raw matter from Fetească albă, Frâncușă, Grasă de Cotnari and Tămâioasă românească**

| Grape variety        | Grape mass (kg) | 100 berries mass (g) | Crushed grapes mass (L) | Stem mass (kg) | Grape marc (kg) | Must (L) | Wine (L) | Structure index |
|----------------------|-----------------|----------------------|-------------------------|----------------|-----------------|----------|----------|-----------------|
| Fetească albă        | 106,5           | 225,01               | 101,5                   | 5              | 35              | 66,5     | 56       | 21,3            |
| Frâncușă             | 82              | 310,6                | 79                      | 3              | 24              | 55       | 47       | 27,3            |
| Grasă de Cotnari     | 85              | 375,77               | 83                      | 2              | 30              | 53       | 43       | 42,5            |
| Tămâioasă românească | 199,5           | 322,7                | 193,5                   | 6              | 67              | 126      | 116      | 33,25           |

Table 2

**Quality characteristics of Fetească albă, Frâncușă, Grasă de Cotnari și Tămâioasă românească musts**

| Grape variety        | Sugars (g/L) | Total acidity (g/L C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> ) | Efficiency in must (%) |
|----------------------|--------------|---|------------------------|
| Fetească albă        | 192          | 7,28  | 62,44                  |
| Frâncușă             | 177,2        | 7,5   | 67,07                  |
| Grasă de Cotnari     | 191,9        | 8,83  | 62,35                  |
| Tămâioasă românească | 203,3        | 6,5   | 63,15                  |

Table 3

**Physical-chemical characteristics of Fetească albă, Frâncușă, Grasă de Cotnari și Tămâioasă românească wines**

| Wine                 | Volatile acidity (g/L C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> ) | Total acidity (g/L C <sub>4</sub> H <sub>6</sub> O <sub>6</sub> ) | Alcoholic conc. (% vol.) | Density (g/cm <sup>3</sup> ) | Reductive substances (g/L) | Total dry extract (g/L) | Non-reductive extract (g/L) | Total SO <sub>2</sub> (mg/L) | Free SO <sub>2</sub> (mg/L) |
|----------------------|--|---|--------------------------|------------------------------|----------------------------|-------------------------|-----------------------------|------------------------------|-----------------------------|
| Fetească albă        | 0.21   | 6.74  | 12.22                    | 0.99166                      | 0.80                       | 20.10                   | 19.30                       | 63.06                        | 29.86                       |
| Frâncușă             | 0.19   | 6.53  | 11.43                    | 0.99303                      | 0.74                       | 21.10                   | 20.36                       | 55.14                        | 27.11                       |
| Grasă de Cotnari     | 0.26   | 6.83  | 12.57                    | 0.99236                      | 0.77                       | 22.90                   | 22.13                       | 73.42                        | 34.73                       |
| Tămâioasă românească | 0.27   | 5.97  | 11.71                    | 0.99237                      | 1.52                       | 20.30                   | 18.78                       | 40.52                        | 17.97                       |

Table 2, presents the characteristics of musts of the 4 grape varieties. Tămâioasă has 203 g /L while Grasă de Cotnari registers 191 g/L, with an equilibrated total acidity 8,83 (g/L C<sub>4</sub>H<sub>6</sub>O<sub>6</sub>) and 6,5 (g/L C<sub>4</sub>H<sub>6</sub>O<sub>6</sub>) respectively. The grape to must conversion rate at all grape varieties was specific to obtaining quality wines, the highest values being registered at Frâncușă, known in literature also as "Vinoasă" due to the structure of the grape berry that allows the accumulation of higher quantities of must compared to other varieties from the same quality category (Rotaru, 2009).

Table 3 presents the physical-chemical characteristics of the 4 wines. All wines are classified as DOC, due to the values of alcoholic concentration and dry extract. Grasă de Cotnari wine has the highest alcoholic concentration (12, 57% vol.) and the highest non-reductive extract (22,9 g/L). The lowest values are found in Frâncușă (11,43% vol.) and Tămâioasă românească (11,7% vol.). The reductive substances are in small quantities, the wines are dry. Total acidity is equilibrated, with an average of 6,5 g/L tartaric acid. As expected, the musts' acidity drops with approx. 1-1,5 g/L tartaric acid in the obtained wines.

## CONCLUSIONS

1. The obtained production levels and compositional characteristics of the grapes prove that the four grape varieties are well adapted to the ecopedoclimatic conditions of Cotnari vineyard, the obtained values being characteristic to the varieties.

2. Although grape harvest was not realised at technological harvest, the obtained wines for 2011 are classified as controlled origin denomination (DOC).

3. Grasă de Cotnari variety proves its quality, the obtained wine having alcoholic concentration, equilibrated acidity and a well structured body, being the etalon variety for Cotnari vineyard.

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# THE EVALUATION OF THE INTEGRATED SYSTEM FOR RED WINES PRODUCING

## EVALUAREA SISTEMULUI INTEGRAT DE PRODUCERE A VINURILOR ROȘII

*FOTESCU Laura<sup>1</sup>, TUDORACHE Aurelia<sup>1</sup>, BRÎNDUȘE Elena<sup>1</sup>*

e-mail: laurafotescu@yahoo.com

**Abstract.** *The integrated system represents the most efficient production system, responding to the current requirements regarding the achievement of quality products, safe in consumption, in a clean and safe environment. The production of red wines is suitable to this system. The studies were realised at the Research and Development Institute for Viticulture and Enology, Valea Calugareasca, during 2006-2010, in order to evaluate the integrated system for red wines producing based on a specific methodology. For defining the competitiveness level of the production system, a number of 45 parameters/indicators were evaluated, from which 2 parameters/indicators for wine quality, 32 parameters/indicators for alimentary safety of wines and 11 parameters/indicators for the environment protection related to the winemaking technology impact. The integrated systems for red wines production was compared with the classical system (used before implementation). The general profile of the classical/integrated production systems is presented into a graphic under radial shape. Analyzing the general profile of the two classical/integrated systems, a quality improvement of most parameters/indicators specific to the integrated system for producing red wines has been noticed.*

**Key words:** integrated system, red wine

**Rezumat.** *Sistemul integrat reprezintă cel mai performant sistem de producție, răspunzând cerințelor actuale privind realizarea produselor de calitate, sigure în consum, într-un mediu curat și protejat. Producția de vinuri roșii este pretabilă acestui sistem. Cercetările efectuate la Institutul de Cercetare-Dezvoltare pentru Viticultură și Vinificație Valea Călugărească în perioada 2006-2010, au avut drept scop evaluarea sistemului integrat de producere a vinurilor roșii pe baza unei metodologii proprii. Pentru definirea nivelului de competitivitate al sistemului de producție, s-a evaluat un număr de 45 de parametrii/indicatori: 2 parametrii/indicatori pentru calitatea vinurilor, 32 parametrii/indicatori pentru siguranța alimentară a vinurilor și 11 parametrii/indicatori pentru protecția mediului față de impactul tehnologiei vinicole. Sistemul integrat de producere a vinurilor roșii a fost comparat cu sistemul clasic (utilizat înaintea implementării). Profilul general al sistemelor de producție clasic/integrat a fost prezentat într-un grafic, sub formă radială. Din analiza profilului general al celor două sisteme clasic/integrat a fost evidențiată o îmbunătățire calitativă pentru majoritatea parametrilor/indicatorilor specifici sistemului integrat de producere a vinurilor roșii.*

**Cuvinte cheie:** sistem integrat, vin roșu

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<sup>1</sup> Research and Development Institute for Viticulture and Enology, Valea Călugărească, Romania

## INTRODUCTION

The integrated system is the most efficient system for production, conceived as a management system based on the objectives which represent the requirements of the system (Fotescu and Tudorache, 2007, Fotescu, 2010). The requirements of the production integrated systems are identified into the legal framework associated to three aspects: the quality of red wines production, the alimentary safety of red wines and the environment protection related to the winemaking technology impact (Rochard et al., 1998; Fotescu et al., 2010). The evaluation highlights the extent to which wine production systems (classical systems/integrated) meet the requirements, using a specific methodology (METEN 0309). The specific methodology for the evaluation of the wines production systems respect the basic principles concerning the establishing of a methodology specified in the national and international scientific papers. The evaluation was performed to prove the efficiency of the integrated system for red wines producing, for the correct implementation and operation of the system.

## MATERIAL AND METHOD

The evaluation of the production systems has been made based upon the studies aiming at:

1. Identification of evaluation the parameters/indicators for three aspects point of view;
2. Establishment of the evaluation classes and points awarded;
3. Data collection;
4. Analysis and data transformation in the parameters/indicators for evaluation;
5. Reporting and evaluation

The methods and the management techniques used for the evaluation of production systems are the following: diagnosis method (used to investigate the main technical, economical, management aspects of the integrated system), the informational system (used for collecting, recording, processing, storage and/or data and integrated system informations transmission) and informatique system (used in automatic processing of information).

## RESULTS AND DISCUSSIONS

The objective evaluation of the red wine production systems was achieved during 2006-2010, at the Institute for Viticulture and Enology, Valea Calugareasca, during 2006-2010 period, using a specific methodology. Model of evaluation is „Planning-Perform-Check-Action”. The Planning alluded to selecting parameters/indicators for evaluation, which may include both parameters/indicators recommended, also news parameters/indicators. The Perform data collection targets for parameters/indicators select, analysis and transformation of dates into informations which describes wines production system performance. The informations were evaluated by comparison and transmission the informations. The check and action is about to analysis and improvement of the system for red wines producing. The evaluation process is sustained.



### 1. The identification of the parameters/indicators for evaluation

In order to characterize a representative system for wine production, the specific parameters/indicators are selected (tab. 1).

Table 1

**The parameters/indicators grouped by aspects used for the production systems evaluation**

| Aspect  | Code indicator | The parameters/indicators used for evaluation                   |                   |
|---|----------------|---|-------------------|
|   |                | Name of indicator   | Specifications    |
| 1. The quality of the red wines production                                | C1             | The synthetic indicator of the quality red wine assortments     |                   |
|   | C2             | The technical level for red wines production                    |                   |
| 2. The alimentary safety of red wines                                     | SA1            | The marques of pollution by pesticides                          | dinocap           |
|   | SA2            |   | dicofol           |
|   | SA3            |   | penconazole       |
|   | SA4            |   | deltamethrin      |
|   | SA5            |   | glyphosate        |
|   | SA6            |   | iprodione         |
|   | SA7            |   | cimoxanil         |
|   | SA8            |   | chlorothalonil    |
|   | SA9            | The concentration of ochratoxin                                 |                   |
|   | SA10           | The heavy metal contamination                                   | cadmium           |
|   | SA11           |   | copper            |
|   | SA12           |   | iron              |
|   | SA13           |   | plumbum           |
|   | SA14           |   | zinc              |
|   | SA15           |   | arsenic           |
|   | SA16           | The cleanliness of the technological equipment                  | BC_01 walls       |
|   | SA17           |   | BC_02 strainers   |
|   | SA18           |   | CRO 7 inside      |
|   | SA19           |   | CRO 5 evacuation  |
|   | SA20           |   | CDI 2 inside      |
|   | SA21           |   | CDI 4 evacuation  |
|   | SA22           |   | CMF 48 inside     |
|   | SA23           |   | PS 1 inside       |
|   | SA24           |   | PS 1 strainer     |
|   | SA25           |   | CMF 3 feeding     |
|   | SA26           | CMF 8 inside  |                   |
|   | SA27           | The conformity of the technological water for food application  | pH                |
|   | SA28           |   | Conductiv at 20°C |
|   | SA29           |   | Turbidity         |
|   | SA30           |   | Coliform bacteria |
|   | SA31           |   | Escherichia coli  |
|   | SA32           | The conformity of the oenological products for food application |                   |
| 3. The environment protection related to the winemaking technology impact | PM1            | The volume of waste water                                       |                   |
|   | PM2            | The characteristics of waste water pollutants                   | pH                |
|   | PM3            |   | Suspended solids  |
|   | PM4            |   | Fixed residue     |
|   | PM5            |   | CCOCr             |
|   | PM6            |   | CBO5              |
|   | PM7            |   | Extractable subs  |

|  |      |   |                         |
|--|------|---|-------------------------|
|  | PM8  |   | Ammonium                |
|  | PM9  |   | Phosphates<br>(P total) |
|  | PM10 |   | Detergents              |
|  | PM11 | The quantity of resulted vegetables waste |                         |

The parameters/indicators of the system are grouped in the table 1 being associated to the following aspects: the quality of the red wines, alimentary safety of wines and the environment protection related to the winemaking technology impact.

The parameters/indicators that meet the requirements associated with the best quality of red wines are: the synthetic indicator of the quality (code C1) and the technical level for red wines production (code C2).

The alimentary safety of red wines was evaluated using specific indicators , as follows: the pollution level of pesticide residue in the production of red wines (SA1-SA8 codes), ochratoxin contamination values (code SA9), the heavy metal contamination (codes SA10-SA15), assessing the state of hygiene of technological equipament (codes SA16-SA26), conformity values of the technological water for food application (codes SA27-SA31) and oenological products values (codes SA32).

The specific indicators for the evaluation of pollution degree environmental related to the winemaking technology impact are: volume of waste water per hectolitre of wine (code PM1), characteristics of waste water pollutants (codes PM2-PM10) and the quantity of vegetable waste (code PM11).

## **2. The establishment of the evaluation classes and points assignment**

The parameters/indicators of the system are grouped in 4 classes of variation.

In the case of expression of the parameters/indicators by numerical values, the range of variation is grouped in the following way: the first class receives 4 points, the second 2 points and the fourth 1 point.

In the case of expression of the parameters/indicators by qualifyings the following unitary correlation are kept: very good 4 points, good 3 points, average 2 points, low 1 point.

Related to “the environment protection related to the winemaking technology impact” the grouping of the parameters/indicators for both classical (CSPRW) and integrated production systems (ISPRW) are presented (fig. 1).

| The parameters/indicators used for evaluation   | classical system<br>(CSPRW) |          |          |          | TOTAL     | integrated system<br>(ISPRW) |           |          |          | TOTAL     |
|---|-----------------------------|----------|----------|----------|-----------|------------------------------|-----------|----------|----------|-----------|
|   | Cl. 1                       | Cl. 2    | Cl. 3    | Cl. 4    |           | Cl. 1                        | Cl. 2     | Cl. 3    | Cl. 4    |           |
|   | 4 pt                        | 3 pt     | 2 pt     | 1 pt     |           | 4 pt                         | 3 pt      | 2 pt     | 1 pt     |           |
| <b>The aspect 3. The environment protection related to the winemaking technology impact</b> |                             |          |          |          |           |                              |           |          |          |           |
| -The volume of waste water  | 0                           | 0        | 0        | x        | 1         | 0                            | x         | 0        | 0        | 3         |
| <i>The score for volume of waste water</i>  | 0                           | 0        | 0        | 1        | 1         | 0                            | 3         | 0        | 0        | 3         |
| -The characteristics of waste water pollutants:   |                             |          |          |          |           |                              |           |          |          |           |
| pH  | 0                           | 0        | x        | 0        | 2         | 0                            | x         | 0        | 0        | 3         |
| Suspended solids  | 0                           | x        | 0        | 0        | 3         | x                            | 0         | 0        | 0        | 4         |
| Fixed residue   | 0                           | x        | 0        | 0        | 3         | 0                            | x         | 0        | 0        | 3         |
| CCOCr   | 0                           | 0        | x        | 0        | 2         | 0                            | 0         | x        | 0        | 2         |
| CBO5  | 0                           | 0        | 0        | x        | 1         | 0                            | 0         | x        | 0        | 2         |
| Extractible suspended   | x                           | 0        | 0        | 0        | 4         | x                            | 0         | 0        | 0        | 4         |
| Ammonium  | 0                           | 0        | x        | 0        | 2         | 0                            | x         | 0        | 0        | 3         |
| Phosphates (total phosphorus)   | x                           | 0        | 0        | 0        | 4         | x                            | 0         | 0        | 0        | 4         |
| Detergents  | x                           | 0        | 0        | 0        | 4         | x                            | 0         | 0        | 0        | 4         |
| <i>The score for characteristics pollutants</i>   | 12                          | 6        | 6        | 1        | 25        | 16                           | 9         | 4        | 0        | 29        |
| -The quantity of resulted vegetables waste  | 0                           | 0        | 0        | x        | 1         | 0                            | 0         | x        | 0        | 2         |
| <i>The score of resulted vegetable waste</i>  | 0                           | 0        | 0        | 1        | 1         | 0                            | 0         | 2        | 0        | 2         |
| <b>The score awarded to the aspect 3</b>  | <b>12</b>                   | <b>6</b> | <b>6</b> | <b>3</b> | <b>27</b> | <b>16</b>                    | <b>12</b> | <b>6</b> | <b>0</b> | <b>34</b> |

Fig. 1. - Gruping parameters/indicators for production systems in classes of variation

The score awarded to the parameters/indicators is different for the two production systems, respectively 27 points for the CSPRW and 34 points for the ISPRW.

### 3. Data collection

Data collections are performed on primary data worksheets.

### 4. The analysis and transformation of the primary data in parameters/indicators for the evaluation

The worksheets primary data are corrected and grouped in the quality classes. An "Evaluation sheet" are realized .

Data analysis is done either by calculating the ratio system, or by defining the system profile.

#### 4.1. The calculation of the system score

The score of the each class is calculated at the level of all the aspects. The calculation is performed by summing the results of the multiplication between the score of the points/classes and number 0 or 1. The number 0 is applied in the case of unmarked box, and the number 1 if the box is marked with X.

The total score represented the sum of the score allocated for each aspect.

#### 4.2. The defining the system profile

A graph of radial type, consisting of a variety of rays which are proportional with the value of the system's parameters/indicators is traced. The extremities of these rays are joined forming the profile of the system.

### 5. The reporting and evaluation

5.1. The evaluation report by points of the CSPRW and ISPRW systems is presented as a SHEET (fig. 2).

The results of the evaluation were the following: total number of the evaluated indicators 45, the grouping of the indicators based on three aspects, number of the evaluation classes 4, the evaluation scale: class 1=4 points, class 2=3 points; class 3=2 points, class 4=1 point.

| The parameters/indicators used for evaluation   | Indicator | CSPRW |       |       |       | TOTAL | ISPRW |       |       |       | TOTAL |
|---|-----------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
|   |           | Cl. 1 | Cl. 2 | Cl. 3 | Cl. 4 |       | Cl. 1 | Cl. 2 | Cl. 3 | Cl. 4 |       |
| <i>The score awarded to the parameters/indicators</i>                                       |           | 4 pt. | 3 pt. | 2 pt. | 1 pt. |       | 4 pt. | 3 pt. | 2 pt. | 1 pt. |       |
| <b>The aspect 1. The quality of the red wines production</b>                                |           |       |       |       |       |       |       |       |       |       |       |
| -synthetic indicator of the quality red wine assortments (N <sub>syn</sub> )                | C1        | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| -technical level for red wines production (N <sub>TE</sub> )                                | C2        | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| <i>The score awarded to the aspect 1</i>  |           | 0     | 0     | 4     | 0     | 4     | 0     | 6     | 0     | 0     | 6     |
| <b>The aspect 2. The alimentary safety of red wines</b>                                     |           |       |       |       |       |       |       |       |       |       |       |
| <i>-markes of pollution by pesticides:</i>  |           |       |       |       |       |       |       |       |       |       |       |
| dinocap   | SA1       | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| dicofol   | SA2       | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| penconazole   | SA3       | 0     | 0     | x     | 0     | 2     | x     | 0     | 0     | 0     | 4     |
| deltamethrin  | SA4       | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| glyphosate  | SA5       | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| iprodione   | SA6       | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| cimoxanil   | SA7       | 0     | x     | 0     | 0     | 3     | x     | 0     | 0     | 0     | 4     |
| chlorothalonil  | SA8       | 0     | x     | 0     | 0     | 3     | x     | 0     | 0     | 0     | 4     |
| <i>The score for markers</i>  |           | 0     | 6     | 12    | 0     | 18    | 12    | 15    | 0     | 0     | 27    |
| -concentration of ochratoxin  |           | 0     | x     | 0     | 0     | 3     | x     | 0     | 0     | 0     | 4     |
| <i>The score for ochratoxin</i>   |           | 0     | 3     | 0     | 0     | 3     | 4     | 0     | 0     | 0     | 4     |
| <i>-heavy metal contamination:</i>  |           |       |       |       |       |       |       |       |       |       |       |
| cadmium   | SA10      | 0     | x     | 0     | 0     | 3     | x     | 0     | 0     | 0     | 4     |
| copper  | SA11      | 0     | x     | 0     | 0     | 3     | x     | 0     | 0     | 0     | 4     |
| iron  | SA12      | 0     | x     | 0     | 0     | 3     | 0     | x     | 0     | 0     | 3     |
| plumbum   | SA13      | 0     | x     | 0     | 0     | 3     | x     | 0     | 0     | 0     | 4     |
| zinc  | SA14      | 0     | x     | 0     | 0     | 3     | 0     | x     | 0     | 0     | 3     |
| arsenic   | SA15      | 0     | x     | 0     | 0     | 3     | x     | 0     | 0     | 0     | 4     |
| <i>The score for heavy metal contamination</i>  |           | 0     | 18    | 0     | 0     | 18    | 16    | 6     | 0     | 0     | 22    |
| <i>-the cleanliness of the technological equipment:</i>                                     |           |       |       |       |       |       |       |       |       |       |       |
| BC_01 walls   | SA16      | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| BC_02 strainer  | SA17      | 0     | x     | 0     | 0     | 3     | 0     | x     | 0     | 0     | 3     |
| CRO 7 inside  | SA18      | 0     | x     | 0     | 0     | 3     | 0     | x     | 0     | 0     | 3     |
| CRO 5 evacuation  | SA19      | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| CDI 2 inside  | SA20      | 0     | x     | 0     | 0     | 3     | 0     | x     | 0     | 0     | 3     |
| CDI 4 evacuation  | SA21      | 0     | 0     | x     | 0     | 2     | 0     | 0     | x     | 0     | 2     |
| CMF 48 inside   | SA22      | 0     | x     | 0     | 0     | 3     | 0     | x     | 0     | 0     | 3     |
| PS 1 inside   | SA23      | 0     | 0     | 0     | x     | 1     | 0     | 0     | x     | 0     | 2     |
| PS 1 strainer   | SA24      | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| CMF 3 feeding   | SA25      | 0     | x     | 0     | 0     | 3     | 0     | x     | 0     | 0     | 3     |
| CMF 8 inside  | SA26      | 0     | x     | 0     | 0     | 3     | 0     | x     | 0     | 0     | 3     |
| <i>The score for cleanliness of equipment</i>   |           | 0     | 18    | 8     | 1     | 27    | 0     | 27    | 4     | 0     | 31    |
| <i>-conformity of the technological water for food application</i>                          |           |       |       |       |       |       |       |       |       |       |       |
| pH  | SA27      | 0     | 0     | x     | 0     | 2     | 0     | 0     | x     | 0     | 2     |
| Conductivity at 20°C  | SA28      | 0     | 0     | 0     | x     | 1     | 0     | 0     | x     | 0     | 2     |
| Turbidity   | SA29      | 0     | 0     | 0     | x     | 1     | 0     | 0     | x     | 0     | 2     |
| Coliform bacteria   | SA30      | x     | 0     | 0     | 0     | 4     | x     | 0     | 0     | 0     | 4     |
| Escherichia coli  | SA31      | x     | 0     | 0     | 0     | 4     | x     | 0     | 0     | 0     | 4     |
| <i>The score for conformity technological water</i>   |           | 8     | 0     | 2     | 2     | 12    | 8     | 0     | 6     | 0     | 14    |
| <i>-conformity of the oenological products for food application</i>                         |           |       |       |       |       |       |       |       |       |       |       |
| <i>The score for conformity oenological products</i>  |           | 4     | 0     | 0     | 0     | 4     | 4     | 0     | 0     | 0     | 4     |
| <i>The score awarded to the aspect 2</i>  |           | 12    | 45    | 22    | 3     | 82    | 44    | 48    | 10    | 0     | 102   |
| <b>The aspect 3. The environment protection related to the winemaking technology impact</b> |           |       |       |       |       |       |       |       |       |       |       |
| <i>-volume of waste water</i>   |           |       |       |       |       |       |       |       |       |       |       |
| <i>The score for volume of waste water</i>  |           | 0     | 0     | 0     | x     | 1     | 0     | x     | 0     | 0     | 3     |
| <i>-characteristics of waste water pollutants:</i>  |           |       |       |       |       |       |       |       |       |       |       |
| pH  | PM2       | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| Suspended solids  | PM3       | 0     | x     | 0     | 0     | 3     | x     | 0     | 0     | 0     | 4     |
| Fixed residue   | PM4       | 0     | x     | 0     | 0     | 3     | 0     | x     | 0     | 0     | 3     |
| COOCr   | PM5       | 0     | 0     | x     | 0     | 2     | 0     | 0     | x     | 0     | 2     |
| CBO5  | PM6       | 0     | 0     | 0     | x     | 1     | 0     | 0     | x     | 0     | 2     |
| Extractable suspended   | PM7       | x     | 0     | 0     | 0     | 4     | x     | 0     | 0     | 0     | 4     |
| Ammonium  | PM8       | 0     | 0     | x     | 0     | 2     | 0     | x     | 0     | 0     | 3     |
| Phosphates (total phosphorus)   | PM9       | x     | 0     | 0     | 0     | 4     | x     | 0     | 0     | 0     | 4     |
| Detergents  | PM10      | x     | 0     | 0     | 0     | 4     | x     | 0     | 0     | 0     | 4     |
| <i>The score for characteristics pollutants</i>   |           | 12    | 6     | 6     | 1     | 25    | 16    | 9     | 4     | 0     | 29    |
| -the quantity of vegetables waste resulted  | PM11      | 0     | 0     | 0     | x     | 1     | 0     | 0     | x     | 0     | 2     |
| <i>The score for the quantity of vegetable waste</i>  |           | 0     | 0     | 0     | 1     | 1     | 0     | 0     | 2     | 0     | 2     |
| <i>The score awarded to the aspect 3</i>  |           | 12    | 6     | 6     | 3     | 27    | 16    | 12    | 6     | 0     | 34    |
| <b>THE TOTAL SCORE/THE ASPECTS</b>  |           | 24    | 51    | 32    | 6     | 113   | 60    | 66    | 16    | 0     | 142   |
| <b>THE MAXIMUM SCORE</b>  |           | 180   |       |       |       |       |       |       |       |       |       |
| <b>THE TOTAL SCORE/THE ASPECTS</b>  |           | 113   |       |       |       |       | 142   |       |       |       |       |

Fig. 2 – Evaluation sheet of the red wine production systems by points

The rating scale of the two production systems reached a maximum of 180 points. The classical production system (CSPRW) registered 113 points, while the integrated production system (ISPRW) 142 points.

5.2. The report regarding the system profile

The profile of red wine production CSPRW/ISPRW systems is a radial graph presented in figure 3.

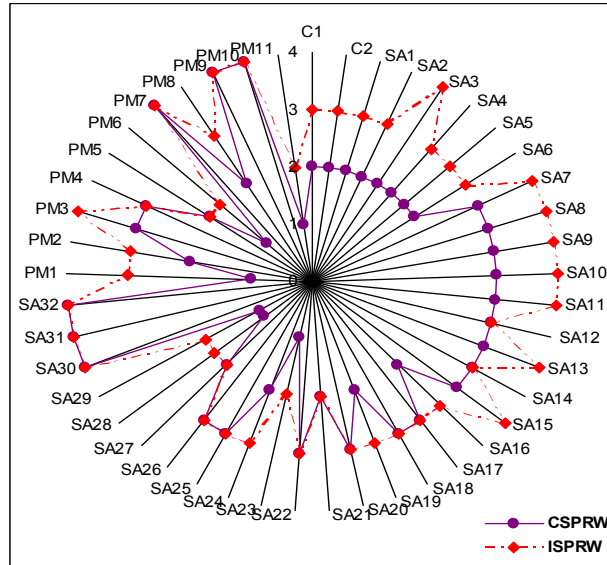


Fig. 3 – The profile of the red wines production systems

The rays are represented by the indicators/parameters ratio of the two CSPRW and ISPRW systems. The profiles of the two systems are obtained by joining the points.

5.3. The report for CSPRW and ISPRW systems analysis at Research and Development Institute for Viticulture and Enology Valea Calugareasca

The verification and the action are performed in order to analyse and improve the system for quality red wines producing. The development stage of the CSPRW/ISPRW compared systems is achieved by the calculation of the relative difference (Table 2).

Table 2

The comparison of the developmental stages of classical/integrated systems

| Stage of development | Relative difference*, % |       |       |
|----------------------|-------------------------|-------|-------|
|                      | optimal                 | CSPRW | ISPRW |
| Exceptional (EXC)    | 90-100                  |       |       |
| Very good (VG)       | 70-90                   |       | 78,89 |
| Good (G)             | 50-70                   | 62,78 |       |

\*Relative difference= the score registered by each system /180x100

The relative difference is expressed as a percentage and represents the results of the multiplication between the score registered from each system and the maximum score reported to 100.

The level of the SIPVR system development is evaluated with the “Very good” qualifying and requires further improvements in order to achieve the “Exceptional” qualifying.

As a result of the systems comparison, SIPVR registered a qualitative improvement for 8 systems parameters/indicators, 5 defining the alimentary safety of wines and 3 the environmental protection related to the winemaking technology impact

The parameters/indicators associated with the alimentary safety of red wines for which ISPRW has registered significant improvements are: the hygiene of equipments (SA21 și SA23 indicators) and the conformity of the technological water (SA27, SA28, SA29 indicators).

The parameters/indicators associated with the environmental protection related to the winemaking technology impact for which ISPRW registered significant improvements are: characteristics of waste water pollutants (PM5 and PM6 indicators) and quantity of resulted vegetable waste (PM11 indicator).

## CONCLUSIONS

1. From a maximum of 180 points, the classical production system (CSPRW) registered 113 points, while the integrated production system (ISPRW) 142 points.

2. The development stage of the CSPRW/ISPRW compared systems was achieved by the calculation of the relative difference.

3. The comparison of the classical/integrated systems put into evidence the superiority of the integrated system. SIPVR registered a qualitative improvement for 8 systems parameters/indicators (5 defining the alimentary safety of wines and 3 defining the environmental protection related to the winemaking technology impact).

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# CORRELATIONS OF SENSORY DATA WITH THE ANALYTICAL POLYPHENOLIC COMPOSITION OF GREEK WINES

## CORELAȚII ÎNTRE DATELE SENZORIALE ȘI COMPOZIȚIA POLIFENOLICĂ ANALITICĂ A VINURILOR GRECEȘTI

*KALLITHRAKA Stamatina*<sup>1</sup>, *KIM D.*<sup>2</sup>, *TSAKIRIS A.*<sup>3</sup>,  
*PARASKEVOPOULOS I.*<sup>3</sup>, *KOTSERIDIS Y.*<sup>1</sup>, *KYRALEOU Maria*<sup>1</sup>, *SOLEAS G.*<sup>2</sup>.  
e-mail: ykotseridis@aua.gr

**Abstract:** *The purpose of this study was to measure the astringency of selected Greek red wines and to assess the relationship between sensory and chemical data. Nine red wines produced by three native Greek grape varieties (Agiorgitiko, Xinomavro and Mandilaria) were used and their astringency and bitterness was assessed by a trained panel. In addition, their astringency was estimated chemically employing the ovalbumin precipitation method. The sensory data showed that Mandilaria was the most astringent variety whereas Agiorgitiko the least. Statistical analysis of the results indicated that the chemical data obtained for astringency significantly correlated with the sensory determinations. In addition significant correlations were obtained between sensory data and wine polyphenolic composition.*

**Key words:** native Greek grape varieties, astringency, polyphenolic composition

**Rezumat:** *Scopul acestui studiu este de a analiza astringența unor vinuri grecești și de a evalua relația dintre datele senzoriale și cele chimice. Nouă vinuri roșii obținute din soiuri tradiționale grecești (Agiorgitiko, Xinomavro and Mandilaria) au fost utilizate, astringența lor fiind analizată cu metoda precipitării ovalbuminei. Datele senzoriale arată ca Mandilaria a fost soiul cu astringența cea mai mare, iar Agiorgitiko cu astringența cea mai redusă. Analiza statistică indică faptul că rezultatele analizelor chimice se corelează semnificativ cu determinările senzoriale. În plus, corelații semnificative au fost obținute între datele senzoriale și compoziția polifenolică a vinurilor.*

**Cuvinte cheie :** soiuri tradiționale grecești, astringență, compoziție polifenolică

### INTRODUCTION

The oral sensation referred to as “astringency”, and most commonly described as “drying”, “roughing” and “puckering” is a primary mouth-feel attributed in red wine. The perception of astringency is a highly dynamic process, changing continuously during ingestion and especially following expectoration or swallowing (Noble, 1995).

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<sup>1</sup> Department of Food Science & Technology, Agricultural University of Athens, Greece

<sup>2</sup> Liquor Control Board of Ontario (LCBO), Toronto, Ontario, Canada

<sup>3</sup> Department of Enology & Beverage Technology, Technological Educational Institute (T. E. I.) of Athens, Greece

Wines that are astringent are termed “tannic”. Wine tannins are polymeric pigments formed by polymerization of flavan-3-ol subunits (Vidal et al., 2003). These polyphenolic compounds form complexes with salivary proteins. Protein-tannin complexes result in the precipitation and/or aggregation of salivary proteins causing them to lose their lubricating properties. The chemical binding between polyphenols and salivary proteins is due to both hydrophobic interactions and hydrogen bonding. The propensity to form precipitates is dependent on the relative concentration of the polyphenol and protein substrates (Gawel, 1998).

The purpose of the proposed research was to measure the astringency of Appellation of Origin Wines originating from three major red varieties (Xinomavro, Mandilaria and Agiorgitiko) cultivated in Greece. In addition, it was of interest to assess the relationship between sensory and chemical data. The outcomes of such studies might provide wine industry an analytical and objective method to estimate astringency of red wines. Finally, exploiting the possible correlations between sensory astringency parameters and the corresponding polyphenolic concentration of the wines would be of practical interest to winemakers since they could improve control over extraction or pressing process and thus improve the quality of the produced wine.

## MATERIAL AND METHOD

**Panelists:** Twelve healthy subjects participated in the experiment. All the subjects were experienced wine assessors and they were all familiar with the simple T-I computerized technique used in the experiment (Kallithraka et.al 1997).

**Wines:** Nine red Appellation of Origin wines were assayed during this study. Three cultivars were studied (*V. vinifera* species): Agiorgitiko, Xinomavro and Mandilaria. Agiorgitiko cultivar were grown in Nemea (Peloponnesus), Xinomavro in Goumenissa (North Greece) and Mandilaria in Paros (Cyclades islands).

**Sensory assessment:** Judges were presented with samples of 10 ml, at room temperature. At time 0s they placed the entire wine sample into their mouth, swirl it for 15 s, and expectorated. A computerized T-I method was used to rate astringency by manipulating a marker using a mouse on a unstructured line scale of 100 mm length, anchored at either end by 0= none and 100 = extreme.

**HPLC determination of anthocyanins:** Analyses were performed, as in Kallithraka, Mohdaly, Makris & Kefalas (2005).

**HPLC determination of individual phenolics:** The individual polyphenolic constituents were determined by HPLC. The chromatography apparatus used was an HP 1090, coupled with an Agilent 1100 diode array detector. The column was a Spherisorb ODS 2, 250 x 4 mm, 5 µm. The chromatographic conditions are described in Makris, Psarra, Kallithraka, & Kefalas, (2003).

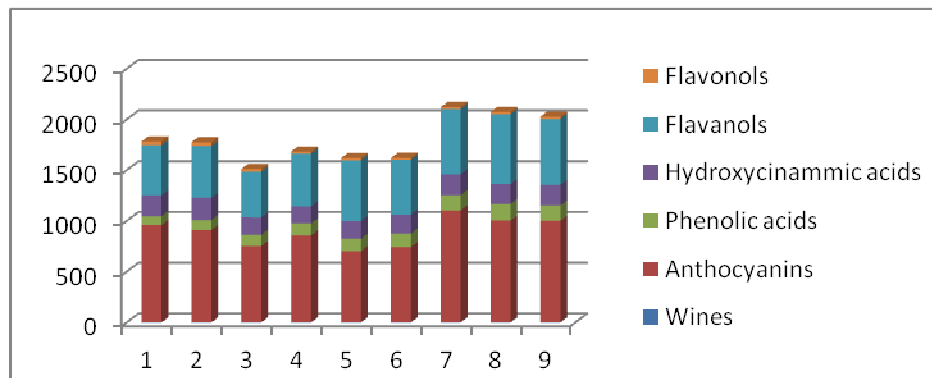
**Total phenols:** Total polyphenol concentration was determined with the Folin-Ciocalteu assay (Arnous, Makris & Kefalas, 2001).

**Ovalbumin precipitation method:** The analysis was performed according to Llaudy, Canals, Canals, Rozes Arola & Zamora (2004) using ovalbumin as precipitation agent and tannic acid solutions as standards.



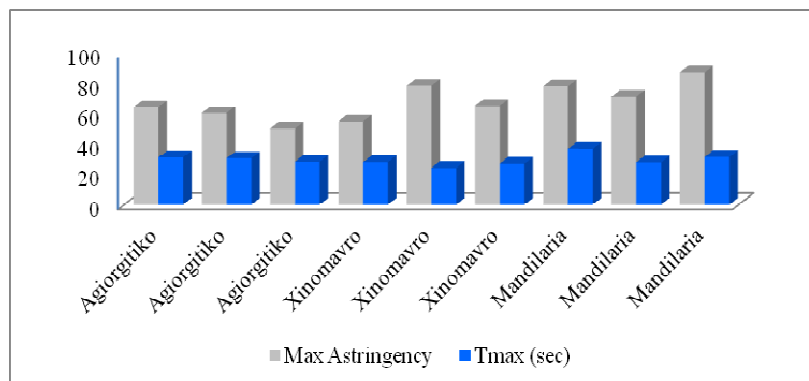
## RESULTS AND DISCUSSIONS

**Phenolic composition of the wines.** The results related to the determination of phenolic compounds in the wines studied are presented as summaries of the concentrations of the individual phenols determined by HPLC: total anthocyanins (TA), total catechins (TC), total hydroxycinnamates (TH), total phenolic acids (TPA) and total flavonols (TF) in figure 1.



**Fig. 1** - Phenolic composition of the wines studied (wines 1-3 are made by Agiorgitiko, 4-6 by Xinomavro and 7-9 by Mandilaria varieties)

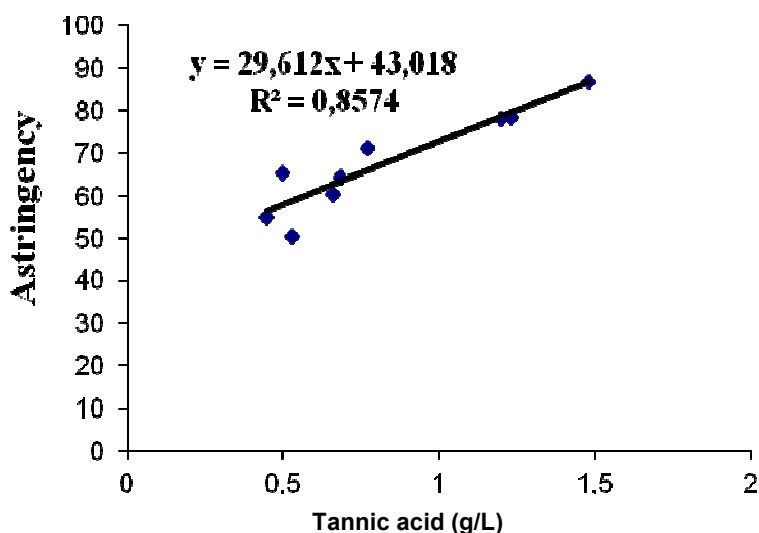
**Sensory determination of astringency.** The T-I data ( $I_{max}$ ,  $T_{max}$ ) for the 12 panelists and the 9 wines tested are presented in Figure 2. Mandilaria wines were scored more astringent on average (78.7) than the wines made by the other two varieties. The less astringent wines were those made by Agiorgitiko (average 58.3 and 53 respectively). The highest time needed to reach astringency's maximum intensity was also observed for Mandilaria wines (31.9 s) followed by Agiorgitiko (29.83 s). Xinomavro wines showed the lowest  $T_{max}$  (26.23 s).



**Fig. 2** - Sensory parameters of the wines

**Estimation of chemical astringency.** The addition of ovalbumin to the tannic acid solutions resulted in tannic acid precipitation. The relationship between these two parameters was logarithmic in agreement with Laudy, Canals, Canals, Rozes Arola & Zamora, (2004). In addition, the slopes obtained from the logarithmic equations and the corresponding tannic acid concentrations were linearly related ( $r^2=0.9821$ ).

Figure 3 shows the relationship between the astringency of the wines assessed by the panel (sensory) and the calculated values of their corresponding tannic acid concentrations (chemical). This graph confirms the clear relationship between the results obtained by the analytical method and the sensory analysis ( $r^2=0.9328$ ).



**Fig. 3** - Relationship between chemical estimated astringency (tannic acid concentration g/L) and sensory assessed astringency

**Correlations with wine polyphenolic compositions.** Linear correlations were obtained by relating mean maximum Intensity of astringency ( $I_{max}$ ) and time to maximum intensity ( $T_{max}$ ) scores of astringency with the corresponding wine phenolic composition (tab. 1). Maximum intensity of astringency was significantly correlated with total wine phenols and total catechins. Hydroxycinnamic acids and flavonols were not found to be related with any of the astringency sensory parameters.

Furthermore, anthocyanins were not found to contribute to astringency maximum intensity in agreement with Landon, Weller, Harbertson & Ross,

(2008). However, a significant correlation was obtained between  $T_{max}$  of astringency and the concentration of wine anthocyanins (table 1).

Table 1

**Pearson's correlation coefficients obtained between polyphenolic composition of the wines examined and sensory data**

|                  | <b>Total Phenols</b> | <b>Total Anthocyanins</b> | <b>Total Catechins</b> |
|------------------|----------------------|---------------------------|------------------------|
| Imax Astringency | 0.8601               | n.s. <sup>2</sup>         | 0.8351                 |
| Tmax Astringency | n.s. <sup>2</sup>    | 0.8351                    | n.s. <sup>2</sup>      |

<sup>1</sup>Correlations are significant at the 0.01 level (two-tailed)

<sup>2</sup>Correlations are not significant at the 0.05 level

Anthocyanins in wines can undergo chemical reactions to form tannin-like polyphenolic compounds. Because these compounds can act as electrophiles or nucleophiles at wine pH, both anthocyanin-flavanol and flavanol-anthocyanin adducts can be formed (Vidal et al., 2004). Given the current model for astringency, a reduction in flavanol hydrophobicity (by incorporation of anthocyanins into their structure) would result in a delay in flavanol-protein interaction and resulting precipitation.

The existence of soluble complexes between salivary proteins and polyphenols in vitro, at mouth temperature and at pH typical of resting saliva, is well documented by Gawel (1998). It is also well documented that  $\pi$ -electrons of anthocyanins can form such complexes with copigments and produce bathochromic and hyperchromic effects. It is thus possible that the formation of such soluble complexes between anthocyanins-flavanols-proteins might retard the development of the astringency sensation as a result of increased viscosity or due to the reduced availability of the astringent compounds. Further experiments are needed to study these complex phenomena and related them to sensory perception.

## CONCLUSIONS

Mandilaria variety appeared to be the most astringent among the three Greek varieties studied. Chemical assessment of astringency was highly correlated with the obtained sensory data. Wine flavanols were strongly correlated with the intensity of astringency and bitterness while anthocyanins were related with  $T_{max}$  of astringency. It was hypothesized that the pigmented polymers of flavanols may lead to a delay in flavanol-protein precipitation.

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# REDUCTION OF PHENOLIC COMPOUNDS LEVEL FROM RED WINE, FOLLOWING TREATMENT WITH MICRO- AND MESOPOROUS MATERIALS

## REDUCEREA CONȚINUTULUI DE COMPUȘI FENOLICI DIN VINUL ROȘU LA TRATAREA CU MATERIALE MICRO- ȘI MEZOPOROASE

LUCHIAN Camelia<sup>1</sup>, COTEA V.V.<sup>1</sup>,  
PATRAȘ Antoanela<sup>1</sup>, NICULAUA M.<sup>2</sup>, SEFTEL Elena<sup>3</sup>  
e-mail: kamelia\_luchian@yahoo.com

**Abstract.** Phenols represent a large and complex group of compounds, with great importance, determining the characteristics and quality of red wines in particular. In this paper we tested the action of micro- and mesoporous materials on the concentration of phenolic compounds in red wines. We performed such experiments, which have demonstrated that micro- and mesoporous materials retain phenolic compounds from wine. Taking into account the maximum rate of retained phenolic compounds on the three studied materials, SBA-15, MCM-41, KIT-6, the results show that all three retain phenolic compounds from wine, the material with the maximum efficiency being SBA-15, with a rate of 19.15% retained phenolic compounds for 8.04 g adsorbent/ L wine.

**Key words:** wine, phenolic compounds, nanomaterials, adsorption

**Rezumat.** Fenolii sunt un grup mare și complex de compuși de importanță deosebită care determină caracteristicile și calitatea îndeosebi a vinurilor roșii. În această lucrare s-a testat acțiunea unor materiale microporoase și mezoporoase asupra concentrației compușilor fenolici din vinurile roșii. Am realizat astfel experimente care au demonstrat că materialele micro și mezoporoase, datorită structurii lor, rețin compușii fenolici din vin. Ținând cont de procentul maxim de compuși fenolici reținut pe cele trei materiale studiate, SBA-15, MCM-41, KIT-6, rezultatele obținute dovedesc că toate trei rețin compușii fenolici din vin, eficiență maximă având materialul SBA-15, cu un procent de 19.15 % compuși fenolici reținuți, pentru 8.04 g adsorbent/L vin.

**Cuvinte cheie:** vin, compuși fenolici, nanomateriale, adsorbție

### INTRODUCTION

Wine collect in its composition more than 1000 compounds, associated in a complex and inconstant way, some come from grapes, in an unchanged state, such as acids: tartaric, malic, citric; carbohydrates, minerals etc. Others are formed during alcoholic fermentation and in other fermentative processes such as alcohols, lactic and succinic acids, etc.; other part is formed by nonfermentative processes (Cotea et. al., 2009).

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup>Oenology Research Center – Iasi Branch of the Romanian Academy, Romania

<sup>3</sup>University of Antwerpen, Laboratory of Adsorption and Catalysis, Belgium

Changing the color, the flavor and the taste of wine are the main oenological problems facing the wine producers during its storage. The change of wine color is the result of phenolic compounds oxidation to quinones (catalyzed by  $\text{Fe}^{2+} / \text{Fe}^{3+}$ ,  $\text{Cu}^{2+}$  and oxidative enzymes), and of condensation reactions between phenolic compounds, with formation of stable colored polymeric structures, from the yellow-brown spectral region (Fabios et. al., 2000; Castro et al., 2001). Phenolic compounds, including phenolic acids (hydroxybenzoic acids and hydroxycinnamic acids), catechins, anthocyanins, procyanidins and flavonols are subject to oxidation processes (Margheri et. al., 1980).

Reducing the concentration of phenolic compounds in wine with adsorbent materials is a frequently used method in winemaking to control color and organoleptic changes (Spagna et. al., 1996; Spagna et. al., 2000).

In 1992, researchers at Mobil Corporation published the siliceous and aluminosiliceous mesoporous molecular sieves synthesis (ordered mesoporous materials) of M41S family (Beck et. al., 1992). Siliceous material MCM-41 is part of the M41S family, together with MCM-48 and MCM-50.

M41S mesoporous materials have pores with diameters between 2.0 nm and 10 nm; specific sizes of mesopores are from 2.0 to 50 nm (meso from Greek means between) (IUPAC, 1972).

Mesoporous silica MCM-41 is a non-acidic and biocompatible material; silica walls are inert to both acid and basic medium, with exception of hydrofluoric acid and concentrated basic solutions. The structure is resistant to abrasion and compression (Corma et. al., 1995).

SBA-15 mesoporous silica is a material obtained by using the structure directing agent, Pluronic P123, triblock copolymer. Structurally, SBA-15 has a 2-D arrangement of tubular channels (Zhao et. al., 1998).

Opposed to MCM-41, SBA-15 can be prepared with mesopores up to 30 nm and is more thermally stable due to higher thickness of the cylindrical pores walls (2-3 nm compared to 0.9-1.1 nm) (Zhao et. al., 1998).

In literature it was reported the synthesis of other mesoporous materials with larger pores, KIT-6, with Ia3d cubic type structure and a network of interconnected channels. Siliceous material KIT-6, has numerous applications in adsorption and catalysis, thanks to unique 3-D structures (Xiaoying et. al., 2002).

## MATERIAL AND METHOD

### 1. SBA-15 synthesis

**Materials:** Tetraethylortosilicate (TEOS) 98% Merck as silica source, amphiphilic nonionic triblock copolymer Pluronic P123 ( $\text{EO}_{20}\text{PO}_{70}\text{EO}_{20}$ , molecular weight 5800) (Aldrich) as structure directing agent (SDA), hydrochloric acid (solution 37%, Merck) and deionized water were used as received in the synthesis of silica SBA-15. The molar ratio of the components was as follows:  $1\text{SiO}_2$ : 0.017 P123: 5.87 HCl: 194  $\text{H}_2\text{O}$  (Zhao et al., 1998). The synthesis was performed using ultrasounds to shorten the reaction time.

### 2. MCM-41 synthesis

**Materials:** Tetraethylortosilicate (TEOS, 98% Merck) as silica source, cetyltrimethylammonium bromide ( $\text{C}_{16}\text{TMAB}$ ) (Aldrich) as structure-directing agent

(surfactant),  $\text{NH}_4\text{OH}$  20% solution (Merck), methanol (Sigma) and deionized water were used, as received, in the synthesis of silica MCM-41. The molar ratio of the components was as follows:  $1\text{SiO}_2$ :  $0.2\text{C}_{16}\text{TMB}$ :  $5.7\text{NH}_3$ :  $113\text{H}_2\text{O}$  (Corma et. al., 1995).

### 3. KIT-6 synthesis

**Materials:** P123 ( $\text{EO}_{20}\text{PO}_{70}\text{EO}_{20}$ , molecular weight 5800) (Aldrich), n-butanol ( $0.8\text{ g/cm}^3$ , Merck), (TEOS, solution 98% Merck) as silica source, hydrochloric acid (solution 37%, Merck) and deionized water were used as received.

Silica KIT-6 was synthesized using Pluronic P123 and n-butanol, a mixture as structure directing agent (Xiaoying et. al., 2002). The molar ratio of the components was as follows: 0.017 P123: 1.3 TEOS: 1.31 BuOH: 1.83 HCl:  $195\text{H}_2\text{O}$

A Cabernet Sauvignon bottled wine originated from Cozmesti area (Romania) and winified in 2009 was selected as a typical wine for the experiments.

Activated carbon (Fisher Chemicals) was used in adsorption process for comparison purpose.

### 4. Characterization of synthesized materials:

**$\text{N}_2$  sorption.** The textural properties were determined with a NOVA 2200 Quanta Chrome Inc.) sorption apparatus. The sample was degassed at  $300^\circ\text{C}$  for 3 hours before the measurement was taken. The BET surface area was calculated based on the adsorption data in the relative partial pressure range of 0.05-0.25. Pore size distribution was determined based on the Barret-Joyner-Halenda (BJH) adsorption curve.

**5. Total index of phenols** from wine was determined spectrophotometrically using a Spectrophotometer Analytik Jena S 200 at 280 nm (D280 – OIV method).

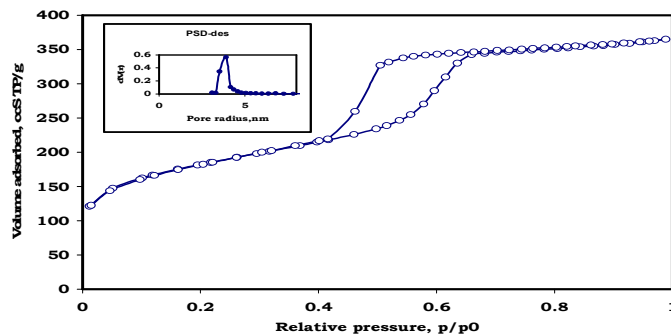
### 6. Adsorption of phenolic compounds on nanoporous materials

Adsorption experiments were conducted at  $5^\circ\text{C}$  for 24 hours adding increased amounts of SBA-15 powder into 50 mL wine. After filtration, the total content of phenols in the liquid phase was determined spectrophotometrically, at 280 nm using the OIV method.

## RESULTS AND DISCUSSIONS

### 1. Physical adsorption, BET

Fig. 1, 2, 3 exhibits the  $\text{N}_2$  adsorption – desorption isotherm at  $-196^\circ\text{C}$  for calcined materials: silica-SBA-15, KIT-6 and MCM-41. Typical isotherm is of Type IV with a hysteresis loop Type H1, a characteristic of mesoporous solids, according to the IUPAC classification. The structural parameters of calcined mesoporous materials are summarized in tab. 1.



**Fig. 1** - The  $\text{N}_2$  adsorption – desorption isotherm at  $-196^\circ\text{C}$  of silica-SBA-15. Inset: the pore size distribution (PSD)

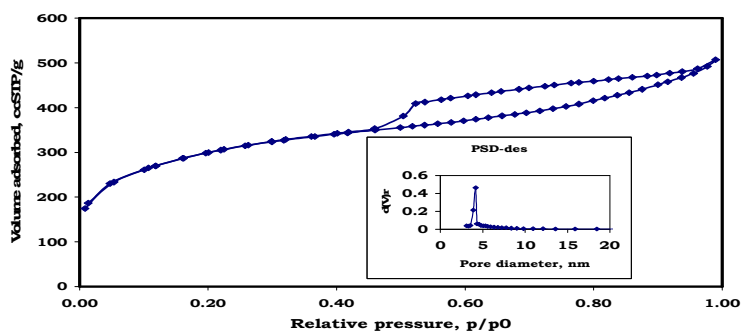


Fig. 2 - The N<sub>2</sub> adsorption – desorption isotherm at -196<sup>0</sup>C of KIT-6. Inset: the pore size distribution (PSD)

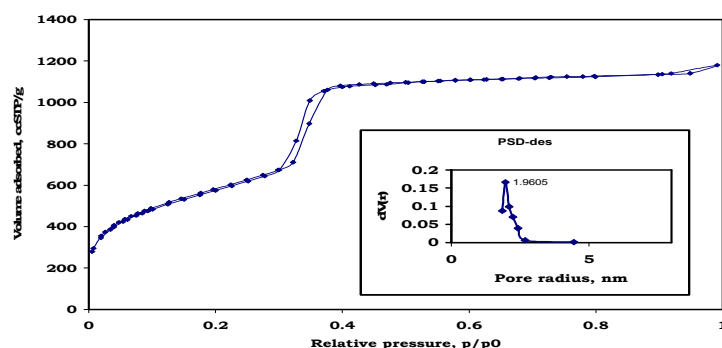


Fig. 3 -- The N<sub>2</sub> adsorption – desorption isotherm at -196<sup>0</sup>C of MCM-41. Inset: the pore size distribution (PSD)

Table 1

Structural parameters of the synthesized nanomaterials derived from nitrogen adsorption

| Adsorbent | S <sub>BET</sub> (m <sup>2</sup> /g) | D <sub>BJH</sub> (nm) | Total pore volume (cm <sup>3</sup> /g) |
|-----------|--------------------------------------|-----------------------|--|
| SBA-15    | 508.6                                | 7.6                   | 0.565                                  |
| KIT-6     | 644,5                                | 4,17                  | 0.761                                  |
| MCM-41    | 2098                                 | 3,12                  | 0,691                                  |

## 2. Adsorption of phenolic compounds on nanoporous materials

The total polyphenols content of the red wine was determined by measuring the absorbance at  $\lambda=280$  nm ( $A_{280}$ ) in quartz cuvettes of 1 cm optical path, compared with deionized water. The calibration curve, using gallic acid solutions of concentration 0; 0,2; 0,4; 0,6; 0,8 mg/L is described by the following equation:

$$y = 0.294x + 0.028 \quad (1)$$

where x is the absorbance value  $A_{280}$  afforded by spectrophotometer and y is the equivalent content of polyphenolic compounds expressed as mg of gallic acid equivalents per L (GAE/L).



The index total polyphenol content for red wine was found as being 1591.13mg/L ( $A_{280} = 54.025$ ). The results of the adsorption experiments are summarized in tab. 2.

Table 2

Variation of total phenols content in red wine with the adsorbent dose

| specification | Amount of adsorbent (g/L) | Absorbance ( $\lambda=280$ nm) | Residual concentration of polyphenols in wine (mg/L) | Polyphenols removed (%) |
|---------------|---------------------------|--------------------------------|--|-------------------------|
| wine          | 0                         | 54.025                         | 1591.13  | 0.00                    |
| SBA-15        | 0.5256                    | 51.050                         | 1503.67  | 5.51                    |
|               | 1.0216                    | 49.785                         | 1466.47  | 7.85                    |
|               | 1.5104                    | 49.570                         | 1460.15  | 8.25                    |
|               | 2.0510                    | 48.950                         | 1441.93  | 9.39                    |
|               | 2.5082                    | 47.645                         | 1403.56  | 11.81                   |
|               | 3.0994                    | 47.050                         | 1386.07  | 12.91                   |
|               | 4.0204                    | 46.520                         | 1370.48  | 13.89                   |
|               | 5.0200                    | 45.875                         | 1351.52  | 15.09                   |
|               | 6.1344                    | 44.690                         | 1316.68  | 17.28                   |
|               | 6.9974                    | 44.370                         | 1307.27  | 17.87                   |
| MCM-41        | 8.0404                    | 43.680                         | 1286.99  | 19.15                   |
|               | 0.518                     | 50.66                          | 1492.23  | 5.27                    |
|               | 1.035                     | 50.65                          | 1491.11  | 5.28                    |
|               | 1.5112                    | 50.24                          | 1479.25  | 6.06                    |
|               | 2.1744                    | 49.12                          | 1446.15  | 8.15                    |
|               | 2.513                     | 48.69                          | 1434.18  | 8.95                    |
|               | 3.0602                    | 48.58                          | 1431.26  | 9.15                    |
| KIT-6         | 4.249                     | 48.53                          | 1429.46  | 9.25                    |
|               | 0.606                     | 33.02                          | 973.21   | 2.6                     |
|               | 1.098                     | 32.93                          | 970.43   | 2,92                    |
|               | 2.138                     | 31.53                          | 929.31   | 7                       |
|               | 3.026                     | 31.05                          | 915.22   | 8.4                     |
| Activ carbon  | 4.1298                    | 30.87                          | 910.23   | 8.9                     |
|               | 0.2808                    | 47.180                         | 1389.89  | 12.67                   |
|               | 0.6594                    | 43.940                         | 1294.63  | 18.67                   |
|               | 1.0130                    | 40.110                         | 1182.03  | 25.76                   |

## CONCLUSIONS

1. Mesoporous materials synthesized and used during the experiments (SBA-15, MCM-41, KIT-6) have been characterized and are in agreement with literature reports.

2. For the synthesis of SBA-15 and KIT-6 materials, has been used an innovative method for synthesis, using ultrasounds.

3. SBA-15, MCM-41, KIT-6 were used for the first time in wine treatment.

4. Taking into account the maximum percentage of phenolic compounds retained on the three studied materials: SBA-15, MCM-41, KIT-6, the results demonstrate that all three retain phenolic compounds from wine, with maximum

efficiency for SBA-15 material with 19.15% retained phenolic compounds, for 8.0404 g adsorbent / L wine.

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# ANALYSIS OF THE PHENOLIC COMPOUNDS THROUGH HPLC IN SOME RED WINES IN IAȘI VINEYARD OBTAINED THROUGH DIFFERENT TECHNOLOGICAL METHODS

## ANALIZA UNOR COMPUȘI FENOLICI PRIN METODE HPLC LA UNELE VINURI ROȘII DIN PODGORIA IAȘI, OBȚINUTE PRIN DIVERSE METODE TEHNOLOGICE

MORARU I.<sup>1</sup>, NICULAUA M.<sup>2</sup>,  
COTEA V.V.<sup>1</sup>, MĂLUȚAN G.<sup>1</sup>, CODREANU Maria<sup>1</sup>  
e-mail: moraruioanlulu@gmail.com

**Abstract.** *In the production year 2010-2011, wines were obtained from four black grape varieties through different wine-making technologies. Basic physical-chemical characteristics were evaluated as well as a series of phenolic compounds: resveratrol, shikimic acid, hydroxycinnamic acids, hydroxybenzoic acids, hydrolysable and non-hydrolysable tannins. Comparing the wines obtained from the 4 technologies, it was registered that the sample from the microwave technology had a higher quantity of phenolic compounds, compared to the other variants.*

**Key words:** resveratrol, shikimic acid, tannins, thermo maceration, microwaves

**Rezumat.** *În anul de producție 2010-2011 s-au obținut dintr-un număr de 4 soiuri de struguri negri din podgoria Iași o serie de vinuri prin diferite tehnologii de vinificație. La aceste probe, pe lângă caracteristicile fizico-chimice de bază s-au evaluat o serie de compuși fenolici, precum: resveratrolul, acidul shikimic, acizi hidroxicinamici, acizi hidroxibenzoici, taninuri hidrolizabile și nehidrolizabile. În urma comparării celor 4 tehnologii s-a observat că variantele obținute prin tehnologia cu microunde și cea cu termomacerare, duc la o creștere a cantității acestor compuși în vinurile analizate.*

**Cuvinte cheie:** resveratrol, acid shikimic, taninuri, termomacerare, microunde

### INTRODUCTION

The amount of tannins present in wines depends on the duration of its contact with the grapes, skins and seeds, as well as the processing way of the grapes. Depending on the amount that is present in wines tannins contribute positively but sometimes negatively to olfactive and gustative attributes of the product (Cotea et al., 2009).

Tannins are phenolic compounds that are found in all of the vine's organs and are responsible for the wine's astringency. They are soluble in water, form coloured compounds and have the capacity of precipitating proteins and inhibiting

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

<sup>2</sup> Oenology Research Center – Iasi Branch of the Romanian Academy, Romania

enzymes' activity, contributing thus, alongside alcohols, to wine conservation (Pomohaci, 2005). Also as antioxidant and antibacterial agent this action has thereby for wines a good preserving property (Țârdea, 2007).

Depending on the version of maceration-fermentation technology used to obtain red wines, the tannins in wine are evaluated by analysing and comparing the results recommending a more efficient version (Moraru, 2011).

Phenolic acids have an important role to interact with anthocyanins in acylation reactions and therefore contributing at the wine aging bouquet formation.

Micro-phenolics compounds (resveratrol and shikimic acid) are present in very small quantities in wines. These compounds do not affect the quality of wines but have beneficial effects on human health (Țârdea, 2007).

## MATERIAL AND METHOD

Four varieties of grape wines were studied, two local (Băbescă neagră – noted Bn and Fetească neagră – noted Fn) and two cosmopolitan varieties (Cabernet Sauvignon - noted CS and Merlot - noted M). The grapes were harvested at technological maturity from Copou and Bucium area. Grapes were divided into four equal parts and were subjected to several types of maceration technology to assess the chemical composition modifications, especially phenolic compounds. Different technological variants of maceration were performed: classical (code - m), microwave maceration (code - mm), thermo- maceration (code - tm) and rotating tanks (code - rm).

At the thermo-maceration option the working temperature was 70 °C for about 30 minutes and in the microwave tests the samples were subjected to 750 W for 15 minutes. Rotating tanks and classical versions had a maceration period of 5 days before end-test of maceration (skin colour invariants during maceration-fermentation).

After its alcoholic fermentation, the wine was racked at room temperature. After 7-8 days the wine was filtered and bottled with the help of an Enomatic Tenco device. Immediately after adding a dose of sulphur dioxide by 40 mg/L per bottle, they were corked with a Mini TS.

For shikimic acid analysis we used the method OIV MA-E-AS313-17-ACSHIK for determining organic acids in wine. Samples were processed on a Shimadzu HPLC system composed of: auto-injector SIL-20AC Shimadzu Prominence series (injection volume: 10 mL, sample temperature 20 °C), quaternary pump LC-20AD Shimadzu Prominence series with five-channel degasser Shimadzu Prominence series DGU-20A5, column oven CTO-20AC Shimadzu Prominence series, diode array detector SPD-M20A Shimadzu Prominence series (scanning range: 200-440 nm), chromatographic system controller CBM-20A Shimadzu Prominence series and the whole system is connected to a desktop computer unit via LAN (local area network). The separation was made through two C18 columns Grace Organic acids (2×POA) 250×4.6 mm and 5 μm stationary phase. The eluent for the separation is a 0.065 M solution of sulphuric acid (H<sub>2</sub>SO<sub>4</sub>) with a flow rate of 0.8 mL/min at a temperature of 20°C.

For analysis of phenolic acids and other phenolic compounds in wine we have been using monolithic separation columns (Castellari et al., 2002) using the same chromatographic separation system described in shikimic acid determination.

## RESULTS AND DISCUSSIONS

Gallic acid (fig. 1) shows high values in all technology variants of wines made from Cabernet Sauvignon, compared with all other wines considered in this study. Maximum value is recorded in the microwave maceration version obtained (83.93 mg/L) and the next most important value of 79.62 mg/L is for the roto-tanks version. The lowest concentration of gallic acid is registered in Băbească neagră variety wine of 1.39 mg/L obtained by thermo-maceration. Values obtained from Merlot wines are between 24.05 mg/L at classical maceration version and 24.48 mg/L for the thermo version. At wines from Fetească neagră variety, values of 11.97 mg/L were obtained for traditional maceration version and 31.91 mg/L for thermo-maceration version.

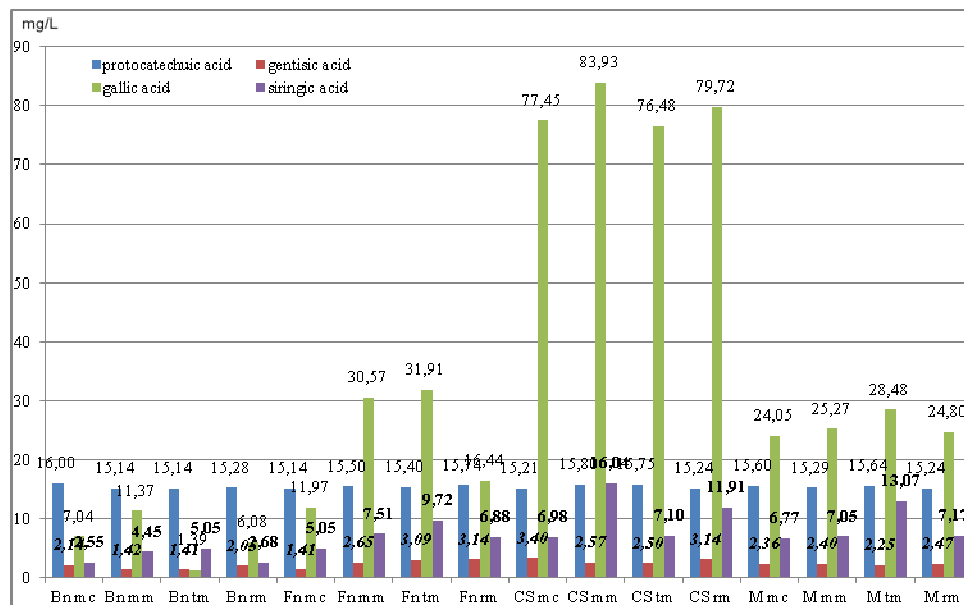


Fig. 1 - Values distribution for *p*-hydroxybenzoic acids at wines in 2010

Protocatechuic acid is invariable in all wines studied regardless of variety or technology. The lowest value (15.14 mg/L) is recorded in Băbească neagră wine (microwave or thermo-maceration variants) and at Fetească neagră (classical maceration) and the highest value (16 mg/L) is from Băbească neagră obtained by classic maceration.

Syringic acid values are between 16.04 mg/L to Cabernet Sauvignon (microwave maceration) and 2.55 mg/L to Băbească neagră version with classical maceration. The Băbească neagră variety wines have low values compared with wines obtained from other varieties studied. Whichever technology used, variants with high values for this acid is in all varieties the thermal variants exploited (microwave and thermo-maceration).

Gentic acid has values that vary between 3.40 mg/L at Cabernet Sauvignon (classic maceration) and 1.41 mg/L in Băbească neagră (thermo-maceration). Also in this case, whichever technology used, it is noted that gentic acid values are similar in all varieties studied.

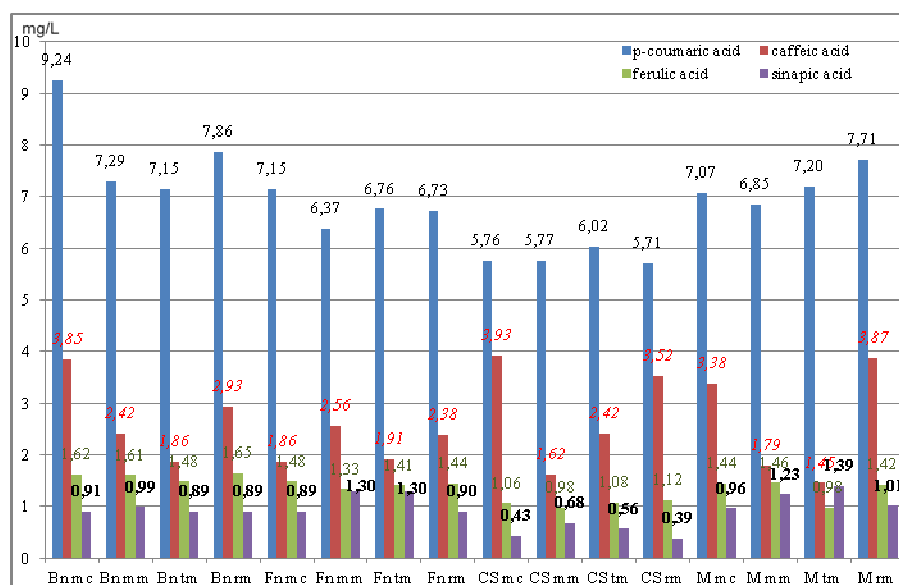


Fig. 2 - Values distribution for cinnamic acids at wines in 2010

The highest value of *p*-coumaric acid (fig. 2) was found in Băbească neagră wine obtained by traditional technology (9.24 mg/L) and the lowest value of 5.71 mg/L at Cabernet Sauvignon (roto-tanks maceration). For all varieties and whatever technological variant used the *p*-coumaric acid values are much higher than the values analyzed for other hydroxycinnamic acids.

The maximum recorded values for caffeic acid (3.93 mg/L) is at Cabernet sauvignon classical maceration version. Lowest value of 1.45 mg/L is found in Merlot obtained by thermo-maceration. Note that the variants have high values obtained by traditional maceration or roto-tanks in detriment to the thermal variations.

Ferulic acid values ranging from 1.65 mg/L obtained by roto-tanks from Băbească neagră version and 0.98 mg/L in Merlot variant obtained by thermo-maceration. In each variety, regardless of technological variant, we see some homogeneity of these values.

Sinapinic acid recorded the highest value of 1.39 mg/L in Merlot thermo-maceration version and the lowest value (0.39 mg/L) at Cabernet sauvignon produced by roto-tanks version. Thermal technology variants recorded higher values in all varieties studied for this kind of substance.

Catechin (fig. 3) has values ranges between 168.42 mg/L at microwave maceration of Băbească neagră variety and 18.6 mg/L in Fetească neagră

(classical maceration). Depending on the variety, the variants obtained by thermo or microwave technology have higher values for this compound.

The highest value for epicatechin (12.32 mg/L) was achieved in the roto-tanks obtained Cabernet sauvignon. Lowest value for maceration of 3.75 mg/L to Fetească neagră microwave version. From the results we can say that only the variety and less the used technological variant would affect the amount of this compound.

Shikimic acid has values between 75.85 mg/L to Fetească neagră (thermo version) and 18.45 mg/L to Băbească neagră (maceration classic). Shikimic acid variation values are very much influenced by variety and less by technology.

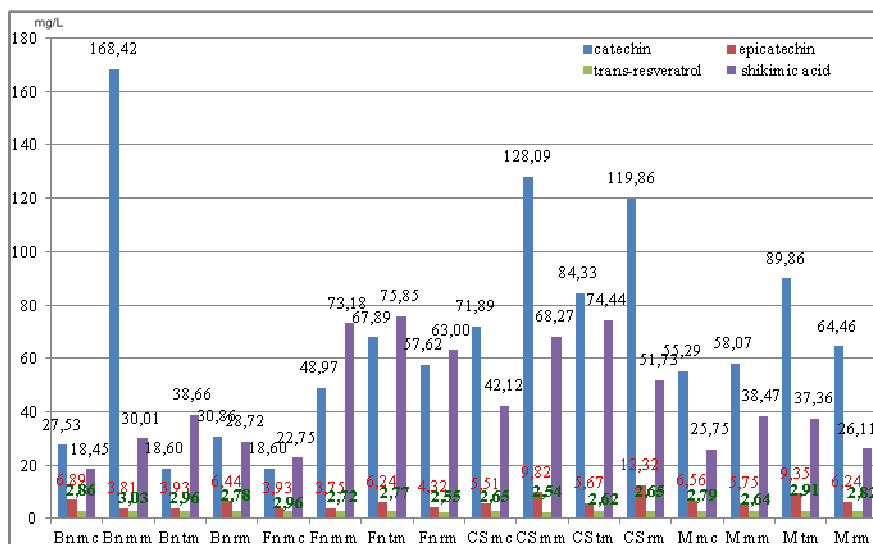


Fig. 3 - Values distribution for catechin, epicatechin, *trans*-resveratrol and shikimic acid at wines in 2010

Trans-resveratrol has very small variation of values, regardless of variety or applied technology. Lowest value of 2.54 mg/L was recorded for Cabernet Sauvignon (microwave version) and the highest value of 3.03 mg/L in Băbească neagră at the same maceration techniques.

## CONCLUSIONS

1. There is a technological facilitation for the accumulation of these compounds in wine by various harder extraction methods (thermo-maceration, microwave maceration and roto-tanks).

2. Cosmopolit varieties are richer in phenolic compounds than local varieties but by using modern methods of maceration-fermentation we can enrich the quality and quantity of wines produced and possibly boosting bioactive character of weaker varieties.

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# CHARACTERIZATION OF ODORANT AREAS IN THREE WINES FROM LOCAL GRAPE VARIETIES FROM REPUBLIC OF MOLDOVA USING GAS CHROMATOGRAPHY – OLFACTOMETRY

## CARACTERIZAREA ZONELOR ODORANTE A TREI VINURI DIN SOIURI AUTOHTONE DIN REPUBLICA MOLDOVA UTILIZÂND GAZ CROMATOGRAFIA – OLFACTOMETRIA

MUSTEAȚĂ G<sup>1</sup>., FURTUNA Natalia<sup>1</sup>, LE FUR Y.<sup>2</sup>  
e-mail: furtuna.utm@gmail.com

**Abstract.** *Three wines from local grape varieties from Republic of Moldova were submitted to both sensory and gas chromatography – olfactometry analyses (GC-O). Through descriptive analysis, a set of aroma attributes has been described, but the volatile compounds responsible for the characteristic sensory notes have not been investigated. In order to identify these odor active compounds, the wines were evaluated using qualitative detection frequency analysis (n=7). The panelists generated in total 697 descriptions distributed in 126 odorant areas (OAs), but only 565 (81 %) distributed in 45 OAs were validated as being representative.*

**Key words:** gas chromatography – olfactometry, detection frequency analysis, odorant area.

**Rezumat.** *Trei vinuri din soiuri autohtone din Republica Moldova au fost supuse analizei senzoriale și gaz cromatografiei – olfactometriei (GC-O). Analiza descriptivă a generat un șir de caracteristici aromatice, însă compușii volatili responsabili de notele aromatice caracteristice nu fuseseră investigați. Pentru identificarea acestor compuși, vinurile au fost evaluate prin metoda frecvențelor de detecție (n=7). Evaluatorii au generat în total 697 descriptori distribuiți în 126 zone odorante, însă numai 565 (81 %) distribuiți în 45 zone odorante au fost validați ca fiind reprezentativi.*

**Cuvinte cheie:** gaz cromatografie – olfactometrie, metoda frecvențelor de detecție, zonă odorantă.

### INTRODUCTION

The gas chromatography-olfactometry (GC-O) is an analytical method that combines the gas chromatography and sensory perception, using the human nose as a detector to assess odor components. The human nose has an odor detection limit of about  $10^{-19}$  moles, making GC-O an extremely valuable and sensitive tool for odor detection (Grosch W., 2000).

After injection, the content of the sample (the extract) is separated by the chromatographic column. Before leaving the column, the effluent is divided into

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<sup>1</sup> Technical University of Moldova, Republic of Moldova

<sup>2</sup> Université de Bourgogne, France

two parts: the smallest is directed to the instrumental detector, usually a flame ionization detector (FID); the largest part is directed to a smelling device (sniffing port) placed at the evaluator's nose height. Therefore, this method provides simultaneously two signals: the chromatogram of the extract and the recording of odor events perceived by assessors (Le Guen et al., 2000).

In order to perform this analysis, three methods can be used: successive dilution of an aroma extract, direct estimation of the intensity and detection frequency of odorant areas.

The odorant areas frequency is correlated to the concentration logarithm of the compound responsible for stimulus. This relationship is based on the hypothesis that, for a certain compound, the perception threshold has a Gaussian distribution. Each assessor must perceive the beginning and the end of the flavor and describe it. The individual aromagrams are summed, yielding the global aromagram where frequency of detection is represented in dependence on time or retention index. In both cases, the olfactometric indices can be used for ranking odorant areas in function of their olfactory impact (Pollien et al., 1997).

The odorant areas obtained via GC-O are characterized by three parameters: olfactometric index, average linear retention index (LRI) or LRI interval and flavor descriptors. All this information is used later in the identification of compounds.

Some studies reinforce that ILR and descriptors are not enough for individualization of odorant areas (Le Fur et al., 2003). Authors relied on the morphology of chromatograms by peak numbering and on the LRI interval. In this study were defined five types of odorant areas:

- 1 – associated with an isolated peak, well separated from other peaks;
- 2 – associated with a part of the chromatogram without peak;
- 3 – extended, associated with a wide peak;
- 4 – located within a very wide peak;
- 5 – associated with two relatively separated consecutive peaks and which descriptors are used to distinguish two successive zones.

## **MATERIAL AND METHOD**

For analysis were used wines made from local grape varieties: Startovyi, Hibernat and Muscat of Ialoveni (harvest 2010) produced at the Practical Scientific Institute of Horticulture and Food Technology from Chişinău.

In order to extract aromatic compounds was used the dichloromethane extraction, based on the method proposed by Moio (Moio et al., 1995).

The olfactometric analysis was performed on 3 extracts by 7 assessors selected in advance and informed that they will analyze three white wines, but no other detail has been specified. The extracts were analyzed by the participants in a different and balanced sequence. Total length of a session was 45 minutes. After injection of the solution into chromatograph column, in order to avoid inhalation of the solvent, the assessor was announced to wait 5 minutes before approaching the nose to the sniffing port (fig. 1).



**Fig. 1** – Sniffing-port (with the glass nose mold), button and microphone for recording

Gas chromatograph Hewlett-Packard 5890 was equipped with split/splitless injector and DB-1701 capillary column. Simultaneous processing of both signals was performed using EZchrom Elite (Agilent Technologies) and AcquiSniff® (© INRA) software. The FID signal was recorded on the computer through channel A and the olfactometric signal through channel B.

Linear retention indices (LRI) of chromatographic peaks and odorant events were calculated using a daily injection of a solution of 13 n-alkanes (from C<sub>7</sub> to C<sub>19</sub>), analyzed under the same chromatographic conditions as the extracts.

The results of each individual data processing were presented in Excel tables where the LRI peak, the assessor codes, the extract codes and their respective descriptors were indicated. Therefore, 21 tables with olfactometric data were obtained (3 wines x 7 assessors), that subsequently were submitted to mathematical processing. Mathematical processing of olfactometric data was performed using Matlab® (The Mathwork Inc.), which implements an iterative mathematical function to get a table that contains the number of detections for each tandem wine/odorant area.

## RESULTS AND DISCUSSIONS

Initially the wines were submitted to sensory analysis sessions (tab. 1). Though considerable dispersion of responses, it was achieved conclusive data. The intensity of wine aroma was appreciated with values within a range from 62.5 to 75 points out of 100.

*Table 1*

**Descriptors set out by tasters during the sensory evaluation**

| The wine           | Types of aromas               |                    |                 |                        |
|--------------------|-------------------------------|--------------------|-----------------|------------------------|
|                    | Floral                        | Fruity             | Vegetal         | Spicy                  |
| Startovyi          | Honey                         | Pear, apple, lemon | Freshly cut hay | Pepper, coconut        |
| Hibernal           | Basil, thyme                  | Pomelo, grapefruit | Herbaceous      | Laurel leaves, paprika |
| Muscat of Ialoveni | Muscat intense, acacia flower | Citrus, pineapple  | Celery          | Nutmeg                 |

The olfactometric study, performed by using frequency detection method, generated 21 individual aromagrams. Global data of olfactometric analysis are cumulated in table 2. The total number of odorant events related to each wine is situated between 228 (Muscat of Ialoveni) and 238 (Hibernal), meaning that for three wines, seven assessors had spotted 697 events. The assessors, with some exceptions, have described each event with only one term, the report terms / events being nearly 1.1.

Table 2

Global data of olfactometric analysis

| The wine           | Total odorant events | Total descriptors | Events without description | % Events without description |
|--------------------|----------------------|-------------------|----------------------------|------------------------------|
| Startovyi          | 231                  | 259               | 22                         | 8,5%                         |
| Hibernal           | 238                  | 272               | 26                         | 9,5%                         |
| Muscat of Ialoveni | 228                  | 250               | 31                         | 12,4 %                       |
| Total 3 wines      | 697                  | 781               | 79                         | 10,1 %                       |

In order to process data obtained by using Matlab® software, it was previously set an eliminatory threshold. This corresponds to the value of first quartile of distribution, i.e., to consider an odorant area as representative it must contain at least 5 odor events. Of the totality of 697 odor events, 565 (81%) were distributed within 45 odorant areas that contain at least 5 events per area. Consequently, the areas with the number of events lower than the eliminatory threshold have been removed (fig. 2).

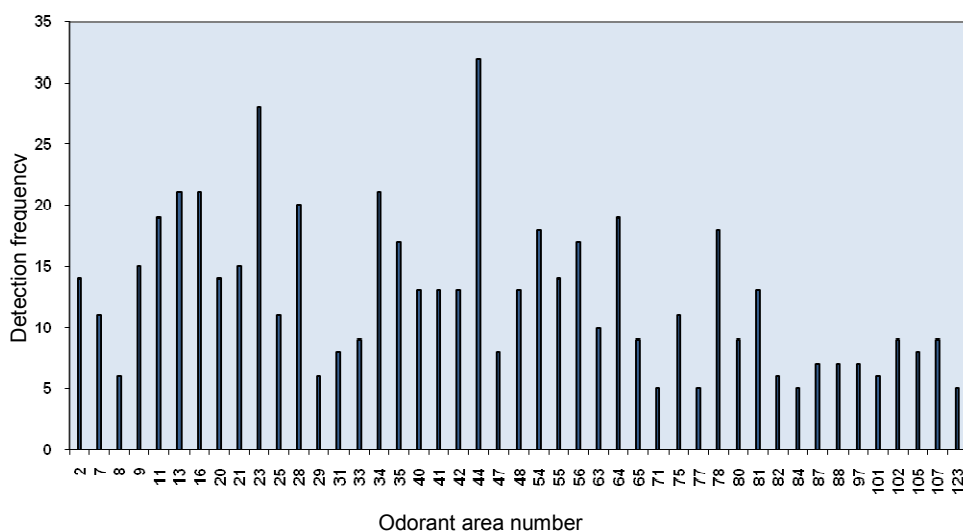


Fig. 2 – Global aromagram of studied wines

The results obtained by GC-O analysis were summarized in table 3. It is provided further identification of odorant compounds responsible for odor events from the described areas.

Table 3

Characteristic of representative odorant areas for studied wines

| Area number* | LRI ** | Detection frequency | Odorant area description                           |
|--------------|--------|---------------------|--|
| 2            | 695    | 14                  | Yoghurt, cream, butter                             |
| 7            | 766    | 11                  | Fruity, solvent                                    |
| 8            | 770    | 6                   | Vinegar, pungent                                   |
| 9            | 778    | 15                  | Fruity, brandy                                     |
| 11           | 816    | 19                  | Fruity, strawberries, pineapple                    |
| 13           | 845    | 21                  | Cocoa, chocolate, yeasty                           |
| 16           | 862    | 21                  | Tutti frutti, strawberries, raspberries            |
| 20           | 906    | 14                  | Fruity, kiwi, pineapple                            |
| 21           | 912    | 15                  | Fruit candy, linden, verbena                       |
| 23           | 938    | 28                  | Peanuts, roasted, banana, pear                     |
| 25           | 957    | 11                  | Cheese   |
| 28           | 1009   | 20                  | Cheese, rancid                                     |
| 29           | 1014   | 6                   | Apple, cheese                                      |
| 31           | 1027   | 8                   | Dried herbs  |
| 33           | 1053   | 9                   | Cooked potatoes, gnocchi                           |
| 34           | 1060   | 21                  | Fruit candy, apple, citrus                         |
| 35           | 1074   | 17                  | Black currant buds                                 |
| 40           | 1149   | 13                  | Flowers  |
| 41           | 1154   | 13                  | Sulfurous, plastic                                 |
| 42           | 1174   | 13                  | Fruity, balsamic                                   |
| 44           | 1194   | 32                  | Lily of the valley, lavender, citrus, marshmallows |
| 47           | 1235   | 8                   | Caramel, chocolate                                 |
| 48           | 1240   | 13                  | Cotton candy, caramel                              |
| 54           | 1284   | 18                  | Honey, rose, lilac                                 |
| 55           | 1292   | 14                  | Flowers  |
| 56           | 1305   | 17                  | Caramel, cotton candy                              |
| 63           | 1350   | 10                  | Cheese, smoky, dusty                               |
| 64           | 1357   | 19                  | Spicy, curry, fennel                               |
| 65           | 1371   | 9                   | Bergamot, citrus                                   |
| 71           | 1432   | 5                   | Licorice   |
| 75           | 1473   | 11                  | Floral, herbaceous                                 |
| 77           | 1489   | 5                   | Chemical, pharmaceutical                           |
| 78           | 1494   | 18                  | Balsamic, clove, curry                             |
| 80           | 1508   | 9                   | Polyfloral honey                                   |
| 81           | 1512   | 13                  | Prune, floral, smoky                               |
| 82           | 1518   | 6                   | Clove  |
| 84           | 1529   | 5                   | Spicy  |
| 87           | 1545   | 7                   | Mineral  |
| 88           | 1550   | 7                   | Floral, herbaceous                                 |

|     |      |   |                       |
|-----|------|---|-----------------------|
| 97  | 1619 | 7 | Fruity, vegetal       |
| 101 | 1644 | 6 | Sulfurous, fermented  |
| 102 | 1662 | 9 | Vanilla               |
| 105 | 1728 | 8 | Mulled wine, balsamic |
| 107 | 1748 | 9 | Coconut               |
| 123 | 1909 | 5 | Fruity, berries       |

\* Odorant areas that contain at least 5 events per area;

\*\* average LRI in DB-1701 capillary column (30 m x 0,32 mm x 1 µm).

## CONCLUSIONS

1. Olfactometry analysis (GC-O) allows the selection of odorant compounds using human analyzer, sequentially combining gas chromatography (instrumental analysis) and sensory perception (subjective analysis), thus being a very precious technique for detection of compounds with higher detection threshold than their concentration in wine, and thereby solving some problems in the aroma analysis.

2. The central method of this research was the olfactometry analysis by using the detection frequency method to generate 21 individual aromagrams, which were later summed into a global aromagram for all three wines.

3 According to mathematical processing of experimental data using Matlab® software, it was established that out of 697 odor events spread in 123 odorant areas, 565 (81%) were distributed within 45 odorant areas that contain at least 5 events per area.

4. By analyzing the global aromagram, it can be concluded that the odorant areas have well separated peaks (odor events), except the odor events of compounds with a perception threshold inferior to the sensorial capacity of assessors, as well as differences between their ability to recognize a flavor.

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# THE STUDY OF THE ANTIRADICALIC ACTIVITY OF SOME POLYPHENOL EXTRACTS OBTAINED FROM *VITIS VINIFERA*

## STUDIUL ACTIVITĂȚII ANTIRADICALICE A UNOR EXTRACTE POLIFENOLICE OBȚINUTE DIN *VITIS VINIFERA*

**NECHITA Ancuța<sup>1</sup>, COTEA V. V.<sup>1</sup>, NECHITA B.<sup>1</sup>**  
e-mail: ancuta.vasile@yahoo.com

**Abstract.** Many polyphenol compounds from medicinal plants and food have an antiradicalic role, mainly in what the digestive tract is concerned because of the enzyme inhibition that catalyses the formation of species of reactive oxygen and/or by capturing them, having a benefic role in preventing and improving aging degenerative affections; that is poor immunity, brain dysfunctions of the nervous system etc. In this context it was studied the antiradicalic activity of three polyphenol extracts that were obtained from the following varieties Negru de Drăgășani, Arcaș and Chambourcine. The antiradicalic properties were evaluated through the inhibition capacity of the organic radical 1,1-diphenyl-2-picryl-hydrazyl (DPPH). Therefore it was determined that the antiradicalic activity of the studied extracts is in general high going to very high, this kind of activity was noticed at Negru de Drăgășani variety.

**Key words:** seed, grape, polyphenols, antiradical activity

**Rezumat.** Mulți compuși polifenolici din plantele medicinale și alimente au un rol antiradicalic în principal în tractul digestiv prin inhibarea enzimelor ce catalizează formarea speciilor oxigen reactive și/sau captarea lor, cu rol benefic în prevenirea și ameliorarea afecțiunilor degenerative ale îmbătrânirii, respectiv imunitate deficitară, disfuncții ale creierului și ale sistemului nervos etc. În acest context a fost studiată activitatea antiradicalică a trei extracte polifenolice obținute din semințele soiurilor Negru de Drăgășani, Arcaș și Chambourcine. Proprietățile antiradicalice au fost evaluate prin capacitatea de inhibare a radicalului organic 1,1-difenil-2-picril-hidrazil (DPPH). Astfel, s-a constatat că activitatea antiradicalică a extractelor studiate este în general mare, spre foarte mare, remarcându-se soiul Negru de Drăgășani.

**Cuvinte cheie:** semințe, struguri, polifenoli, activitate antiradicalică

### INTRODUCTION

Many studies concerning the antioxidant and the caption activity of the free-radicals by the polyphenol compounds from plants have highlighted the key role of the free-radicals in many fundamental cellular activities and have suggested that the oxidative stress may be important for the pathology of the common diseases (Fukumoto et al., 2000; Shih et al., 2007; Elisia et al., 2008).

Many polyphenol compounds from medicinal plants and food have an antioxidant role, mainly in the digestive tract through the inhibition of the

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

enzymes that catalyze the formation of reactive species of oxygen and/or their caption with a benefic role in the prevention and the improvement of aging degenerative affections; that is poor immunity, brain dysfunctions and dysfunctions of the nervous system etc. (Ames et al., 1993; Okezie and Aruoma, 1998). Recent research has shown that the active oxygen radicals participate at the carcinogenesis. There was also signaled the contribution of the oxygenated radicals concerning the initiation and the development of the tumors but also the inhibant effect of the antioxidants that have the properties to capture the free-radicals (Matito et al., 2003). In this context, the researches from this paper aim at highlighting the antiradicalic activity of some polyphenol extracts obtained from *Vitis vinifera*.

## MATERIAL AND METHOD

The polyphenol extracts that were studied were obtained from grape seeds from Arcaş, Negru de Negreşti and Chambourcine. The extraction procedure was done in a continuous system in the Soxhlet device, being used ethanol as a solvent in a proportion of 1/10 (vegetal material (g)/ solvent (mL)). For the preliminary characterization of the polyphenol extracts were determined the total polyphenols using the Folin-Ciocalteu method and the index of tanoid matters (ITM) through the method that was established by Bourzex. Also, by analyzing the HPLC (chromatography of high performance liquids) were indentified and quantized a series of stilbens (trans-resveratrol), non-hydrolyzable tannins (catechin and epicatechin) as well as some flavones (rutin and quercetin).

It was evaluated the capacity of antiradicalic properties of polyphenol extracts to inhibit the organic radical 1,1-diphenil-2picryl-hydrazyl (DPPH) (Brand – Williams et al., 1995). Therefore, on the basis of the concentrations of DPPH radical that remained unknown in the methanolic solution, the dilution was identified as being efficient, ED 50, meaning that dilution of the extract that reduces with 50% the quantity of DPPH radical from the initial solution (D0 dilution).

## RESULTS AND DISCUSSIONS

Concerning the evaluation of the antiradicalic properties, the polyphenol extracts that were studies were first submitted to a process of characterization, the results being written in table 1

Table 1

The characterization of the polyphenol extracts obtained from grape seeds

| Parametrii analizați                     | Arcaş extract | Negru de Drăgășani extract | Chambourcine Extract |
|--|---------------|----------------------------|----------------------|
| Analyzed parameters                      | 2.53          | 1.90                       | 2.75                 |
| Total polyphenols, g echiv. galic acid/L | 38.4          | 31.6                       | 41.6                 |
| Index of tanoid matter                   | 2.427         | 2.435                      | 2.433                |
| Trans-resveratrol, mg/L                  | 3.949         | 9.319                      | 2.162                |
| Catechin, mg/L                           | 3.691         | 3.584                      | 3.890                |
| Epicatechin, mg/L                        | 1.074         | 0.749                      | 1.125                |
| Rutin, mg/L                              | 1.752         | 1.752                      | 1.529                |

From the values that were obtained it is noticed a reduced variability of the chemical constants depending on the taxonomy of the vegetal matter (seeds) that was submitted to the extraction procedure. However it is noticed the polyphenolic



extract that was obtained from Negru de Drăgășani, which has even higher values for catechin (9,319 mg/L), in comparison with the other two polyphenol extracts that are studied.

The analysis of the antiradical activity of the extracts was based on developing more dilutions, from D0 to D4, to which was measured their capacity to reduce to half the quantity of added DPPH• radicals. It is known the fact that if the effective dilution – DE50 is higher, then the antiradical activity is superior. For each determination it was eliminated from the calculus the oxidation of the diphenyl-picryl-hydrazyl radical from the methanolic solution under the influence of factors like environment, air, light and temperature. Therefore it was used a “blind” sample, that contains only methanolic solution in which case it was traced its antiradicalic power until it was obtained a plateau for each of the 5 dilutions.

In the case of the seeds extract from Negru de Drăgășani were used five dilutions, the results that were obtained previously to the reading of the spectrophometer are presented in table 2.

Table 2

**Antiradicalic activity of the seeds extract from Negru de Drăgășani**

| Quantity of analyzed extract | Equivalent dilution | Read absorbance | DPPH concentration | % DPPH remained |
|------------------------------|---------------------|-----------------|--------------------|-----------------|
| D0 0 μL extract              | control sample      | 0.955           | 0.078432           | 0               |
| D1 7,5 μL extract            | 1/533               | 0.057           | 0.004880           | 93.7780655      |
| D2 5 μL extract              | 1/800               | 0.422           | 0.034776           | 55.6611458      |
| D3 2,5 μL extract            | 1/1600              | 0.654           | 0.053778           | 31.433405       |
| D4 1 μL extract              | 1/4000              | 0.774           | 0.063607           | 18.901815       |

From the figure 1 it may be observed that for a concentration of 50% DPPH that remained are needed 4,5 micro liters of extract that correspond with a dilution of 1/888. This dilution of 1/888 is equal or higher than the specific values for a red wine.

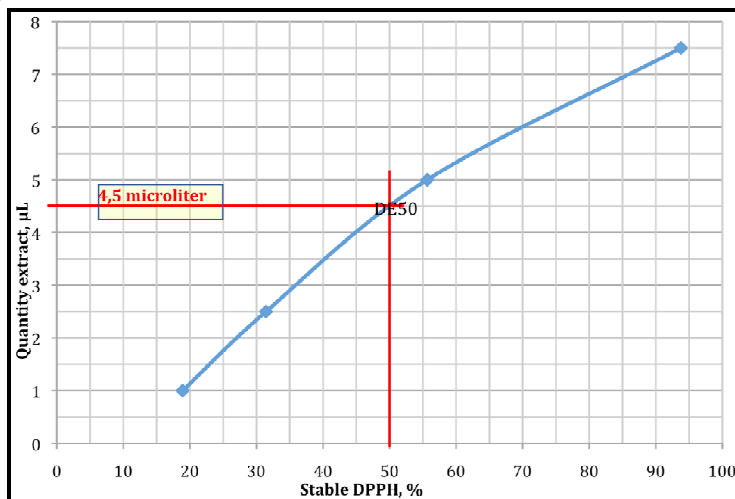


Fig. 1 – Efficient dilution (ED50) for the seeds extract obtained from Negru de Drăgășani

In the case of the seeds extract from Arcaș it is noticed that for a

concentration of 50% DPPH that remained are necessary 7,3 microliters of extract, which correspond with a dilution of 1/689 (table 3, figure 2). This dilution of 1/689 is equal or even higher than the specific values for a red wine.

Table 3

**Antiradical activity of the seeds extract from Arcaş**

|    | Quantity of analyzed extract | Equivalent dilution | Read absorbance | DPPH concentration | % DPPH remained |
|----|------------------------------|---------------------|-----------------|--------------------|-----------------|
| D0 | 0 µL extract                 | control sample      | 1               | 0.082118           | 0               |
| D1 | 12,5 µL extract              | 1/320               | 0.045           | 0.003897           | 95.25424        |
| D2 | 7,5 µL extract               | 1/533               | 0.484           | 0.039854           | 51.46721        |
| D3 | 5 µL extract                 | 1/800               | 0.699           | 0.057464           | 30.02254        |
| D4 | 2,5 µL extract               | 1/1600              | 0.88            | 0.072289           | 11.96912        |

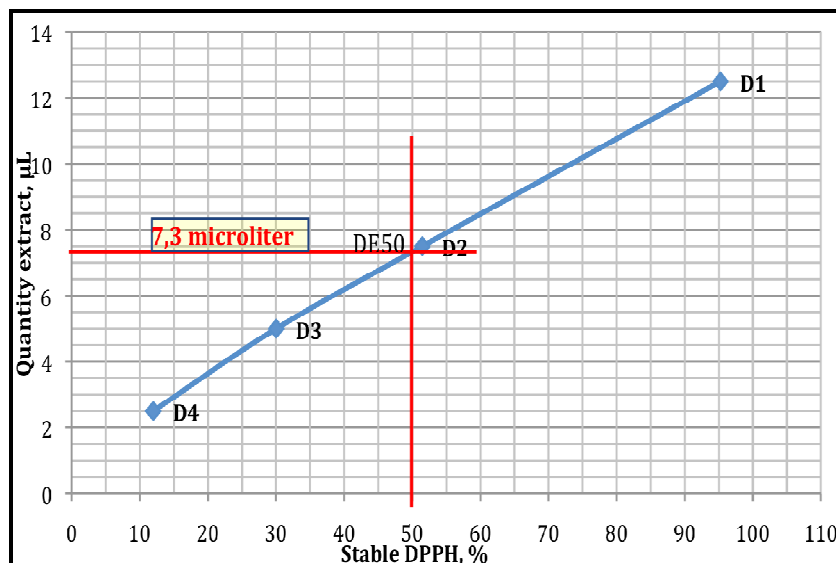


Fig. 2 – Efficient dilution (ED50) for the seeds extract obtained from Arcaş

Also, in the case of the seed extract from Chambourcine it is noticed that for a concentration of 50% DPPH that remained are necessary 5,6 microliters of extract, which correspond with a dilution of 1/714 (table 4, figure 3). And in this case, the dilution of 1/714 is equal to or even higher than the specific values for a red wine.

Table 4

**Antiradical activity of the seeds extract from Chambourcine**

|    | Quantity of analyzed extract | Equivalent dilution | Read absorbance | DPPH concentration | % DPPH remained |
|----|------------------------------|---------------------|-----------------|--------------------|-----------------|
| D0 | 0 µL extract                 | control sample      | 0.958           | 0.078678           | 0               |
| D1 | 7,5 µL extract               | 1/533               | 0.26            | 0.021507           | 72.66443        |
| D2 | 5 µL extract                 | 1/800               | 0.549           | 0.045178           | 42.57844        |
| D3 | 2,5 µL extract               | 1/1600              | 0.8             | 0.065737           | 16.4484         |
| D4 | 1 µL extract                 | 1/4000              | 0.873           | 0.071716           | 8.848821        |

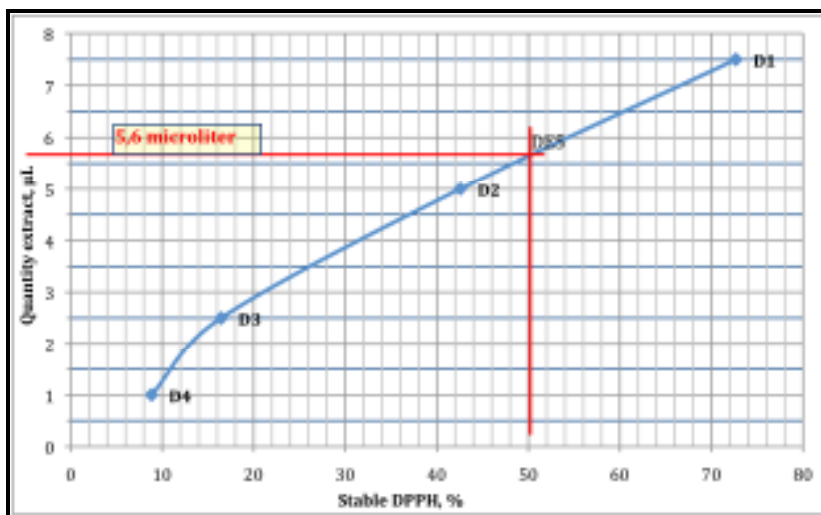


Fig. 3 – Efficient dilution (ED50) for the seeds extract obtained from Chambourcine

The antiradicalic power of the extracts that were studied is generally higher. Although it is important to know that the blockage of the free radicals does not take place immediately but in a higher or a smaller interval, a thing that allows certain reactions of the chemical compounds from the polyphenol extracts. This element is important mainly because of the evaluation of the biological effects of the extracts, that is the activities of the tissues from the body.

## CONCLUSIONS

1. The results of the preliminary characterization process of the polyphenol extracts that were obtained from varieties of seeds like Arcaş, Negru de Drăgăşani and Chambourcine, justify the study of the antiradicalic properties.
2. The antiradicalic activity of the extracts that were studied is high going on very high, it is similar with the one of the red wines, being noticed the extract that was obtained from the seeds of Negru de Drăgăşani.

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# PRELIMINARY CHARACTERISATION OF POLYPHENOLIC EXTRACTS FROM GRAPE SKINS

## CARACTERIZAREA PRELIMINARĂ A UNOR EXTRACTE POLIFENOLICE OBTINUTE DIN PIELIȚELE DE STRUGURI

NECHITA Ancuța<sup>1</sup>, COTEA V. V.<sup>1</sup>, NICULAU M.<sup>2</sup>, NECHITA B.<sup>1</sup>

e-mail: ancuta.vasile@yahoo.com

**Abstract.** In order to characterize the polyphenolic extracts obtained from grape skins, with function in the maintenance of the metabolic equilibrium and of the human organism's state of health, it was performed the HPLC analysis (high performance liquid chromatography) through which have been identified and quantified a series of phenolic acids, stilbenes ((trans-resveratrol), certain non-hydrolysable tannins (catechin and epicatechin). In addition, it was also performed the anthocyanic profile for each polyphenolic extract. There were studied the polyphenolic extracts obtained from the grape skins of seven vine varieties, of which four were autochthon (Fetească neagră, Băbească neagră, Arcaș, Negru de Drăgășani), two varieties of international brand (Cabernet Sauvignon, Merlot) and the variety with increased resistance Chambourcine. The results obtained justify the continuation of the research regarding the biologically active properties of the polyphenolic extracts that were studied.

**Key words:** *Vitis vinifera*, grapes, skins, polyphenols, anthocyanins.

**Rezumat.** În vederea caracterizării extractelor polifenolice, obținute din pielețele de struguri, cu rol în menținerea echilibrului metabolic și a stării de sănătate a organismului uman, s-a realizat analiza HPLC (cromatografie de lichide de înaltă performanță) prin care au fost identificați și cuantificați o serie de acizi fenolici, stilbeni (trans-resveratrolul) unele taninuri nehidrolizabile (catechina și epicatechina). De asemenea, s-a realizat și profilul antocianic al fiecărui extract polifenolic. Au fost studiate extractele polifenolice obținute din pielețele de strugurii a șapte soiuri de viță de vie, dintre care patru autohtone (Fetească neagră, Băbească neagră, Arcaș, Negru de Drăgășani), două din sortimentul internațional (Cabernet Sauvignon, Merlot) și soiul cu rezistență sporită Chambourcine. Rezultatele obținute justifică continuarea cercetărilor cu privire la proprietățile biologice active ale extractelor polifenolice studiate.

**Cuvinte cheie:** *Vitis vinifera*, struguri, pielețe, polifenoli, antociani.

### INTRODUCTION

The polyphenolic compounds constitute one of the most representative classes of secondary metabolites in plants, having an essential role in the insurance of normal growth and development of the plants, some of them contributing to the pigmentation and the defence against pathogen agents and of predators (Lapornik et al., 2005; Tsao et al., 2003; Balasundram et al., 2006).

It is known that the grapes store complex mixtures of polyphenolic

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

<sup>2</sup>Oenology Research Center – Iasi Branch of the Romanian Academy, Romania

compounds, readily accessible and relatively easy to be extracted. These compounds are found especially in the seeds and skin of grapes from where they are taken over in grape juices and wines, during the maceration process (Merton S. et.al., 2003). If the grape seeds constitute the most important source of polyphenolic compounds of polymeric type (21 - 27 GAE/g fresh vegetal mass, numbering between 60 and 70% out of the total extractable polyphenolic compounds), the grape skins contain significantly less total polyphenols and polymers, but these are more variable, being included between 0,1 și 5 mg GAE/g fresh grape skins. Polymeric polyphenols are found in variable quantity of 6–43% out of the total polyphenols from the grape skins. At the same time, it must be specified that the distribution of these compounds is not equal, varying according to the genetic, climatic factors and the development of the vegetal segments, etc. (Fregoni et al., 2004).

The research from this paper attempt to realize the characterization of certain extracts obtained from the grape skins through the identification and quantification of the most important polyphenolic compounds from their composition.

## **MATERIAL AND METHOD**

The polyphenolic extracts have been obtained from the grape skins of certain autochone vine varieties as well as of varieties of international range (Fetească neagră, Băbească neagră, Arcaș, Negru de Drăgășani, Cabernet Sauvignon, Merlot and Chambourcine). After grinding, at dimensions of 1-2 mm, the vegetal materials were degreased with ethanol. The extraction procedure was intermittent in stationary regime at a temperature of only 30 °C, the ethyl alcohol being used as solvent in a proportion of 1/10 (vegetal material (g)/solvent (mL)). For characterization, the polyphenolic extracts have been analyzed through high performance liquid chromatography (HPLC), being identified and quantified a series of phenolic acids, stilbenes (trans-resveratrol), non-hydrolysable tannins (catechin and epicatechin), certain flavones (rutin and quercitin), as well as a series of anthocyanic compounds.

## **RESULTS AND DISCUSSIONS**

In order to evaluate the beneficial properties of the polyphenolic extracts obtained from grape skins in the maintenance of the metabolic equilibrium and of the human organism's state of health, these were submitted to a process of preliminary characterization. Among the polyphenolic compounds, phenolic acids are the most common for vegetal extracts, these being found as mixtures: hydroxybenzoic and hydroxycinnamic acids (tab. 1 and 2).

In the case of analyzed extracts, the content of hydroxybenzoic acids varied between wide limits (tab. 1). The most important hydroxybenzoic acid, gallic acid was identified in very small quantities (1,095 – 1,514 mg/L), right below the detection limit (the case of extracts obtained from the grape skins of Fetească neagră and Băbească neagră). Although in the specialty literature it is mentioned the fact that salicylic acid is formed during the process of alcoholic fermentation, it was identified in the polyphenolic extracts obtained from grape skins in

considerable amounts, in the case of the varieties Băbească neagră (37,804 mg/L) and Chambourcine (30,971 mg/L). In the analyzed polyphenolic extracts there have also been identified in small quantities other hydroxybenzoic acids, such as: p-hydroxybenzoic acid, m-hydroxybenzoic acid and vanillic and gentisic acid.

Table 1

**The hydroxybenzoic acids identified in the polyphenolic extracts obtained from the grape skins of the varieties taken study**

| Vine varieties     | p-hydroxy benzoic acid, mg/L | m-hydroxy benzoic acid, mg/L | salicylic acid, mg/L | vanillic acid, mg/L | gallic acid, mg/L | acid siringic, mg/L | gentisic acid, mg/L |
|--------------------|------------------------------|------------------------------|----------------------|---------------------|-------------------|---------------------|---------------------|
| Fetească neagră    | 0.013                        | 0.206                        | 1.622                | 1.025               | -                 | 1.072               | 0.959               |
| Negru de Drăgășani | -                            | 0.200                        | 1.637                | 0.959               | 1.148             | 5.948               | 0.835               |
| Arcaș              | 0.088                        | -                            | 0.995                | 1.443               | 1.514             | 0.432               | 0.732               |
| Merlot             | 0.022                        | 0.268                        | 2.665                | 1.499               | 1.139             | 2.624               | 1.095               |
| Cabernet Sauvignon | 0,005                        | 0.195                        | 2.938                | 0.997               | 1.095             | 1.579               | 1.187               |
| Băbească neagră    | 0.204                        | 0.242                        | 37.804               | 1.101               | -                 | 4.154               | 3.219               |
| Chambourcine       | 0.077                        | -                            | 30.971               | 0.877               | 1.184             | 2.798               | 2.116               |

Through HPLC analysis of the polyphenolic acids there have been also identified a series of hydroxycinnamic acids, respectively caffeic, p-coumaric, ferulic, sinapic and chlorogenic acid (tab. 2).

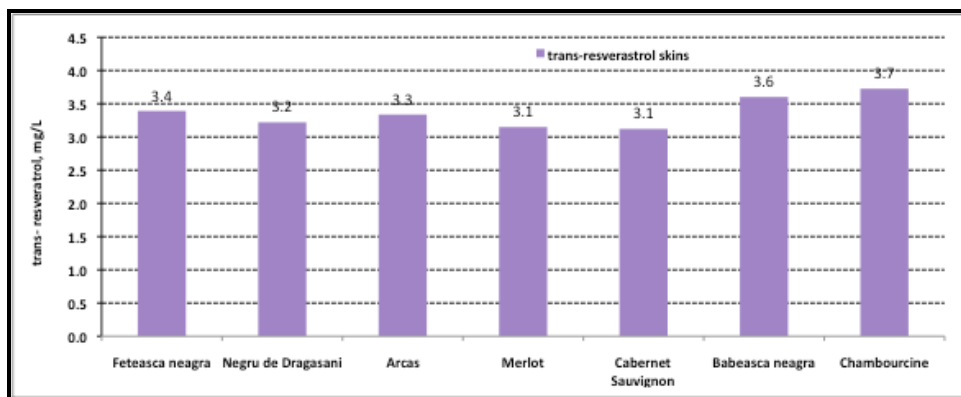
Table 2

**The hydroxycinnamic acids identified in the polyphenolic extracts obtained from grape skins of varieties taken study**

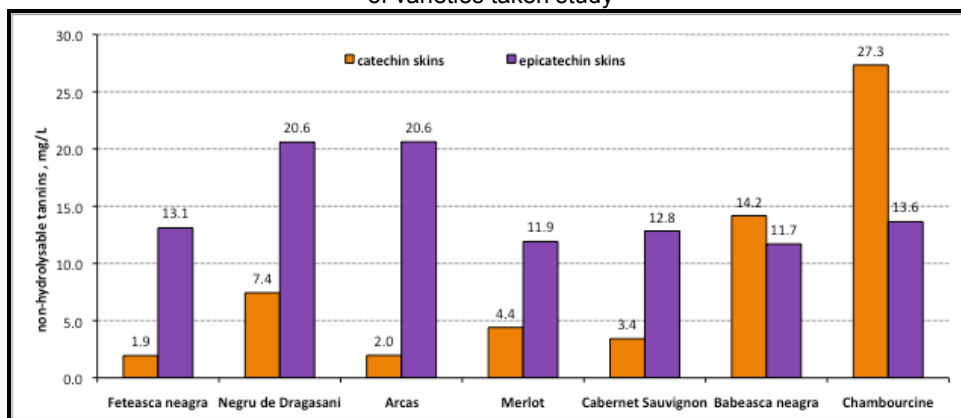
| Vine varieties     | caffeic acid, mg/L | p-coumaric acid, mg/L | ferulic acid, mg/L | sinapic acid, mg/L | chlorogenic acid, mg/L |
|--------------------|--------------------|-----------------------|--------------------|--------------------|------------------------|
| Fetească neagră    | -                  | 5.943                 | 0.987              | 0.738              | 3.348                  |
| Negru de Drăgășani | -                  | 7.494                 | 0.978              | 1.229              | 4.970                  |
| Arcaș              | -                  | 7.613                 | 0.968              | 1.732              | 3.513                  |
| Merlot             | -                  | 6.307                 | 1.175              | 1.726              | 3.280                  |
| Cabernet Sauvignon | -                  | 5.989                 | 1.106              | 1.205              | 3.282                  |
| Băbească neagră    | 0.532              | 7.420                 | 1.774              | 3.273              | 4.019                  |
| Chambourcine       | 0.547              | 6.882                 | 1.850              | 2.573              | 3.676                  |

Analyzing the data, one can notice the fact that no matter the variety of which extracts were obtained, the contents in hydroxycinnamic acids does not show significant variations, the values being very close one to another. Besides the phenolic acids, in the extracts obtained from grape skin of varieties taken into account for the present study there was also identified the trans-resveratrol. From the graphical representation of the obtained data (fig. 1), one can notice a low variation of the concentration of trans-resveratrol between 3,12 and 3,73 mg/L.

Furthermore, there were highlighted some non-hydrolysable (condensed) tannins, respectively catechin and epicatechin (fig. 2). As the contents of catechin are concerned, one can notice the high range of variation, from 1,95 mg/L in the case of Fetească neagră to 27,33 mg/L in the case of Negru de Drăgășani. As epicatechin is concerned, higher values were noticed at Negru de Drăgășani (20,60 mg/L) and Arcaș (20,62 mg/L), in the case of other varieties the values being close one to another (between 11,70 and 13,64 mg/L).

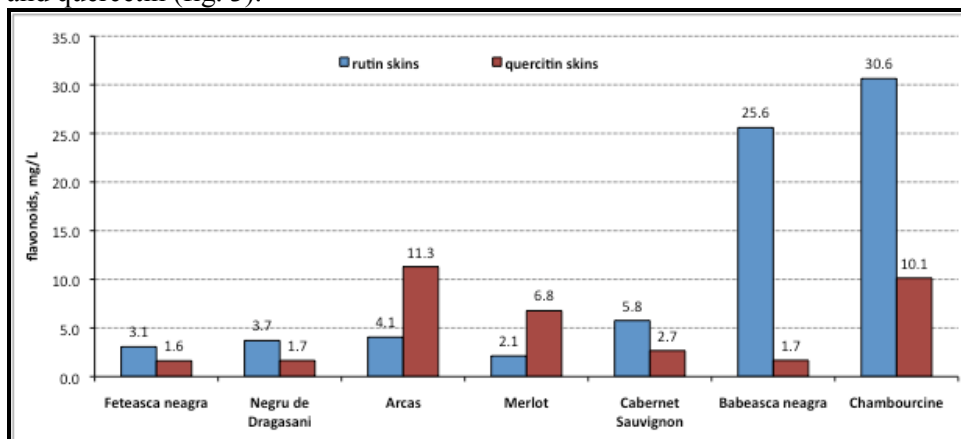


**Fig. 1** - The contents of trans-resveratrol of polyphenolic extracts obtained from grape skin of varieties taken study



**Fig. 2** – Variation of the concentration of non-hydrolysable tannins in polyphenolic extracts obtained from the grape skins of varieties study

Among flavonoids, through the HPLC analysis there were identified rutin and quercetin (fig. 3).



**Fig. 3** – Variation of the contents of flavonoids of polyphenolic extracts obtained from grape skin of varieties taken study



Both rutin and quercetin have been identified having significant quantities. It is specially remarked the extract obtained from grape skins of the variety Chambourcine with 30,64 mg/L rutin and 10,12 mg/L quercetin.

The identification of the anthocyanic composites from polyphenolic extracts was done based on chromatograms. For each chromatogram there were identified and then calculated the relative proportions of the following anthocyanins: delphinidin-3-monoglucoside (Dp), cyanidin-3-monoglucoside (Cy), petunidin-3-monoglucoside (Pt), peonidin-3-monoglucoside (Po) malvidin-3-monoglucoside (Mv), peonidin-3-monoglucoside acetilate (Po-a), malvidin-3-monoglucoside acetilate (M-a), peonidin-3-monoglucoside coumaroylated (Po-cm), malvidin-3-monoglucoside coumaroylated (M-cm).

From the obtained chromatograms were extracted the areas of signals (in mAU\*s) of anthocyanins from extracts obtained from grape skins for the seven varieties taken into account for the present study. As an evaluation of each anthocyanin from the anthocyanic extract based on areas is more difficult to achieve, the interpretation of results was done easily from the perspective of percentage proportions of areas of the main anthocyanins from extracts (tab. 3).

Table 3

**Main anthocyanins (%) identified in the extracts obtained from grape skins**

| Main anthocyanins identified (%) | Fetească neagră | Negru de Drăgășani | Arcaș | Merlot | Cabernet Sauvignon | Băbească neagră | Chambourcine |
|----------------------------------|-----------------|--------------------|-------|--------|--------------------|-----------------|--------------|
| Delphinidin                      | 1,34            | 1,25               | 2,01  | 5,97   | 7,47               | 19,29           | 24,24        |
| Cyanidin                         | 1,17            | 1,10               | 1,03  | 3,19   | 3,90               | 14,59           | 17,64        |
| Petunidin                        | 8,41            | 6,67               | 6,48  | 8,09   | 9,41               | 12,56           | 14,42        |
| Peonidin                         | 21,21           | 13,97              | 11,50 | 19,14  | 13,90              | 9,99            | 7,42         |
| Malvidină                        | 48,87           | 48,25              | 31,99 | 31,24  | 33,64              | 26,62           | 27,27        |
| Peonidin-acetilate               | 0,04            | 0,73               | 4,17  | 5,17   | 3,77               | 3,10            | 0,93         |
| Malvidin-acetilate               | 2,81            | 5,58               | 21,04 | 16,97  | 19,44              | 9,71            | 4,04         |
| Coumaroylated peonidin           | 3,28            | 3,31               | 4,53  | 3,16   | 1,58               | 0,73            | 0,36         |
| Coumaroylated malvidin           | 12,87           | 19,14              | 17,24 | 7,07   | 6,91               | 3,42            | 3,69         |
| ΣAnt.-acet.<br>+ Ant.-coum.      | 19,00           | 28,76              | 46,98 | 32,38  | 31,69              | 16,96           | 9,02         |
| ΣAnt.-acet./<br>ΣAnt.-cum.       | 0,18            | 0,28               | 1,16  | 2,16   | 2,74               | 3,09            | 1,23         |

From the analysis of the results obtained it is noticed that in all extracts prevails malvidin, aspect which confirms the date in the specialty literature. A discrimination parameter in the case of black grapes can be, as in the case of wines, the sum of the esterified anthocyanins (acetylated and coumaroylated), as well as in the report between these (acetylated anthocyanins/coumaroylated anthocyanins). The sum of the acetylated and coumaroylated anthocyanins (peonidin-3-monoglucoside acetylated (Po-a), malvidin-3-monoglucoside acetylated (M-a), peonidin-3-monoglucosid coumaroylated (Po-cm), malvidin-3-monoglucoside coumaroylated (M-cm), varied between 46,98 % at the variety Arcaș and 9,02% at the variety Chambourcine. The report between acetylated anthocyanins and the coumaroylated ones presents much smaller values, which varied from 0,18% at the variety Fetească neagră to 3,09% at the variety Băbească neagră.

## CONCLUSIONS

1. The identification of active principles (phenolic acids, stilbenes, tannins, flavones, etc.) of the polyphenolic extracts obtained from the grape skins contributes to the evaluation of the oenological potential of the vine varieties and, implicitly, to the evaluation of beneficial properties in the maintenance of the metabolic equilibrium and the human organism's state of health.

2. As regards the phenolic acids it was noticed that the gallic acid was identified in small quantities, between 1,095 – 1,514 mg/L, right below the detection limit in the case of extracts obtained from the grape skins of Fetească neagră and Băbească neagră. The salicylic acids presented great variation limits between 0,995 mg/L (Arcaș) and 37,804 mg/L (Băbească neagră). In reduced quantities there have also been identified other hydroxybenzoic acids, such as: *p*-hydroxybenzoic acid, *m*-hydroxybenzoic acid, such as vanillic and gentisic acid. Furthermore, irrespective of the variety from which were obtained the extracts, the content of hydroxycinnamic acids (caffeic, *p*-coumaric ferulic, sinapic) did not presented significant variations, the values being very close to each other.

3. The polyphenolic extracts which were analyzed present a small variation of the *trans*-resveratrol's concentration between 3,12 and 3,73 mg/L, and a great variation range regarding the catechin from 1,95 mg/L at Fetească neagră to 27,33 mg/L at the variety Negru of Drăgășani. In addition, rutin as well as quercetin were identified in significant quantities, being remarked especially the extract obtained from the grape skins of the variety Chamburcine with 30,64 mg/L rutin and 10,12 mg/L quercetin.

4. The analysis of the anthocyanic profile highlights the fact that among all the studied extracts prevails malvidin, aspect which confirms the data from the specialty literature.

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# STUDY ON MATURATION DYNAMICS AND POTENTIAL OF THE PHENOLIC COMPOUNDS IN THE LOCAL VARIETIES OF RED GRAPES

## STUDIUL PRIVIND DINAMICA MATURĂRII ȘI POTENȚIALUL COMPUȘILOR FENOLICI ÎN SOIURILE AUTOHTONE ROȘII

**OBADĂ Leonora<sup>1</sup>, MINDRU A.<sup>1</sup>, RUSU E.<sup>1</sup>,  
GOLENCO Lidia<sup>1</sup>, CIBUC Mariana<sup>1</sup>, GROSU Olga<sup>1</sup>**  
e-mail: oenologie\_vdo@mail.ru

**Abstract:** The aim of researches is the study of the dynamics and the evaluation of the phenolic compounds potential of the grain black grapes of local varieties namely: Rară neagră, Bătută neagră, Brează, Ciorcuță neagră, Fetească neagră, Kopceac, Negru de Akkerman, Negru de Căușeni, Seină and Tămâioasă de Bohotin the harvest of 2011 grown in the Central region of the Republic of Moldova. The Codrinschi variety grown in the central region (the land Stăuceni) and South (the land Causeni) has also been studied. The variety Merlot served as a control. Carbohydrates, organic acids and phenolic complex potential at different stages of maturation process were determined. It was determined that after the phenolic complex potential and compared with control variety Merlot the investigated varieties may be classified as follows: varieties with high content in phenolic compounds varieties with medium content and varieties with low content in phenolic compounds.

**Key words:** local varieties, maturation dynamics, carbohydrates, titratable acids, phenolic substances.

**Rezumat** Scopul cercetărilor îl constituie studiul dinamicii maturării și evaluarea potențialului compușilor fenolici la strugurii cu bob negru aparținând soiurilor autohtone: Rară neagră, Bătută neagră, Brează, Ciorcuță neagră, Fetească neagră, Kopceac, Negru de Akkerman, Negru de Căușeni, Seină și Tămâioasă de Bohotin din recolta anului 2011, cultivați în regiunea Centru a Republicii Moldova. De asemenea, a fost studiat și soiul Codrinschi cultivat în regiunea Centru (plaiul Stăuceni) și regiunea Sud (plaiul Pleșeni). Ca martor s-a folosit soiul Merlot. Au fost determinate glucidele, acizii organici și potențialul complexului fenolic la diferite etape ale procesului de maturare. S-a constatat că, după potențialul complexului fenolic și în raport cu soiul martor Merlot, soiurile investigate pot fi grupate în felul următor: cu conținut ridicat de compuși fenolici, cu conținut mediu și cu conținut scăzut în compuși fenolici.

**Cuvinte cheie:** soiuri autohtone, dinamica maturării, glucide, acizi titrabili, substanțe fenolice.

## INTRODUCTION

Lately several countries highlights from the base assortment of varieties, some so called "national".

Currently Moldovan range of grape varieties with black bean consists mainly of French classics, as well as from clones of these varieties like Cabernet

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<sup>1</sup> Scientifico-Practical Institute of Horticulture and Food Technologies, Chișinău, Republic of Moldova

Sauvignon, Merlot, Pinot Noir. It is difficult to create a national symbol of the existing assortment of wine.

In the current tough competition from several countries worldwide wine market with great diligence promotes wines from indigenous varieties. An example may serve Georgia and Saperavi, Rkațiteli varieties, Bulgaria - Mavrud, Rubin and Melnic, România – Tamâioasă, Fetească Neagră, Azerbaijan - Şirvanşah, Baian publication. (Rusu et al., 2008, Crăciun, 2008). Many local varieties like Rară neagră, Fetească neagră Tamâioasă de Bohotin etc., had a mark on history of the country and created typicity of wine vineyards.

Currently we have some local red varieties, which are invaluable, but unfortunately they have not undergone thorough research on the potential of phenolic substances and colorants.

The purposes of these investigations is the study of aging dynamics and assess, the potential of phenolic compounds of grapes with black bean of local varieties in the climatic conditions of 2011, which would enable optimization of their processing technology to obtain red wines.

## **MATERIAL AND METHOD**

Researches were performed at the Scientific-Practical Institute of Horticultural and Food Technologys (SPIHFT) Republic of Moldova in 2011 on Rară neagră grapes varieties (Babească neagră), Batută neagră, Breaza, Ciorcuță neagră, Fetească neagră, Kopceak, Negru of Akkerman, Negru of Căușeni, Seină and the Tamâioasă of Bohotin grown ampelographic collection of SPIHFT, Central region, where they were provided the same culture conditions. When it was affiliated Codrinschi local variety grown in the central region (Stauceni) and South (Pleseni). Served as a witness Merlot, grown on plantations SPIHFT (central region).

As research objects grapes served with black bean named local varieties harvested and analyzed at different stages of maturation - the first fruits before the technological maturation. The grapes were made the following determinations: carbohydrate content, titratable acids, phenolic substances and anthocyanins. Sugars and titratable acids content was determined according to standard methods, phenolic substances - according to colorimetric method with Folin-Ciocalteu reagent, anthocyanins - colorimetric method. Research has been conducted in laboratory Oenology and Wine with Designation of Origin in collaboration with the laboratory genetic fund and improvement of the SPIHFT.

## **RESULTS AND DISCUSSIONS**

Evolution of carbohydrate content and titratable acidity expressed as tartaric acid during ripening grape varieties indigenous black beans is presented in table 1.

The data presented can be seen that local varieties under investigation differ in different capacities carbohydrate accumulation and reduced titratable acidity. Thus, on September 7, 2011 with the highest carbohydrate content outlined Codrinschi varieties (South region) - 194 g/L, the Tamâioasă of Bohotin, Negru of Căușeni, Kopceak - 183-187g /L.

Table 1

## Carbohydrate content and titratable acidity during ripening grape varieties local black grain under 2011

| Variety name                         | 7 September                            |             | 15 September                           |             | 20 September                           |             | 26 September                           |             | 29 September                           |             |
|--------------------------------------|--|-------------|--|-------------|--|-------------|--|-------------|--|-------------|
|                                      | Titratable acidity (g/L tartaric acid) | sugar (g/L) | Titratable acidity (g/L tartaric acid) | sugar (g/L) | Titratable acidity (g/L tartaric acid) | sugar (g/L) | Titratable acidity (g/L tartaric acid) | sugar (g/L) | Titratable acidity (g/L tartaric acid) | sugar (g/L) |
| Rară neagră                          | 11,5                                   | 173         | 10,2                                   | 207         | 9,8                                    | 212         | 9,2                                    | 217         | 8,4                                    | 228         |
| Brează                               | 8,0                                    | 155         | 7,5                                    | 165         | 7,1                                    | 177         | 6,4                                    | 188         | 6,0                                    | 192         |
| Kopceak                              | 9,0                                    | 183         | 8,1                                    | 200         | 7,5                                    | 203         | 7,0                                    | 213         | -                                      | -           |
| Bătută neagră                        | 7,4                                    | 130         | 7,1                                    | 135         | 5,8                                    | 140         | 5,6                                    | 155         | 5,5                                    | 160         |
| Seină                                | 7,4                                    | 153         | 7,3                                    | 160         | 7,2                                    | 180         | 7,0                                    | 200         | 6,5                                    | 205         |
| Fetească neagră                      | 9,3                                    | 175         | 7,6                                    | 194         | 7,5                                    | 210         | 7,3                                    | 227         | 7,2                                    | 250         |
| Ciorcuță neagră                      | 7,4                                    | 177         | 7,3                                    | 187         | 7,0                                    | 195         | 6,5                                    | 197         | -                                      | -           |
| Tămâioasă of Bohotin                 | 8,9                                    | 187         | 8,6                                    | 205         | 8,3                                    | 247         | -                                      | -           | -                                      | -           |
| Negru of Akkerman                    | 6,8                                    | 128         | 6,5                                    | 145         | 6,0                                    | 153         | 5,5                                    | 164         | -                                      | -           |
| Negru of Căușani                     | 10,2                                   | 185         | 9,0                                    | 200         | 8,9                                    | 208         | 8,8                                    | 215         | -                                      | -           |
| Codrinschi (Pleșeni) South region    | 7,9                                    | 194         | 7,6                                    | 202         | 7,6                                    | 212         | 6,5                                    | 230         | 6,3                                    | 240         |
| Codrinschi (Stăuceni) Central region | 14,9                                   | 170         | 10,7                                   | 190         | 8,8                                    | 202         | 8,3                                    | 213         | 8,2                                    | 215         |
| Merlot (control)                     | 9,0                                    | 167         | 8,8                                    | 192         | 8,4                                    | 212         | 8,2                                    | 218         | -                                      | -           |

The varieties Negru of Akkerman and Bătută neagră carbohydrates have the lowest level, 130 g/L, and varieties Brează, Seină, Merlot (control), Codrinschi (Central region), Rară neagră, Ciorcuță neagră stand with an average carbohydrate. On titratable acidity may be mentioned that at that time the highest value is observed in varieties Codrinschi (Central region), Rară neagră, and Negru of Căușani. The other varieties, including witness Merlot variety, this index is included within the 7.4 to 9.3 g/L.

On September 15, 2011 there is an increased accumulation of carbohydrates in Rară neagră varieties, Merlot and Codrinschi (Stăuceni) - 35, 25 and 20 g/L respectively. Varieties Bătută neagră, Seină, Brează, Ciorcuță neagră is characterized by a weak capacity to increase carbohydrates during this period - with only 5-10 g/L.

In the titratable acidity can be seen that a more significant decrease in the proportion of 1.2 to 4.7 g/L is observed in the varieties with the highest value of this index: Negru de Căușani, Rară neagră, Fetească neagră, Codrinschi (Stăuceni) (table 1). To the other varieties titratable acidity decreased more slowly, and some varieties: Seină, Ciorcuță neagră, Negru of Akkerman remained basically the same.

On September 20, 2011 Tămăioasă of Bohotin variety of incense recorded the highest carbohydrate content from all investigated varieties 247 g/L, representing titratable acidity 8.3 g/L. Rară neagră varieties, Fetească neagră, Negru of Căușani, Codrinschi (Pleșeni) and Merlot (control) have a carbohydrate content of between 208 and 212 g/L, and titratable acidity varies between 7.5 and 8.9 g/L except by Rară neagră variety, characterized by the highest value of this index (9.8 g/L) on nomination.

It should be noted that, just as in the previous period, the lowest carbohydrate content is observed in varieties Bătută neagră (140 g/L), Negru of Akkerman (153 g/L) and Brează (177 g/L). They also have the lowest values of titratable acidity accordingly 5.8 - 6.0 and 7.1 g/L. In other varieties under investigation carbohydrates varies within the 180-203 g/L, and titratable acids between 7.0 and 8.8 g/L.

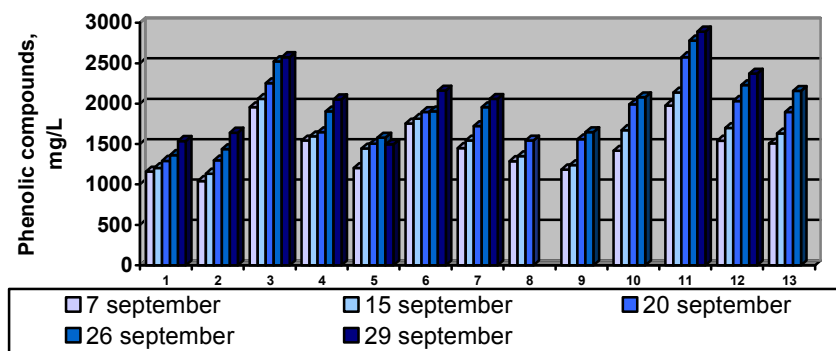
At the end of September (the technological maturation) in local varieties Fetească neagră, Codrinschi (Pleșeni), Rară neagră carbohydrate content reached values of 250, 240 and 228 g/L respectively, and titratable acidity decreased to 6.3 - 7.2 g/L, except Rară neagră variety, which has the highest index value of the varieties under study - 8.4 g/L.

It should be noted that the varieties under investigation, is characterized by a low potential for accumulation of carbohydrate varieties Bătută neagră (160 g/L), Negru of Akkerman (164 g/L) and Brează (192 g/L), with and lowest titratable acidity, which varies between 5.5 and 6.3 g/L.

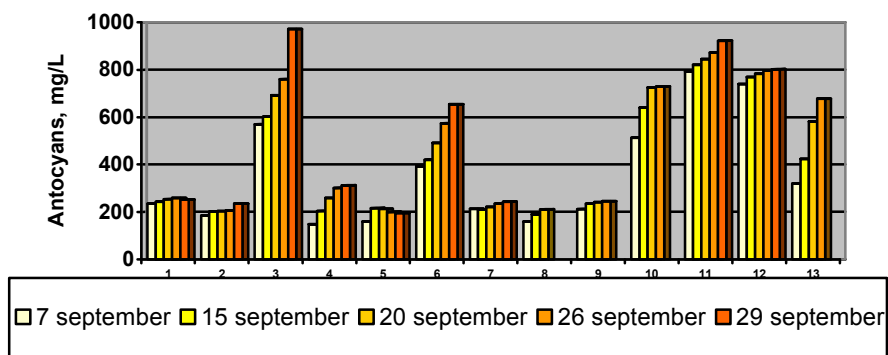
Carbohydrate content in the witness Merlot variety (218 g/L) was recorded in varieties Codrinschi (Stăuceni), Negru of Căușani and Kopceak.

In figures 1 and 2 are presented data on the evolution of phenolic substances and anthocyanins in varieties subject investigations.

**Fig. 1 - Evolution of phenolic substances during ripening grapevine varieties in the local climatic conditions of 2011**



**Fig. 2 - Evolution anthocyanins during ripening grapevine varieties in the local climatic conditions of 2011**



Research shows that local varieties are characterized by very different potential of phenolic complex. At the beginning of maturation in varieties Codrinschi (Pleşeni) and Kopceak is registered the highest values of phenolic substances and anthocyanins content - 1978 and 793 mg/L, 1957 and 571 mg/L, appropriate, and varieties Breaza, Rară neagră, Negru of Akkerman, Seină and Tămâioasă of Bohotin have the lowest values of these indices, which range between 1042 and 1284 mg/L for phenolic substances and 159-235 mg/L for anthocyanins. In the variety Codrinschi (Stauceni) may be mentioned that compared with the same variety grown in South phenolic substances and anthocyan content is lower and consists 1573 and 740mg / L.

Fetească neagră variety show with an average content in phenolic compounds – 1754 mg/L phenolic substances and 392 mg/L anthocyanins, are placed closer to reference variety Merlot - 1505 mg/L and 320 mg/L respectively. With an average content of phenolic substances (1416 mg/L) and advanced in anthocyanins (514 mg/L) is distinguished variety of Negru of Causeni.

It should be noted that Ciorcuță neagra and Bătută neagră varieties have a medium level of phenolic substances - 1545 and 1448 mg/L and relatively low anthocyanins – 148 and 213 mg/L corresponding.

From fig. 2 may find that more intense accumulation of phenolic compounds in the climatic conditions of 2011 are recorded in the second half of September. It should be noted that throughout the ripening grape varieties investigated had different storage capacities and phenolic substances and anthocyanins. Research shows that close to the reference variety Merlot as phenolic and anthocyanins content of the substances are Fetească neagră, Negru of Căușani and Bătută Neagră. Codrinschi varieties (Pleşeni) and Kopceak are characterized by the highest potential of phenolic compound. In Rară neagră and Tămâioasă of Bohotin varieties content of phenolic and coloring substances is less than the Merlot.

After phenolic compound potential (phenolic substances / anthocyanins, mg/L) varieties are placed subject to investigations as follows:

- With a high content (more than 600 coloring mg/L and phenol - more than 2000 mg/L): Codrinschi (Pleşeni) – 2891/923; Kopceak - 2524/761; Codrinschi (Stăuceni) - 2371/803; Fetească neagră – 2163/655; Merlot – 2158/678; Negru of Căușeni – 2078/730;

- Average (coloring 300-600 mg/L and phenol - 1600 to 2000 mg/L: Bătută neagră – 2054/312;

- Small containing (coloring below 300 mg/L and phenol – below 1960 mg/L): Ciorcuță neagră – 1957/235; Negru of Akkerman – 1648/245; Rară neagră (Center region) – 1540/252; Tămâioasă de Bohotin – 1545/211; Brează – 1436/206; Seină – 1582/200.

## CONCLUSIONS

1. Investigated local red varieties are distinguished by the carbohydrate content, titratable acidity and potential of phenolic compounds. Codrinschi varieties (Stăuceni) Rară neagră and Negru de Căușeni are outlined with higher value of titratable acidity - 8.2 to 8.8 g/L.

2. After potential phenolic compound and compared with reference variety Merlot varieties studied can be grouped as follows:

a) varieties with a high content in phenolic compounds - Codrinschi, Kopceak, Fetească neagră, Negru of Causeni; b) varieties with medium contain of phenolic compounds (the witness) – Bătută neagră; c) varieties with low content of phenolic compounds – Tămâioasă de Bohotin, Brează, Ciorcuță neagră, Seină, Negru of Akkerman, Rară neagră (center wine region).

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# RESEARCH ON IMPROVING THE PRODUCTION TECHNOLOGY OF THE RED WINE OBTAINED FROM THE LOCAL VARIETY CODRINSCHI IN REPUBLIC OF MOLDOVA

## CERCETĂRI PRIVIND AMELIORAREA TEHNOLOGIEI DE PRODUCERE A VINULUI ROȘU DIN SOIUL AUTOHTON CODRINSCHI ÎN REPUBLICA MOLDOVA

*RUSU E.<sup>1</sup>, OBADĂ Leonora<sup>1</sup>, COVALCIUC Olga<sup>1</sup>*  
e-mail: oenologie\_vdo@mail.ru

**Abstract.** *Are presented research results on the influence of different technological processes used in domestic Codrinschi species of grapes, harvested in the central region of Moldova on the extraction of phenolic compounds and chemical composition of wine: maceration-fermentation of the pulp(control); adjusting the ratio of solid and liquid phase by separating the wort preventively ; thermal treatment of pulp; using enzyme preparations and wine produced from the same species harvested in the South, Crocmaz village by maceration-fermentation of the pulp.*

**Key words:** Grapevine variety Codrinschi, maceration-fermentation, Central region, South region, Republic of Moldova

**Rezumat.** *În lucrare sunt prezentate rezultatele cercetărilor privind influența diferitor procedee tehnologice utilizate la prelucrarea strugurilor de soiul autohton Codrinschi, recoltați din regiunea Centru a Republicii Moldova asupra extragerii compușilor fenolici și compoziției chimice a vinurilor. Variantele folosite au fost: macerarea-fermentarea pe boștină (martor); reglarea raportului dintre faza solidă și lichidă prin separarea inițială a mustului; tratarea termică a mustuielii; utilizarea preparatelor enzimatice, precum și vinul obținut din același soi recoltat din regiunea Sud, plaiul Crocmaz prin macerarea-fermentarea mustuielii.*

**Cuvinte cheie:** soi autohton Codrinschi, macerarea-fermentarea, regiunea Centru, regiunea Sud, Republica Moldova

### INTRODUCTION

In present, wines marketing competition in the world is very hard. It depends on the quality and effectiveness of promoting them. The varieties of wines established in many countries consist mainly of classic French grape varieties. Also, at this chapter, more and more countries highlights the basic grade of some grape varieties, so called "National", on whom are created the wine brand (Rusu, 2006, Pomohaci et al., 2000). In this context, is attractive experience gained in the last few years of Georgia, that are promoted with a great eagerness wines obtained from the varieties of wines as Saperavi and Rcațiteli, but in Bulgaria more popular are wines obtained from native varieties as Mavrud, Rubin and Melnik.

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<sup>1</sup> Scientifico-Practical Institute of Horticulture and Food Technologies, Chișinău, Republic of Moldova

The Republic of Moldova has some new varieties for the selection of National Institute of Winegrowing and Winemaking, and local varieties which have a value that could not be calculated. On a large scale, their use would contribute to create identity moldavian wines (Apruda et al., 1992, Țuțuc et al., 1998).

From local varieties of red wines, Codrinski variety represents a great interest which until now has not been the subject of extensive research in the development of relevant technologies of processing grapes.

The purpose of the work is development of a technology of processing grapes from the variety Codrinschi and chemical composition analysis of the wine, especially as regards the content well as phenolic compounds.

## **MATERIAL AND METHOD**

For research has been used Codrinschi variety grown in the Center region, village Stauceni, as well as in the South region, village Purcari.

Experimental samples of wine were obtained through the use of the following technological process: maceration and fermentation (control); initial separation of musts to 10 % (variant I); idem 20% (variant II); thermal treatment of must at 70°C during 30 minutes (variant III); use of enzymatic preparations Enovin Color (variant IV) and Trenolin Color (variant V). The South region grapes-maceration and fermentation at 25-28°C (variant VI).

Processing of grapes has been carried out in accordance with the conditions of the Institute of Winegrowing and Winemaking of harvest - 2009; physico-chemical analyzes have been made by according to OIV methods.

## **RESULTS AND DISCUSSIONS**

The investigation results of physico-chemical composition of samples obtained by different technological process are shown in the table 1. Data obtained indicates that red wines of the Center region have an alcoholic strength of between 12.3 and 13.6 % vol.

Research has shown that wines produced from the Codrinschi variety are distinguished by content in organic acids, whitely values between 6.8 and 8.0 g/L. In red wines investigated the pH Index varies from 3,20 to 3,38. Total soluble salts have large quantities in the Codrinschi wine obtained using enzymes.

Red wines were investigated to the content of substances phenolic, antocianic and chromatic indices of color. It has been found that the antocianic concentration differs in dependency on the process used and the place of origin of the grapes and reaches values from 264 to 422 mg/l in the Center region. Content higher in antocian certifying in the Codrinschi wine from Crocmaz, the South region 528 mg/L.

Research has shown that extraction of phenolic compounds is a function of technological process applied to it. The wine samples obtain in Center region are distinguished by a different content of phenolic compounds, having values between 1288 and 1803 mg/L.

Table 1

## Physico-chemical indices in red wines obtained from the variety Codrinschi from the harvest of 2009

| Tehnology for grape variety | Alcohol concentration, %vol | Mass concentration of |                     |                    |                             |                        |                              | Staining intensity, D <sub>420</sub> +D <sub>520</sub> | Hue, D <sub>420</sub> / D <sub>520</sub> | pH   | Total Soluble salt, mg/L | Conductivity, µS/cm |
|-----------------------------|-----------------------------|-----------------------|---------------------|--------------------|-----------------------------|------------------------|------------------------------|--|--|------|--------------------------|---------------------|
|                             |                             | Reducing sugar, g/L   | Titribile acid, g/L | Volatile acid, g/L | Sulfuric Dioxid total, mg/L | Fenolic compound, mg/L | Antocian concentration, mg/L |  |  |      |                          |                     |
| Control                     | 13,2                        | 1,4                   | 8,0                 | 0,26               | 57                          | 1494                   | 323                          | 1,92   | 0,48                                     | 3,20 | 849                      | 1698                |
| Variant I                   | 13,4                        | 1,2                   | 7,1                 | 0,39               | 65                          | 1648                   | 359                          | 2,35   | 0,57                                     | 3,28 | 864                      | 1728                |
| Variant II                  | 13,1                        | 1,1                   | 7,1                 | 0,26               | 69                          | 1803                   | 422                          | 2,50   | 0,56                                     | 3,24 | 867                      | 1741                |
| Variant III                 | 13,0                        | 2,1                   | 6,8                 | 0,26               | 70                          | 1751                   | 402                          | 2,30   | 0,53                                     | 3,28 | 839                      | 1676                |
| Variant IV                  | 12,3                        | 1,4                   | 7,2                 | 0,46               | 55                          | 1288                   | 264                          | 1,27   | 0,55                                     | 3,38 | 894                      | 1790                |
| Variant V                   | 12,4                        | 3,3                   | 7,3                 | 0,33               | 58                          | 1751                   | 402                          | 1,94   | 0,49                                     | 3,33 | 896                      | 1798                |
| Variant VI (Crocma)         | 13,6                        | 1,7                   | 7,2                 | 0,39               | 60                          | 2421                   | 528                          | 2,55   | 0,59                                     | 3,22 | 865                      | 1728                |

An advanced content of phenolic substances provides maceration and fermentation must with initial separation of musts in the ratio of 20 %, thermal treatment of must and use enzyme Trenolin Color. The content of phenolic substances and hue substances depends on the technological process applied to it. Values of more than 400 mg/L have been recorded in variants II, III and V. The intensity of the color samples experimental referred to those obtained by partial separation of musts and termomaceration of pulp, which have values between 2.3 and 2.5 and can be considered as typical color of red wines. The intensity of the color in control wine and the one obtained by the treatment with the Enovin Color enzymes is less than 2.0 and the color of these wines has less feature of this category of red wines.

That sample of the wine produced from grapes grown in South region is distinguished from those of the Center region by physico-chemical composition and especially by the content as phenolic compounds - 2420 mg/L and 528 mg/L antocians. It has more intense color, the value which is 2.55, hue is the 0,59.

## CONCLUSIONS

1. Physico-chemical composition and especially the content well as phenolic compounds of the wine made from the local variety Codrinschi depends on the region of origin of the grapes.

2. Climatic Conditions characteristic for Center region what keeps energy resources (heat and radiation) are not optimal for building up sufficient phenolic compounds in grapes of Codrinschi variety, that is why obtaining a deep colored red wine can be provided by the application of technological process more effective, such as partial separation of musts in the ratio of up to 20%, or by using enzymes to the Enovin Color type, that intensifies phenolic compounds extraction.

3. In the South wine-growing zone, due to build-up grapes to a quantity of more advanced phenolic compounds, producing Codrinschi red wine variety can be achieved through by using the classic process of maceration and fermentation for 4-5 days.

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# STUDY ON THE INFLUENCE OF VARIOUS MACERATION-FERMENTATION TECHNIQUES ON THE COLOUR OF RED WINES FROM FETEASCĂ NEAGRĂ GRAPES

## STUDIUL PRIVIND INFLUENȚA DIFERITELOR TEHNICI DE MACERARE-FERMENTARE ASUPRA CULORII VINURILOR ROȘII OBTINUTE DIN STRUGURI DE FETEASCĂ NEAGRĂ

ZAMFIR C.I.<sup>1</sup>, COTEA V.V.<sup>1</sup>, ODĂGERIU G.<sup>2</sup>,  
NICULAUA M.<sup>2</sup>, COLIBABA Cintia<sup>1</sup>  
e-mail: czamfir@uaiasi.ro

**Abstract.** *The typicity of wines colour is a difficult but important problem that can be solved by correct quantification of a large segment of aspects. The objective of the present study was to evaluate the colour of red wines produced from Fetească Neagră grape variety from 3 Romanian vineyards, through different maceration procedures (traditional, thermo, roto-tank and carbonic). The identification of anthocyanins in wines made from the Romanian traditional grape variety Fetească Neagră was carried out and their profile was determined using HPLC. The relationship of anthocyanin profile and specific characteristics with different maceration treatments was investigated. The results showed that the different maceration treatments exerted important differences on the content of anthocyanins and important variations in the colour of Fetească neagră wines. The different kinds of maceration and the location of vineyards influenced the percentage of each anthocyanin in the specific profile of Fetească Neagră wine.*

**Key words:** anthocyanins, Fetească neagră, maceration, colour difference.

**Rezumat.** *Tipicitatea culorii vinurilor este o problemă dificilă, dar importantă, care poate fi rezolvată prin cuantificarea corectă a unui segment larg de aspecte. Obiectivul prezentului studiu a fost de a evalua culoarea vinurilor roșii obținute din soiul de struguri Fetească neagră recoltat din 3 podgorii din România și vinificat prin proceduri diferite de macerare (clasic, termomacerare, cisterne rotative și macerație carbonică). S-a avut în vedere identificarea cu ajutorul HPLC a antocianilor și a profilului acestora în vinurile obținute din soiuri de struguri tradițional românesc Fetească neagră. A fost investigată relația dintre profilul antocianilor și caracteristicile specifice induse de diferitele tratamente de macerare folosite. Rezultatele au arătat că diferitele tratamente de macerare prezintă influențe semnificative asupra conținutului de antociani și induc variații importante în culoarea vinurilor de Fetească neagră. Variantele de macerare studiate și locația podgoriilor influențează procentul de participare al fiecărui antocian în profilul specific pentru vinul Fetească neagră.*

**Cuvinte cheie:** antociani, Fetească neagră, macerare, diferență de culoare

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<sup>1</sup> University of Agriculture and Veterinary Medicine of Iasi, Romania

<sup>2</sup> Oenology Research Center – Iasi Branch of the Romanian Academy, Romania

## INTRODUCTION

Anthocyanins are pigments of red, blue and purple colours, mainly occurring in cellular vacuoles of grape skin (Odăgeriu et al, 2007). Anthocyanins are important compounds for characterization of red grape varieties; they are chemical markers in distinguishing varietal red wines (Zamfir et al, 2008). It is known that the mutual relations of anthocyanins (the anthocyanins profile) belongs to the vine variety, even though their absolute content in ripe grapes varies a lot and depends on factors that concern the climatic factors, such as intensity of light and temperature (Odăgeriu et al, 2008). Although the wine anthocyanins composition is firstly determined by the genetic factor of the grape sort, the vinification parameters also have an important impact. It was shown that the maceration parameters have a significant influence on extraction of anthocyanins from grape skins (Zamfir et al, 2009). The conditions of maceration, fermentation and maturation of wine influence the anthocyanins composition, which is very significant, because the total concentration and composition of anthocyanins determine the colour of red wines (Cotea et al, 2007).

Feteasca neagra is a Romanian local red variety of *Vitis silvestris*, which acquires its superior quality in the Iasi, Dealu Bujorului and Panciu vineyards where wine with a protected geographic origin are produced. This grape variety is very important for production of high-quality red wines.

## MATERIAL AND METHOD

The experiments were done during September 2011 – March 2012, at the Oenology Laboratory of the University of Agricultural Studies and Veterinary Medicine “Ion Ionescu de la Brad” Iasi.

Vinification was carried out on 1000 kg of the Fetească neagră grape variety by random sampling in the 3 vineyards. The grapes were harvested at the full maturity.

After destemming and crushing three quarters of the total quantity- vineyard, pectolytic enzymes were added to better extract the colour. Maceration was performed in four ways: the classical maceration, roto-tanks maceration, thermo-maceration and carbonic maceration.

Treatment 1 (V1). Classical maceration: 50 kg of pomace was kept in static vessels, selected yeasts for fermentation were added, the cap was punched 3 times / day, for 3 days. After pressing, the wine was transferred into classic glass vessels, to complete its alcoholic and malolactic fermentation. The maceration process proceeded at a temperature of maximum 25 °C.

Treatment 2 (V2). Thermo-maceration: The pomace (50 kg) was divided in three. Two thirds of the obtained must was heated up to 80 °C and then transferred onto the pomace, bringing the temperature of the total mass up to 60 °C. When everything cooled, a 3 day maceration followed, with 3 times / day homogenization. After pressing, the wine was transferred into classic glass vessels, to complete its alcoholic and malo-lactic fermentation.

Treatment 3 (V3). Roto-tanks maceration: 50 kg of pomace was kept in roto-tanks, selected yeasts for fermentation were added, the content was homogenized 3 times/ day for 5 minutes, clockwise and anti-clock wise. The roto-tanks maceration lasted for 3 days. After pressing, the wine was transferred into classic glass vessels,

to complete its alcoholic and malolactic fermentation. The maceration process proceeded at a temperature of maximum 29 °C.

Treatment 4 (V4). Carbonic maceration: The selected grapes (50 kg), the remaining fourth of the total quantity were transferred into a mini carbonic maceration tank. This had at the bottom a grill under which fermenting must of Fetească neagră (with the same physical-chemical characteristics as the one above) was kept. The grapes are positioned without being crushed. After 12 days, the obtained mass is destemmed and pressed; fermenting yeasts are added, the wine being transferred into classic glass vessels, to complete its alcoholic and malolactic fermentation

Chromatic parameters of the analysed wine samples were calculated according to CIE Lab 76 method, taking into consideration the registered absorption spectrum for each wine sample (Odăgeriu et al., 2007, 2008; Zamfir et al., 2008; Zamfir, 2009). A Specord S200 spectrometer and calculator were used. An automated registration and classification of absorption spectrums was copied in a file. To minimize analysis errors when determining absorbencies, specific vials were used, with an optical characteristic of 1.0 cm. The spectres were processed with a soft realised within the research group, for obtaining the chromatic parameters (L, a, b, C, H°), colour intensity (I) and hue (N).

The colour differences were also calculated with the  $\Delta E$  2000 formula, it was considered that, for values of  $\Delta E$  smaller than the unity, the colours of two wines are seen as identical, or otherwise said, they cannot be sensorial differentiated.

Analysis of anthocyanins was performed in a Hewlett-Packard HP-1100 high-performance liquid chromatography. The injected sample volume was 20  $\mu$ L. Separation of anthocyanins was carried out at the column C18 (250 mm  $\times$  4,6 mm, 5  $\mu$ m particle size) at 25 °C. The chromatographic method conditions were as follows: mobile phase flow rate: 1,2 mL/min; DAD detection in the visible at 518 nm; mobile phase A: water:formic acid:acetonitrile 87:10:3; mobile phase B: water:formic acid:acetonitrile 40:10:60, with the elution program being a gradient starting from 6% to 60% to mobile phase B for 35 min. Anthocyanins were identified in correlation to the retention time, elution sequence, and UV-VIS spectral properties.

For statistical evaluation of data obtained we applied Multifactor ANOVA statistical tests type. The Multifactor ANOVA procedure is designed to construct a statistical model describing the impact of two or more categorical factors  $X_j$  on a dependent variable  $Y$ . Tests are run to determine whether or not there are significant differences between the means of  $Y$  at the different levels of the factors and whether or not there are interactions between the factors. In addition, the data may be displayed graphically in various ways, including a multiple scatter plot, a means plot, and an interaction plot.

## RESULTS AND DISCUSSIONS

Classification of the obtained wines according to their colour is similar to the order given by tracings of the absorption curves of the studied wines.

The order established by the values of total phenolic compounds of studied wines is the same with the hierarchy established based on anthocyanins' content, the order established by absorption spectrums of each wine and the one drawn by digital simulation of wines' colour (tab. 1).

Table 1

Values of chromatic parameters of wines from Fetească neagră

| No. | Grape variety        | Colour computerised simulation | L     | a     | b     | C     | H     |
|-----|----------------------|--------------------------------|-------|-------|-------|-------|-------|
| 1   | F.N.-V2-D. Bujorului |                                | 23.29 | 56.04 | 37.06 | 67.18 | 33.48 |
| 2   | F.N.-V2-Adamachi     |                                | 24.16 | 56.57 | 36.12 | 67.12 | 32.56 |
| 3   | F.N.-V1-D. Bujorului |                                | 24.25 | 57.19 | 39.18 | 69.32 | 34.41 |
| 4   | F.N.-V3-D. Bujorului |                                | 24.39 | 56.69 | 38.85 | 68.72 | 34.42 |
| 5   | F.N.-V1-Adamachi     |                                | 25.51 | 57.60 | 39.16 | 69.65 | 34.21 |
| 6   | F.N.-V2-Panciu       |                                | 27.47 | 59.69 | 40.59 | 72.18 | 34.22 |
| 7   | F.N.-V3-Adamachi     |                                | 28.63 | 59.05 | 36.35 | 69.34 | 31.62 |
| 8   | F.N.-V3-Panciu       |                                | 31.23 | 60.16 | 33.46 | 68.84 | 29.08 |
| 9   | F.N.-V1-Panciu       |                                | 33.45 | 62.50 | 37.93 | 73.11 | 31.25 |
| 10  | F.N.-V2-Uricani      |                                | 37.05 | 61.47 | 31.09 | 68.89 | 26.83 |
| 11  | F.N.-V1-Uricani      |                                | 41.55 | 61.99 | 29.71 | 68.74 | 25.60 |
| 12  | F.N.-V3-Uricani      |                                | 42.85 | 64.31 | 29.13 | 69.80 | 22.87 |
| 13  | F.N.-V4-D. Bujorului |                                | 45.68 | 62.69 | 27.49 | 68.45 | 23.68 |
| 14  | F.N.-V4-Adamachi     |                                | 56.55 | 47.91 | 22.43 | 52.90 | 25.09 |
| 15  | F.N.-V4-Panciu       |                                | 58.59 | 45.89 | 18.28 | 49.03 | 21.24 |
| 16  | F.N.-V4-Uricani      |                                | 62.26 | 44.06 | 13.78 | 46.16 | 17.36 |

Analysing the colour differences obtained with the  $\Delta E$  2000 formula, the most rigorous one, it can be observed that the majority of wines can be sensorial differenced with some exceptions: variant F.N.-V1-Dealu Bujorului and variant F.N.-V3-Dealu Bujorului and F.N.-V1-Adamachi and variant F.N.-V2-Dealu Bujorului with variant F.N.-V2-Adamachi (fig. 1).

| Nr. crt. | SORUL                   | Nr crt | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    | 16    |
|----------|-------------------------|--------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|-------|
| 1        | F.N.-V1-Dealu Bujorului | 1      | 0,00  | 0,93  | 7,39  | 15,01 | 1,07  | 1,35  | 2,47  | 11,16 | 0,17  | 3,75  | 6,34  | 16,41 | 18,95 | 29,44 | 32,16 | 36,91 |
| 2        | F.N.-V1-Adamachi        | 2      | 0,93  | 0,00  | 6,48  | 14,13 | 1,79  | 1,60  | 1,57  | 10,29 | 0,86  | 2,91  | 5,52  | 15,53 | 18,07 | 28,60 | 31,33 | 36,11 |
| 3        | F.N.-V1-Panciu          | 3      | 7,39  | 6,48  | 0,00  | 7,89  | 7,98  | 7,28  | 5,08  | 4,29  | 7,32  | 3,88  | 2,90  | 9,27  | 11,83 | 22,81 | 25,61 | 30,38 |
| 4        | F.N.-V1-R2-Uricani      | 4      | 15,01 | 14,13 | 7,89  | 0,00  | 15,42 | 14,64 | 12,88 | 4,00  | 14,92 | 11,33 | 8,93  | 1,49  | 4,02  | 15,54 | 17,98 | 21,90 |
| 5        | F.N.-V2-Dealu Bujorului | 5      | 1,07  | 1,79  | 7,98  | 15,42 | 0,00  | 0,87  | 3,33  | 11,52 | 1,08  | 4,17  | 6,03  | 16,80 | 19,35 | 29,89 | 32,56 | 37,27 |
| 6        | F.N.-V2-Adamachi        | 6      | 1,35  | 1,60  | 7,28  | 14,64 | 0,87  | 0,00  | 2,95  | 10,72 | 1,31  | 3,42  | 5,81  | 16,02 | 18,58 | 29,23 | 31,89 | 36,60 |
| 7        | F.N.-V2-Panciu          | 7      | 2,47  | 1,57  | 5,08  | 12,88 | 3,33  | 2,95  | 0,00  | 9,10  | 2,42  | 2,08  | 4,57  | 14,27 | 16,81 | 27,38 | 30,17 | 34,98 |
| 8        | F.N.-V2-R2-Uricani      | 8      | 11,16 | 10,29 | 4,29  | 4,00  | 11,52 | 10,72 | 9,10  | 0,00  | 11,07 | 7,43  | 4,95  | 5,35  | 7,99  | 19,47 | 22,09 | 26,50 |
| 9        | F.N.-V3-Dealu Bujorului | 9      | 0,17  | 0,86  | 7,32  | 14,92 | 1,08  | 1,31  | 2,42  | 11,07 | 0,00  | 3,08  | 6,26  | 16,32 | 18,86 | 29,33 | 32,05 | 36,80 |
| 10       | F.N.-V3-Adamachi        | 10     | 3,75  | 2,91  | 3,88  | 11,33 | 4,17  | 3,42  | 2,08  | 7,43  | 3,68  | 0,00  | 2,61  | 12,72 | 15,30 | 26,16 | 28,88 | 33,66 |
| 11       | F.N.-V3-Panciu          | 11     | 6,34  | 5,52  | 2,50  | 8,93  | 6,03  | 5,81  | 4,57  | 4,95  | 6,26  | 2,61  | 0,00  | 10,28 | 12,91 | 24,07 | 26,75 | 31,49 |
| 12       | F.N.-V3-R2-Uricani      | 12     | 16,41 | 15,53 | 9,27  | 1,49  | 16,80 | 16,02 | 14,27 | 5,35  | 16,32 | 12,72 | 10,28 | 0,00  | 2,74  | 14,48 | 16,79 | 20,57 |
| 13       | F.N.-V4-Dealu Bujorului | 13     | 18,95 | 18,07 | 11,83 | 4,02  | 19,35 | 18,58 | 16,81 | 7,99  | 18,86 | 15,30 | 12,91 | 2,74  | 0,00  | 11,63 | 13,81 | 17,46 |
| 14       | F.N.-V4-Adamachi        | 14     | 29,44 | 28,60 | 22,81 | 15,54 | 29,89 | 29,23 | 27,38 | 19,47 | 29,33 | 26,16 | 24,07 | 14,48 | 11,63 | 0,00  | 2,78  | 6,74  |
| 15       | F.N.-V4-Panciu          | 15     | 32,16 | 31,33 | 25,61 | 17,98 | 32,56 | 31,89 | 30,17 | 22,09 | 32,05 | 28,88 | 26,75 | 16,79 | 13,81 | 2,78  | 0,00  | 4,00  |
| 16       | F.N.-V4-R2-Uricani      | 16     | 36,91 | 36,11 | 30,38 | 21,90 | 37,27 | 36,60 | 34,98 | 26,50 | 36,80 | 33,66 | 31,49 | 20,57 | 17,46 | 6,74  | 4,00  | 0,00  |
| Nr. crt. | SORUL                   | Nr crt | 1     | 2     | 3     | 4     | 5     | 6     | 7     | 8     | 9     | 10    | 11    | 12    | 13    | 14    | 15    | 16    |

Fig. 1 - Nomogram of colour difference calculation between studied wines by  $\Delta E$  2000.

Area percentages values of each main anthocyan are the only ones based on which the studied wines can be differenced, with some exceptions like F.N.-V1-Dealu Bujorului, F.N.-V1-Adamachi and F.N.-V3-Dealu Bujorului. These presented the same value ( $0,45 \pm$  standard deviation for each wine sample) when the ratio between the sum of participation percentage of acetylated anthocyan and the sum of participation percentage of coumarate anthocyan was calculated. The allure of the three chromatograms is very similar, situation explained by the



use of the same grape variety, respectively Fetească neagră. These wines are the ones that were mentioned above as having no difference from a sensorial point of view, differences calculated with  $\Delta E$  2000 formula.

All the other variants were identified as different from a sensorial point of view, they have different values of the ratios between the sum of participation percentage of acylated anthocyanins and the sum of participation percentage of coumarate anthocyanins, as well as different values of the ratios of the area percentages' sum of monoglicosidic anthocyanins and area percentages' sums of acylated and coumarate anthocyanins. Even though the chromatograms had similar allures, they could be differentiated as shown above.

After applying statistical tests ANOVA to the different types of wine made from Fetească neagră variety, harvested in different vineyards and who have applied several maceration-fermentation technology to show any influence on the characteristics that give them colour, it showed that the vineyard of origin of these grapes exert a distinct significant influence only on the proportions of participation of two anthocyanins, delphinidin-3-monoglicozide respectively petunidin-3-monoglicozide, while the other anthocyanins not show any effect on proportions calculated.

*Table 2*

**Results of the ANOVA tests on main anthocyanins from wines obtained from Fetească neagră grape sort**

| <b>ANTHOCYANS</b>                        | <b>F - the influence of vineyard</b>                           | <b>F-crit</b> |
|--|--|---------------|
| Delfinidin-3-monoglicozide               | 6,300**  | 5,412         |
| Cyanidin-3-monoglicozide                 | 0,907 ns   | 3,259         |
| Petunidin-3-monoglicozide                | 6,229**  | 5,412         |
| Peonidin-3-monoglicozide                 | 1,310 ns   | 3,259         |
| Malvidin-3-monoglicozide                 | 1,980 ns   | 3,259         |
| Peonidin-3-monoglicozide acylated        | 3,085 ns   | 3,259         |
| Malvidin-3-monoglicozide acylated        | 2,491 ns   | 3,259         |
| Peonidin-3-monoglicozide coumarate       | 0,917 ns   | 3,259         |
| Malvidin-3-monoglicozide coumarate       | 1,178 ns   | 3,259         |
| $\Sigma gl / (\Sigma acil + \Sigma cum)$ | 0,942 ns   | 3,259         |
| $\Sigma acil / \Sigma cum$               | 0,922 ns   | 3,259         |
|  | <b>F - the influence of maceration-fermentation technology</b> | <b>F-crit</b> |
| Delfinidin-3-monoglicozide               | 18,095*  | 10,804        |
| Cyanidin-3-monoglicozide                 | 3,498***   | 3,490         |
| Petunidin-3-monoglicozide                | 17,443*  | 10,804        |
| Peonidin-3-monoglicozide                 | 9,216**  | 5,953         |
| Malvidin-3-monoglicozide                 | 6,881**  | 5,953         |
| Peonidin-3-monoglicozide acylated        | 8,909**  | 5,953         |
| Malvidin-3-monoglicozide acylated        | 0,162 ns   | 3,490         |
| Peonidin-3-monoglicozide coumarate       | 6,428**  | 5,953         |
| Malvidin-3-monoglicozide coumarate       | 4,626***   | 3,490         |
| $\Sigma gl / (\Sigma acil + \Sigma cum)$ | 0,800 ns   | 3,490         |
| $\Sigma acil / \Sigma cum$               | 1,073 ns   | 3,490         |

ns - no statistically significant influence where P-value  $\square$  p = 0.05;

\* - Very significant statistical influence where P-value  $\square$  p = 0.001;

\*\* - Distinct significant statistical influence where P-value  $\square$  p = 0.01;

\*\*\* - Significant statistical influence where P-value  $\square$  p = 0.05.

When evaluating the influence of maceration-fermentation technology on participation percentage values of anthocyanins, it is noted that the calculated proportions reports on anthocyanins malvidin-3-acylated monoglycozide not exercise any significant influence, the rest on other anthocyanins were showed statistical significant influence (cyanidin-3-monoglycozide and malvidin-3-monoglycozide coumarate), distinct significant influence (peonidin-3-monoglycozide, malvidin-3-monoglycozide, peonidin-3-acylated and peonidin-3-coumarate), and very significant influence (delphinidin-3-monoglycozid and petunidin-3-monoglycozid) (tab. 2).

## CONCLUSIONS

Considering the use of maceration fermentation techniques adequate to composition characteristics and to sanitary parameters of the crop, wines that “simulate” very well the colour prints of wines obtained from other vineyards or other maceration-fermentation technologies could be obtained.

A clear influence of the origin of the harvest is registered on the percentage values of the anthocyanins in Fetească neagră wines. These observations certify the fact that the compared wines are different from a maceration-fermentation technological point of view and also from the origin area of the grapes (viticultural centers or different vineyards).

As a general conclusion, testing the percentage value of petunidin-3-monoglycoside is the main factor that can differentiate wines according to origin vineyard or viticulture centre.

Of course, there are also other instrumental determinations, that, together with sensorial evaluations are useful and can generate high precision results, but they cannot be used by themselves. Wine’s colour appreciation is strongly connected to all factors that can influence the physical-chemical and sensorial state of the studied wines.

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# COMPARATIVE STUDIES REGARDING THE INFLUENCE OF FILM TREATMENTS ON APPLE FRUIT QUALITY OF GENEROUS AND STARKRIMSON VARIETIES STORED REFRIGERATED

## STUDII COMPARATIVE PRIVIND INFLUENȚA UNOR TRATAMENTE PELICULARE ASUPRA CALITĂȚII FRUCTELOR DE MĂR DIN SOIURILE GENEROS ȘI STARKRIMSON PĂSTRATE FRIGORIFIC

*ANGHEL Roxana Mihaela*<sup>1</sup>  
e-mail: roxanam77@yahoo.fr

**Abstract.** Among the newest procedures to prevent quality degradation during storage of horticultural products include the treatments with coating film. Films are edible and provide external tissue fortifications protecting the original product structure and texture, can prevent loss of moisture, are semi-permeable to gas, allowing controlled gas exchange between the product and the external environment. Also, provides a sterile surface, preventing losses due to pathogen attack. After harvest, before refrigerated at 2°C, the apples of varieties Generous and Starkrimson were treated with three types of film: film of wax, film of carboxymethyl cellulose and chitosan film. Physico-chemical analysis, after seven months of cold storage, have demonstrated that fruits treated has a higher quality compared to the control, with better firmness and high dry soluble substance and organic acids.

**Key words:** statistical analysis, edible films, fruits quality

**Rezumat.** Printre cele mai noi procedeele de prevenire a degradării calității produselor horticole pe timpul depozitării se numără și tratamentele de acoperire a suprafeței cu peliculă inertă. Peliculele sunt comestibile și pot oferi fortificații ale țesuturilor externe, protejând structura și textura inițială a produsului, pot preveni pierderile de umiditate, sunt semipermeabile pentru gaze, permițând schimbul controlat de gaze dintre produs și mediul exterior. De asemenea oferă o sterilitate de suprafață, prevenind pierderi importante datorită atacului de agenți patogeni. După recoltare, înainte de a fi introduse în celulele frigorifice, la temperatura de 2°C, merele din soiurile Generos și Starkrimson au fost tratate cu trei tipuri de peliculă: peliculă de ceară, peliculă din carboximetilceluloză și peliculă din chitosan. Analizele fizico-chimice efectuate după șapte luni de păstrare frigorifică au demonstrat că fructele tratate pelicular prezintă o calitate superioară față de martor, printr-o fermitate mai bună și un conținut ridicat în substanță uscată solubilă și acizi organici.

**Cuvinte cheie:** analiza statistică, pelicule comestibile, calitatea fructelor

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

## INTRODUCTION

The quality of the apples stored refrigerated is given, mainly, by physical-chemical parameters: acidity, dry soluble substance, structo-textural firmness, respiration intensity, degree of hydrolysis of starch (Anghel, 2011).

Values give us clues about the food and dietetics value of fruit studied, at the end of the storage period (Anghel, 2011).

In this study we proposed to analyze the influence of films treatments on quality of two varieties apple fruit, by statistical interpretation data from physico-chemical analysis, concerning the preserved fruit store.

By comparing the results obtained shall be established if the treatment carried out was of any significance or not statistically.

## MATERIAL AND METHOD

The analyzed material was represented by the results of chemical analysis and physical measurements of two varieties of apple, Starkrimson and Generos, stored during by October 2010-April 2011 at the cold storage Sârca, SCDPP Iasi (Anghel, 2011).

Fruits were applied a treatment, with the following films: wax, chitosan and cellulose from carboxymethyl.

These variants were made a series of chemical and physical measurements, in order to assess the quality of fruit preserve, both during and at the end of this period.

The results obtained from these determinations have been processed statistically, with Fisher test, variance analysis – using ANOVA, in Excel application. This application is used for testing significant difference between multiple environments (Oancea, 2007).

Analysis of variance aims to analyse any differences that arise between the variants considered: to study the effect of film treatment on the quality of fruit, compared with blank variant (Jităreanu, 2006).

Statistical analysis was performed on the results of the following qualitative parameters: acidity, dry soluble substance and structo-textural firmness.

## RESULTS AND DISCUSSIONS

As a result of the determinations in April 2011 (tab. 1), the values obtained for quality parameter dry soluble substance content ( $^{\circ}\text{Bx}$ ) were:

**The dry soluble substance content ( $^{\circ}\text{Bx}$ ) fruit of the Generos variety**

*Table 1*

| <b>Variant</b>               | <b>Repetition 1</b> | <b>Repetition 2</b> | <b>Repetition 3</b> |
|------------------------------|---------------------|---------------------|---------------------|
| Blank                        | 12,0                | 11,9                | 12,1                |
| Treatment with wax film      | 14,0                | 13,9                | 14,1                |
| Treatment with chitosan film | 13,6                | 13,5                | 13,7                |
| Treatment with CMC film      | 13,8                | 13,7                | 13,9                |

Influence of films treatments on the dry soluble residue content, at the end of the period of storage is represented as follows:

*Table 2*

**Statistical results treatment of matching the values of dry soluble substance in the apple of Generos variety**

| Variant                      | F   | F crit   | P-value | Influence |
|------------------------------|-----|----------|---------|-----------|
| Treatment with wax film      | 600 | 7,708647 | 0,00001 | ***       |
| Treatment with chitosan film | 384 | 7,708647 | 0,00003 | ***       |
| Treatment with CMC film      | 486 | 7,708647 | 0,00002 | ***       |

\*\*\* - very significant influence

As a result of the determinations in April 2011 (tab. 3), the values obtained for quality parameter titrable acidity (g mailc acid/100 g product) were:

*Table 3*

**The titrable acidity content (g mailc acid/100 g product) fruit of the Generos variety**

| Variant                      | Repetition 1 | Repetition 2 | Repetition 3 |
|------------------------------|--------------|--------------|--------------|
| Blank                        | 0,36         | 0,35         | 0,37         |
| Treatment with wax film      | 0,40         | 0,39         | 0,41         |
| Treatment with chitosan film | 0,38         | 0,39         | 0,37         |
| Treatment with CMC film      | 0,39         | 0,38         | 0,40         |

As regards the content of organic acids (tab. 4) noted that the operations carried out in this variety had a meaning less important.

*Table 4*

**Statistical results treatment of matching the values of titrable acidity in the apple of Generos variety**

| Variant                      | F    | F crit   | P-value  | Influence |
|------------------------------|------|----------|----------|-----------|
| Treatment with wax film      | 24   | 7,708647 | 0,00805  | **        |
| Treatment with chitosan film | 6    | 7,708647 | 0,070484 | Ns        |
| Treatment with CMC film      | 13,5 | 7,708647 | 0,021312 | *         |

\*\* - distinctly significant influence

Ns – insignificant influence

\* - significant influence

The most effective treatment proved to be the film of wax, with a distinctly significant influence, followed by treatment with carboxymethyl cellulose film that had a significant influence. Treatment with chitosan film showed no statistical significant influence, the organic acids content of apple fruit from variety Generous.

Apple's structo-textural firmness (UP), measured in the last mount of cold storage (tab. 5) presented the following values:

*Table 5*

**Structo-textural firmness values (UP) of fruit from Generos variety**

| Variant                      | Repetition 1 | Repetition 2 | Repetition 3 |
|------------------------------|--------------|--------------|--------------|
| Blank                        | 42           | 41           | 43           |
| Treatment with wax film      | 38           | 39           | 37           |
| Treatment with chitosan film | 38           | 39           | 37           |
| Treatment with CMC film      | 39           | 40           | 38           |

Wax film and chitosan films treatments (tab. 6) were statistically distinct significant differences from the blank, while treatment with carboxymethyl cellulose film had only a significant influence on fruit firmness of the apple of Generos variety.

Table 6

**Statistical results treatment of matching the values of structo-textural firmness in the apple of Generos variety**

| Variant                      | F    | F crit   | P-value  | Influence |
|------------------------------|------|----------|----------|-----------|
| Treatment with wax film      | 24   | 7,708647 | 0,00805  | **        |
| Treatment with chitosan film | 24   | 7,708647 | 0,00805  | **        |
| Treatment with CMC film      | 13,5 | 7,708647 | 0,021312 | *         |

\*\* - distinctly significant influence

\* - significant influence

The fruit of Starkrimson variety (tab. 7) were obtained the following values of soluble dried substance (<sup>0</sup>Bx):

Table 7

**The dry soluble substance content (<sup>0</sup>Bx) fruit of the Starkrimson variety**

| Variant                      | Repetition 1 | Repetition 2 | Repetition 3 |
|------------------------------|--------------|--------------|--------------|
| Blank                        | 12,4         | 12,3         | 12,5         |
| Treatment with wax film      | 14,6         | 14,7         | 14,5         |
| Treatment with chitosan film | 14,4         | 14,2         | 14,6         |
| Treatment with CMC film      | 14,4         | 14,3         | 14,5         |

Table 8

**Statistical results treatment of matching the values of dry soluble substance in the apple of Sarkrimson variety**

| Variant                      | F   | F crit   | P-value   | Influenta |
|------------------------------|-----|----------|-----------|-----------|
| Treatment with wax film      | 726 | 7,708647 | 0,0000112 | ***       |
| Treatment with chitosan film | 600 | 7,708647 | 0,0000164 | ***       |
| Treatment with CMC film      | 600 | 7,708647 | 0,0000164 | ***       |

\*\*\* - very significant influence

For fruit of Starkrimson variety (tab 8) the influence of film treatment on dry soluble substance content was highly statistically significant.

Total organic acids content (g malic acid /100 g product) Starkrimson variety of fruits (tab 9) is as follows:

Table 9

**The titrable acidity content (g malic acid/100 g product) fruit of the Starkrimson variety**

| Variant                      | Repetition 1 | Repetition 2 | Repetition 3 |
|------------------------------|--------------|--------------|--------------|
| Blank                        | 0,20         | 0,21         | 0,19         |
| Treatment with wax film      | 0,25         | 0,24         | 0,26         |
| Treatment with chitosan film | 0,21         | 0,20         | 0,22         |
| Treatment with CMC film      | 0,22         | 0,23         | 0,21         |

Film wax treatment had a distinct significant influence on organic acids content of apple fruit from Starkrimson variety (tab.10).

The other two treatments analyzed - film of chitosan, respectively film carboxymethyl cellulose, showed statistically significant influences.

Table 10

**Statistical results treatment of matching the values of titrable acidity in the apple of Sarkrimson variety**

| Variant                      | F    | F crit   | P-value  | Influence |
|------------------------------|------|----------|----------|-----------|
| Treatment with wax film      | 37,5 | 7,708647 | 0,003602 | **        |
| Treatment with chitosan film | 1,5  | 7,708647 | 0,287864 | Ns        |
| Treatment with CMC film      | 6    | 7,708647 | 0,070484 | Ns        |

\*\* - distinctly significant influence

Ns – insignificant influence

The structo-textural firmness of the fruit from variety Starkrimson in cold storage last month (tab. 11) has the following values:

Table 11

**Structo-textural firmness values (UP) of fruit from Starkrimson variety**

| Variant                      | Repetition 1 | Repetition 2 | Repetition 3 |
|------------------------------|--------------|--------------|--------------|
| Blank                        | 38           | 39           | 37           |
| Treatment with wax film      | 33           | 32           | 34           |
| Treatment with chitosan film | 35           | 36           | 34           |
| Treatment with CMC film      | 37           | 36           | 38           |

Table 12

**Statistical results treatment of matching the values of structo-textural firmness in the apple of Sarkrimson variety**

| Variant                      | F    | F crit   | P-value  | Influence |
|------------------------------|------|----------|----------|-----------|
| Treatment with wax film      | 37,5 | 7,708647 | 0,003602 | **        |
| Treatment with chitosan film | 13,5 | 7,708647 | 0,021312 | *         |
| Treatment with CMC film      | 1,5  | 7,708647 | 0,287864 | Ns        |

\*\* - distinctly significant influence

\* - significant influence

Ns – insignificant influence

In the case structo-textural firmness of apple fruit (tab. 6) we see that the applied treatments have varying degrees of influence.

Treatment with wax film has the most influence on this parameter.

## CONCLUSIONS

1. All treatments were generally applied significant influence on apple fruit studied, regardless of the variety of origin;

2. The soluble solids content to say that these treatments had the greatest impact, statistical analysis showing that all films have very significantly influenced the content of this parameter on both varieties studied;

3. The organic acid content, film treatments had better effect in Generous variety;

4. Regarding structo-textural firmness of apple fruit was significantly influenced significantly distinct, except carboxymethyl cellulose treatment in Starkrimson variety;

5. Generally, we can say that film treatments were applied different effectiveness depending on the variety, so the variety Generous relatively good results were obtained for all treatments, but the variety Starkrimson carboxymethyl cellulose film had no statistical influence than in terms of soluble substances content.

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# STUDIES REGARDING THE INFLUENCE OF HEAT WATER TREATMENT ON APPLE FRUIT QUALITY OF GENEROUS AND STARKRIMSON VARIETIES STORED REFRIGERATED

## STUDII PRIVIND INFLUENȚA TRATAMENTUL TERMIC CU APĂ CALDĂ ASUPRA CALITĂȚII FRUCTELOR DE MĂR DIN SOIURILE GENEROS ȘI STARKRIMSON PĂSTRATE FRIGORIFIC

**ANGHEL Roxana Mihaela<sup>1</sup>**  
e-mail: roxanam77@yahoo.fr

**Abstract.** Treatment with hot water is used by increasing numbers of organic fruit producers, to prevent the development of pathogens in storage. Also, by exposing the fruit to high temperatures, are diminishes some maturation processes, was reduced ethylene production due to inhibition of enzymes. Before refrigerated at 2<sup>0</sup>C, the apples of varieties Generos and Starkrimson were treated hot water. For not suffer a thermal shock, fruits were initially immersed in water at 30<sup>0</sup>C for 5 minutes, then in water at 50<sup>0</sup>C for 3 minutes. After seven months of cold storage were analyzed physico-chemically, the results were compared with untreated fruit. Qualitative parameters analyzed indicated that treatment with hot water has a significant influence in maintaining quality of apple fruits in cold storage.

**Key words:** hot water treatment, quality fruits, cold storage

**Rezumat.** Tratamentul termic cu apă caldă este utilizat în proporție tot mai mare de către producătorii de fructe ecologice, pentru a preveni dezvoltarea de agenți patogeni în depozite. De asemenea, prin expunerea fructelor la temperaturi ridicate, se diminuează unele procese de maturare, fiind redusă producția de etilenă, datorită inhibării unor enzime. Înainte de a fi introduse în celulele frigorifice la temperatura de 2<sup>0</sup>C, fructele din soiurile Generos și Starkrimson au fost supuse unui tratament termic cu apă caldă. Pentru a nu suferi un șoc termic, fructele au fost imersate inițial în apă la temperatura de 30<sup>0</sup>C timp de 5 minute, apoi în apă la temperatura de 50<sup>0</sup>C, timp de 3 minute. După șapte luni de păstrare frigorifică au fost analizate fizico-chimic, rezultatele fiind comparate cu fructele netratate. Parametrii calitativi analizați au indicat faptul că tratamentul termic cu apă caldă a avut o influență deosebită în menținerea calității merelor în depozitul frigorific.

**Cuvinte cheie:** tratament cu apă caldă, calitate fructe, păstrare frigorifică

### INTRODUCTION

The quality of the apples stored refrigerated is estimated by physical-chemical parameters: acidity, dry soluble substance, structo-textural firmness, respiration intensity, degree of hydrolysis of starch (Anghel, 2011).

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

The exposure of fruit to high temperature, for short time, is to alleviate some maturation processes.

Ethylene production is reduced due to inhibition of enzymes. Even if, by this treatment, the fruit undergoes a mild heat shock, at the end of the storage period has a high quality fruit from the blank.

In this study we proposed to analyze the influence of heat treatment with hot water on quality of two varieties apple fruit, by statistical interpretation data from physico-chemical analysis, concerning the preserved fruit store.

By comparing the results obtained shall be established if the treatment carried out was of any significance or not statistically.

## MATERIAL AND METHOD

The analyzed material was represented by the results of chemical analysis and physical measurements of two varieties of apple, Starkrimson and Generos, stored during by October 2010-April 2011 at the cold storage Sârca, SCDPP Iasi. (Anghel, 2011)

Fruits were applied heat treatment with hot water. For not suffer a heat stroke, fruits were initially immersed in water at 30°C for 5 minutes, then in water at 50°C for 3 minutes.

After seven months of cold storage were analyzed physico-chemically, the results were compared with untreated fruit. (Anghel, 2011)

The results obtained from these determinations have been processed statistically, with Fisher test, variance analysis – using ANOVA, in Excel application. This application is used for testing significant difference between multiple environments. (Oancea, 2007)

Analysis of variance aims to analyse any differences that arise between the variants considered: to study the effect of film treatment on the quality of fruit, compared with blank variant. (Jităreanu, 2006).

Statistical analysis was performed on the results of the following qualitative parameters: acidity, dry soluble substance and structo-texturale firmness.

## RESULTS AND DISCUSSIONS

After measurements in April 2011 (tab. 1 and tab. 3), values for quality parameters analyzed, respectively soluble dry substance (<sup>0</sup>Bx), total acidity (g malic acid /100 g product) and structo-textural firmness (UP) were as follows:

Table 1

**Results obtained in the last month of storage in storage of fruit from Generos variety**

| Variant  | Repetition 1 | Repetition 2 | Repetition 3 |
|--|--------------|--------------|--------------|
| <b>Soluble dry substance content (<sup>0</sup>Bx)</b>            |              |              |              |
| Blank  | 12,0         | 11,9         | 12,1         |
| Heat treatment with hot water                                    | 14,0         | 13,9         | 14,1         |
| <b>Titration acidity content ( g malic acid /100 g product )</b> |              |              |              |
| Blank  | 0,36         | 0,35         | 0,37         |
| Heat treatment with hot water                                    | 0,39         | 0,40         | 0,38         |
| <b>Structo – textural firmness value (UP)</b>                    |              |              |              |
| Blank  | 42           | 41           | 43           |
| Heat treatment with hot water                                    | 39           | 40           | 38           |

Influence of hot water treatment on fruit quality of Generos variety is presented in tab. 2:

Table 2

**ANOVA test results concerning the influence of hot water treatment of apple fruit quality parameters of the Generos variety**

| Quality parameter           | F    | F crit    | P-value  | Influence | Significance     |
|-----------------------------|------|-----------|----------|-----------|------------------|
| Soluble dry substance       | 600  | 7,7086497 | 0,00001  | ***       | very significant |
| Titration acidity           | 13,5 | 7,708647  | 0,021312 | *         | significant      |
| Structo – textural firmness | 13,5 | 7,708647  | 0,021312 | *         | significant      |

Data analysis concerning the influence of heat treatment with hot water on the apple fruit variety Generos (tab 2) reveals that this treatment had a very significant influence on the soluble dry substance content, the coverage probability of 99.9 %, the null hypothesis is rejected because p-value <0.001.

Regarding the content of organic acids and Structo-textural firmness, we can say that hot water treatment applied to apple fruit had a significant influence at a degree of trust of 95% of the test (because p value = 0, 021 312 <p = 0,05). In these cases the null hypothesis was rejected.

Table 3

**Results obtained in the last month of storage in storage of fruit from Starkrimson variety**

| Variant   | Repetition 1 | Repetition 2 | Repetition 3 |
|---|--------------|--------------|--------------|
| <b>Soluble dry substance content (°Bx)</b>                      |              |              |              |
| Blank   | 12,4         | 12,3         | 12,5         |
| Heat treatment with hot water                                   | 14,8         | 14,7         | 14,9         |
| <b>Titration acidity content ( g acid malic/100 g product )</b> |              |              |              |
| Blank   | 0,20         | 0,21         | 0,19         |
| Heat treatment with hot water                                   | 0,23         | 0,22         | 0,24         |
| <b>Structo – textural firmness value (UP)</b>                   |              |              |              |
| Blank   | 38           | 39           | 37           |
| Heat treatment with hot water                                   | 34           | 33           | 35           |

Table 4

**ANOVA test results concerning the influence of hot water treatment of apple fruit quality parameters of the Starkrimson variety**

| Quality parameter           | F    | F crit   | P-value    | Influence | Significance           |
|-----------------------------|------|----------|------------|-----------|------------------------|
| Soluble dry substance       | 864  | 7,708647 | 0,00000797 | ***       | very significant       |
| Titration acidity           | 13,5 | 7,708647 | 0,021312   | *         | significant            |
| Structo – textural firmness | 24   | 7,708647 | 0,00805    | **        | distinctly significant |

In the case the analysis of the influence of heat treatment with hot water of fruits belonging Starkrimson variety (tab. 4), we can see that for the three quality parameters studied were obtained different results.

Therefore, on the soluble dry substance content, the treatment was, of statistical point of view, very significant influence, with a high degree of probability, the null hypothesis is rejected as  $p$  value  $<0.001$ .

Of content in the organic acids, heat treatment had a significant influence on the coverage probability of 95% ( $p$  value = 0.021312 as  $<p = 0.05$ ). Null hypothesis was rejected.

Was presented a very significant statistical influence of heat treatment on the firmness of fruit. Null hypothesis was rejected at a probability of 99% coverage.

## CONCLUSIONS

1. From the statistical analysis performed can be seen that heat treatment with hot water had no influence on the quality parameters studied.

2. Null hypothesis was rejected for all parameters studied the fruit of both varieties, at different degrees of trust of the test.

3. Regarding the treatment effect on content to soluble dry substance, we can say that the point was very significant statistically, the highest trust this test (99.9%), whatever the variety of which came from fruits.

4. Concerning of content to the organic acids, the influence was significant at a probability of 95% for both varieties studied

5. The influence of hot water over structo-textural firmness was different, depending on variety.

6. To the Generos variety is observed significant influence on the coverage at 95% probability and a distinct significant influence of Starkrimson variety, at a 99% probability of coverage.

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# ANALYSIS AND CHARACTERIZATION OF ANTHOCYANINS IN *MORUS NIGRA* L. FRUITS, HARVESTED FROM IASSY AREA, ROMANIA

## ANALIZA ȘI CARACTERIZAREA CONȚINUTULUI ÎN ANTOCIANI AL FRUCTELOR SPECIEI *MORUS NIGRA* L. RECOLTATE DIN ZONA IAȘI, ROMÂNIA

FILIMON V. R.<sup>1</sup>, NICULAU M.<sup>2</sup>, COȚOVANU Roxana<sup>1</sup>  
e-mail: razvan\_f80@yahoo.com

**Abstract.** Used for centuries in folk medicine worldwide, black mulberries have an important content of anthocyanins, still unused to their real technological potential. Were analyzed the ethanolic extracts of *Morus nigra* L. fruit (mulberry) harvested from the experimental field of UASVM Iași, being determined spectrophotometrically total content of anthocyanins and phenolic compounds. The anthocyanin profile of the extracts was performed by HPLC-DAD technique and for an objective assessment of extracts color, were calculated chromatic parameters ( $L^*a^*b^*$ ), based on VIS spectrum. It was found a high anthocyanins content ( $159.26 \pm 0.17$  mg/100 g fruit, expressed as equivalent of cy-3-gl), the main representative of anthocyanin profile was cyanidin-3-O-glucoside, with a rate of participation over 70% of total area. Knowing the type and quantities of pigment available in horticultural products, can be appreciate the technical and functional qualities (preventive and curative) of fruits, and also the structure and stability of the color obtained.

**Keywords:** *Morus nigra* L., Iassy area, anthocyanins, HPLC-DAD, chromatic parameters.

**Rezumat.** Folosite în medicina tradițională mondială de secole, dudulele negre au un conținut important în antociani, încă neutilizat la adevăratul potențial tehnologic. Au fost analizate extractele etanolice obținute din fructele speciei *Morus nigra* L. provenite din câmpul experimental al USAMV Iași, fiind determinate spectrofotometric cantitățile totale de antociani și compuși fenolici. De asemenea, a fost realizat profilul antocianic al extractelor prin tehnica HPLC-DAD, iar pe baza spectrului VIS au fost calculați parametrii cromatici ( $L^*a^*b^*$ ) ai acestora, pentru o evaluare obiectivă a culorii extractelor. A fost identificat un conținut ridicat în antociani ( $159.26 \pm 0.17$  mg cianidină-3-O-glucozid/100 g fruct.), principalul reprezentant al profilului fiind cianidină-3-O-glucozid, cu un procent de participare de peste 70 % din aria totală corespunzătoare antocianilor. Cunoscând tipul și cantitățile de pigment disponibile în produsele horticoale, se pot aprecia calitățile tehnologice și funcționale (preventive și curative) ale fructelor, dar și structura și stabilitatea culorii obținute.

**Cuvinte cheie:** *Morus nigra* L., arealul Iași, antociani, HPLC-DAD, parametri cromatici.

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

<sup>2</sup>Oenology Research Center – Iasi Branch of the Romanian Academy, Romania

## INTRODUCTION

Interest in natural pigments increased gradually in the last decade, as a consequence of global legislation in a permanent update and consumers reticence to synthetic food colorants. It became an immediate need to identify new sources of vegetal pigments, non-toxic and non-polluting such as anthocyanins, phenolic compounds that belong to flavonoid class, responsible for the red-blue-violet nuances of fruits, flowers, and certain vegetables. Anthocyanins are now considered the most reliable alternative in replacement of some red-blue synthetic food colorants (Liu et al., 2004).

Anthocyanins extracted from the fruits of *Morus nigra* L. currently have limited applications in food industry, although possess a very good stability (at 80 °C), being used as color additives in confectionery products or carbonated beverages (Mazza and Miniati, 1993), but beyond their coloring capacity, they were reported as owning a large number of beneficial effects on the human body being considered anti-cancer, vaso-protective, anti-inflammatory and neuro-protective agents (Chen et al., 2006, Wu et al., 2011).

Black mulberries contain significant amounts of anthocyanins, with values within the range of 25.3 and 83.0 mg cy-3-gl/100 g fresh fruit (Yang and Tsai, 1994; Ozgen et al., 2009) or between 147.68 and 2725.46 mg cy-3-gl/L juice (Liu et al., 2004). Were identified in the black mulberries extract four main anthocyanins, cyanidin-3-O-rutinoside (cy-3-rut), cyanidin-3-O-glucoside (cy-3-gl), pelargonidin-3-O-rutinoside (pg-3-rut), pelargonidin-3-O-glucoside (pg-3-gl) (Du et al., 2008; Qin et al., 2010). It is obvious that, whatever the area of origin or method of analysis was, cy-3-gl remains the main pigment in *Morus nigra* L.

## MATERIAL AND METHOD

Fruits were harvested from the experimental field of the University of Agricultural Sciences and Veterinary Medicine (UASVM) Iassy, V. Adamachi farm, at maturity of consumption, identified as the moment when the fruit turn its color from green to dark purple, 28-30 July, 2010 (Yang and Tsai, 1994; Özgen et al., 2009).

Were determined certain physico-chemical properties of the fruits at harvest: the average mass of a fruit (g), the moisture content (%), soluble dry substance (°Bx), titratable acidity, ascorbic acid (titration with 2,6-DI), pH, reducing sugars (Schoorl method), total dry substance (%), as factors that influence the anthocyanin content.

Mulberries were stored at  $-18 \pm 2^\circ\text{C}$  until extraction (7 days), anthocyanin transformation being considered as minimal for fruits kept in a frozen state (Mazza, Miniati, 1993). Were performed two phases of extraction at an interval of 12 hours, until the depletion of vegetal material. Extraction system used was ethanol-HCl-water (96:1:3), the final ratio between plant material and solvent was 1:5 (m/v) (5 g fruits/25 mL solvent). Determination of total monomeric anthocyanins content (ACY) was performed by the pH differential method (J. AOAC Int., 2005):

$$A (\text{absorbance}) = (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 0.68} - (A_{520 \text{ nm}} - A_{700 \text{ nm}})_{\text{pH } 3.56};$$

Results were expressed as mg equivalent cy-3-gl/100 g fruit:

$$\text{ACY (mg cy-3-gl, mg/L)} = (A \times \text{MW} \times \text{DF} \times 10^3) / (\epsilon \times l);$$

where: ACY - total anthocyanin content (cy-3-gl equivalents, mg/L), A - absorbance, MW - molecular weight (449.2 g/mol for cy-3-gl); DF - dilution factor; l - optical pathlength (1 cm);  $\epsilon$  - molar extinction coefficient ( $26\ 900\text{L}\cdot\text{mol}^{-1}\cdot\text{cm}^{-1}$  for cy-3-gl);  $10^3$  - factor for conversion from g to mg.

To obtain the total phenolic compounds content (TPC) was used Folin-Ciocalteu photocolometric method, by measuring absorbance at  $\lambda_{\text{max}}$  (765 nm) (Singleton and Rossi, 1965), results were expressed in grams of gallic acid equivalents (g GAE) /100g fresh fruits. Measurements were made using a UV-VIS spectrophotometer, Analytik Jena Specord 200, the results represent the average of three determinations, having calculated standard deviation ( $\pm$ ), and the coefficient of variability (CV) or uniformity of the data obtained.

Measuring colors in CIE-76 system consists in determination of  $L^*$  (brightness coordinate or psychometric clarity),  $a^*$  (red-green coordinate) and  $b^*$  (yellow-blue coordinate), and parameters: C (chromaticity or color saturation), H (tone), Intensity ( $A_{420}+A_{520}+A_{620}$ ) and color tint ( $A_{420}/A_{520}$ ) (OIV, 2012). For an objective assessment, extract color was simulated using the software Digital Colour Atlas<sup>®</sup> 3.0 Demo version.

Using a Shimadzu LC 20 liquid chromatograph, with a Hypersil ODS C18 separation column (25 cm length) at 20 °C, was performed the separation of anthocyanins. Elution was achieved at a flow rate of 1.2 mL/min. As eluent A was used H<sub>2</sub>O:HCOOH:CH<sub>3</sub>CN (87:10:3) and as eluent B, H<sub>2</sub>O:HCOOH:CH<sub>3</sub>CN (40:10:50), increasing eluent B from 6% to 60%. Anthocyanin compounds were individualized with a diode array detector (DAD) Shimadzu at  $\lambda$ 518 nm, and their identification was made according to Ozgen et al., 2009 and Qin et al., 2010.

## RESULTS AND DISCUSSIONS

Physico-chemical properties of *Morus nigra* fruits, showed lower values than those offered by the literature, regarding the titratable acidity, at a pH 3.01 and a content of reducing sugars in juice of 22.7 mg glucose/100 g fruits (tab. 1).

Table 1

The physico-chemical properties of black mulberries samples

| Sample             | M. fr.*<br>(g)     | M.*<br>(%)          | T. ac.*<br>(g m. a.) | Asc. ac.*<br>(mg %) | Rd. sg.*<br>(gl. %) | pH<br>(units)      | SDS*<br>(°Bx)       | TDS*<br>(%)         |
|--------------------|--------------------|---------------------|----------------------|---------------------|---------------------|--------------------|---------------------|---------------------|
| <i>Morus nigra</i> | 1,72<br>$\pm 0,15$ | 81,62<br>$\pm 1,12$ | 0,56<br>$\pm 0,02$   | 14,84<br>$\pm 0,54$ | 22,72<br>$\pm 0,32$ | 3,01<br>$\pm 0,03$ | 12,92<br>$\pm 0,11$ | 18,38<br>$\pm 0,22$ |

**Legend:**\*M. fr. - average mass of fruit; M (%) - moisture; T. ac. (g m.a.) - titratable acidity (g malic acid /100g); Asc. ac. (mg %) - ascorbic acid (mg/100 g); Rd. sg. (gl. %) - reducing sugars (mg glucose/100 g fruits); SDS (°Bx) - soluble dry substance (°Brix); TDS (%) - total dry substance (%); **CV<10; p-value>0,05.**

The content of ascorbic acid was considered low compared with other species rich in anthocyanins, only 14.84 mg/100 g fruits. The coefficient of variability (CV) was in all cases less than 10 (CV<10), indicating that the data are homogeneous and behave uniform according to the studied parameters.

After the interpretation of the absorption spectra at  $\lambda$  520 nm, specific of anthocyanins (fig. 1) and  $\lambda$  765 nm for phenolic compounds with reducing properties, were determined total quantities of monomeric anthocyanins (ACY) and total phenolic compounds (TPC), summarized in table 2. Average values of these parameters were found to be higher than the data available in the literature for this specie, presented in the introductory chapter.

Percentage of total anthocyanins participation at total phenolic compounds, was 14.61% (tab. 2). This value is important in the context of evaluation on the biologically active capacity of *Morus nigra*L. fruits.

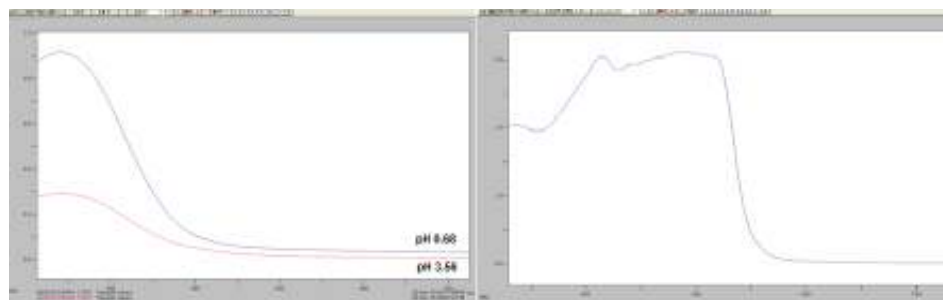


Fig. 1-Overlay of absorbance spectra at  $\lambda$  520 nm, pH 0.68 and 3.56

Fig. 2-VIS absorption spectrum of the black mulberry extract

Table 2

ACY and TPC values of fresh black mulberry extract

| Sample           | ACY<br>(mg cy-3-gl/100g) | TPC<br>(g GAE/100g)      | ACY of TPC<br>(%) |
|------------------|--------------------------|--------------------------|-------------------|
| Black mulberries | 159.26±1.17 <sup>a</sup> | 1.0902±0.05 <sup>a</sup> | 14.61             |

CV<5; <sup>a</sup>p-value>0.05

To determine the stability of extracts and the anthocyanin losses by enzymatic and oxidative degradation, spectrophotometric measurements were repeated after 12 months storage of extracts at low temperature (4 °C) and in the dark. Mean values calculated are presented in table 3.

It is noted that the recorded values revealed a loss of anthocyanin pigments by degradation of 25.78 % and only 14.37 % for total phenolic compounds. These values are considered to be at a low level, indicating the high stability of mulberry anthocyanins. Degradation of anthocyanins can be attributed partially to acidic hydrolysis that may occur in the extract as a result of low pH (<1.0).

Table 3

ACY and TPC values of black mulberry extract after 12 months storage

| Sample           | ACY<br>(mg cy-3-gl/100g) | TPC<br>(g GAE/100g)      | ACY of TPC<br>(%) |
|------------------|--------------------------|--------------------------|-------------------|
| Black mulberries | 118.20±0.84 <sup>a</sup> | 0.9335±0.08 <sup>a</sup> | 10.32             |

CV<5; <sup>a</sup>p-value>0.05

Anthocyanin profile of the extract includes four main anthocyanins, in which aglicons are represented by cyanidin and pelargonidin (fig. 3). Anthocyanin that participated in the highest proportion at black mulberry profile was cy-3-rut (70.30%), followed by cy-3-gl (29.65%), the others identified compounds influence in a very small proportion the anthocyanins area (tab. 4). By the analysis of UV-VIS spectra of peaks, obtained through chromatographic analysis, can be observed the increase in



spectral line at  $\lambda$  270-280 nm, specific to all phenolic compounds and at  $\lambda$  515-530 nm, specific only to anthocyanin compounds (fig. 4, fig. 5).

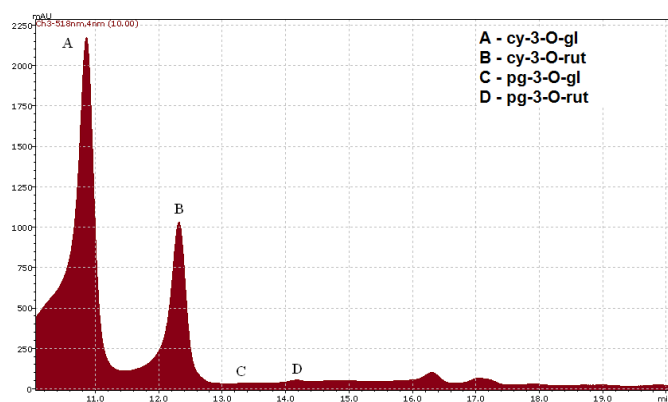


Fig. 3-HPLC chromatogram of black mulberries ethanolic extract

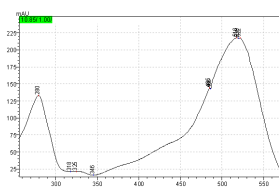


Fig. 4-UV-VIS spectra of cy-3-gl

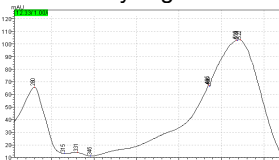


Fig. 5- UV-VIS spectra of cy-3-rut

Table 4

Percentages of area corresponding to anthocyanins identified in black mulberries

| Species               | cy-3-gl | cy-3-rut | pg-3-gl | pg-3-rut | Total  |
|-----------------------|---------|----------|---------|----------|--------|
| <i>Morus nigra</i> L. | 70.30   | 29.65    | 0.01    | 0.03     | 100.00 |

Color parameters of the black mulberries extract were calculated after obtaining the absorption spectrum in the visible domain (fig. 2). In table 5 were summarized the chromatic parameters values, average psychometric clarity (40.66) being pointed out also by the visual analysis of the extract.

Table 5

Chromatic parameters of black mulberries extracts

| Species               | Color simulation       | $L^*$ | Color coordinates |       | $C^*$ | $H^\circ$ | $H^*$ | $I^*$ |
|-----------------------|------------------------|-------|-------------------|-------|-------|-----------|-------|-------|
|                       |                        |       | $a^*$             | $b^*$ |       |           |       |       |
| <i>Morus nigra</i> L. | [Red color simulation] | 40.66 | 73.86             | 65.71 | 98.68 | 41.66     | 5.24  | 0.69  |

Legend: \* $L$  – Brightness (clarity): 0 (opaque) - 100 (transparently);  $a$  – red (+) - green (-);  $b$  – yellow (+) - blue (-);  $C$  – Saturation;  $H^\circ$  – Tone;  $H$  – Color tint;  $I$  – Intensity of color.

The parameter  $a^*$  has significant positive value (73.86), indicating the presence of red color given by the participation of anthocyanins, fact also sensorially observed by the analysis of extract color simulation (table 5). Sensation of bright red is more pronounced because of color hue, value which is closer to zero (5.24), the color being slightly influenced by yellow nuances. Considering that at  $H^\circ=90^\circ$  the corresponding color is yellow, we noticed an approaching to the angle of  $0^\circ$  of this parameter (41.66°), dominated by the blue shades. There is a general correlation between luminosity ( $L^*$ ) and color intensity ( $I^*$ ), thus in the intensely colored extracts, the psychometric clarity ( $L^*$ ) is lower, due to a higher phenolic content, which is observed also in this situation.

## CONCLUSIONS

1. Alcoholic extracts of *Morus nigra L.* fruits denoted an enormous therapeutic and commercial potential still insufficiently exploited.
2. The content of anthocyanins and total phenolic compounds had values higher than those existing in the literature for this species, the percentage of anthocyanins participation at the total phenolic compounds, was over 14 %. After 12 months of cold storage, ACY and TPC values remained high, suggesting the stability of these compounds, as an important technological feature.
3. Anthocyanin profile comprises four anthocyanins, represented by the cyanidin and pelargonidin, with rutinoside and glucoside forms, cyanidin-3-O-glucoside being the main anthocyanin of the profile.
4. Chromatic parameter  $a^*$ , presented significant positive values, indicating the presence of red color in the extract given by the participation of anthocyanins in colour composition.

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**ASSESSMENT ON THE POLYPHENOLIC BIOACTIVE  
POTENTIAL IN FRUITS OF SOME BLACKCURRANT  
(*RIBES NIGRUM L.*) VARIETIES GROWN IN IASSY AREA,  
NE OF ROMANIA**

**EVALUAREA POTENȚIALULUI BIOACTIV POLIFENOLIC AL  
FRUCTELOR UNOR SOIURI DE COACĂZ NEGRU (*RIBES NIGRUM L.*)  
CULTIVATE ÎN AREALUL MUNICIPIULUI IAȘI, NE ROMÂNIEI**

**FILIMON V. R.<sup>1</sup>, NICULAU M.<sup>2</sup>,  
COȚOVANU Roxana<sup>1</sup>, ARION Cristina<sup>1</sup>, BĂETU Alina<sup>1</sup>**  
e-mail: razvan\_f80@yahoo.com

**Abstract.** In recent years a large number of epidemiological studies have suggested remarkable benefits to human health by an high intake of fresh or processed (juice, jam etc.) blackcurrants. Fruits of *Ribes nigrum L.* are a important source of biologically active compounds, mostly belonging to the class of polyphenols, especially anthocyanins, which give the red-violet color of the fruits epicarp. The purpose of the study is the determination the total amount of anthocyanins and polyphenols, identification of the main anthocyanins (HPLC-DAD) and the participation percentage of each representative to the anthocyanin profile of ethanolic extracts obtained from fruits of two blackcurrant varieties, grown in the NE area of Romania. Measurements have revealed an important anthocyanin content in both analyzed varieties (319.97±1.89 mg cy-3-gl/100 g fruit, at Ronix variety and 286.41±1.19 mg cy-3-gl/100 g fruit, at Abanos variety), being identified four major anthocyanins, delphinidin and cyanidin with their glycosidic forms, glucoside and rutinoside.

**Key words:** blackcurrant, anthocyanin, polyphenolic, Iassy area, HPLC

**Rezumat.** În ultimii ani un număr mare de studii epidemiologice au sugerat beneficii remarcabile aduse sănătății umane printr-un consum ridicat de coacăze negre în stare proaspătă sau prelucrate (sucuri, gem etc). Fructele speciei *Ribes nigrum L.* reprezintă o sursă importantă de compuși biologic activi, aparținând clasei polifenolilor, în special antociani, ce conferă culoarea roșie-violet a fructelor. Scopul acestui studiu este determinarea cantităților totale de antociani și polifenoli, identificarea principalilor compuși antocianici (HPLC-DAD) și a procentului de participare a fiecărui reprezentant la profilul antocianic al extractelor etanolicе obținute din fructele a două soiuri de coacăz negru, cultivate în zona de NE a României. Determinările au relevat un conținut important în antociani a celor două soiuri analizate (319,97±1,89 mg cy-3-gl/100 g fruct la soiul Ronix și 286,41±1,19 mg cy-3-gl/100 g fruct la soiul Abanos), fiind identificați patru antociani majoritari, reprezentați de delfinidină și cianidină cu formele lor glicozidice, glucozid și rutinozid.

**Cuvinte cheie:** coacăze negre, antociani, polifenoli, arealul Iași, HPLC

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

<sup>2</sup>Oenology Research Center – Iasi Branch of the Romanian Academy, Romania

## INTRODUCTION

Early European traditional medicine mentions the use of black currant fruit to treat arthritis, gout, inflammation, stomach pains, diseases of the kidneys, as a diuretic and remedy for fatigue (Matsumoto et al., 2005). Although black currants contain a large number of nutrients, including fiber, minerals, essential fatty acids and vitamins (ascorbic acid), another class of bioactive compounds is considered the main responsible for numerous health benefits suggested by the studies conducted in the last decade, including the prevention of cancers, of some cardiovascular and inflammatory diseases, vision disorders, and this is the class of phenolic compounds (Bishayee et al., 2010).

Anthocyanins are the largest group of water soluble vegetable pigments, belonging to the flavonoid class, chemically being derivatives of flavylium salt (2-phenylbenzopyrylium) (Ghosh and Konishi, 2007).

The pigment extracted from black currant juice, administered to human subjects, favored rapid visual adaptation to darkness (Davies, 2004). Delphinidin and cyanidin, two of the main aglycones of black currants, have been identified as the most effective inhibitor of low density lipoprotein oxidation (Cacace and Mazza, 2003), indicating the important functional value of *Ribes nigrum L.* anthocyanins. In black currants, anthocyanin content identified by various authors fall within the range of 80-810 mg/100 g fruits, anthocyanin profile being composed from four main anthocyanins, glycosides of delphinidin and cyanidin (Mazza and Miniati, 1993, Slimestad and Solheim, 2002, Gould et al., 2009).

## MATERIAL AND METHOD

Anthocyanin content (ACY) and total phenolic compounds (CFT) of ethanolic extracts obtained from fruits of two varieties of black currant (*Ribes nigrum L.*), Abanos and Ronix, created in 1999 and 2000 respectively, by the Research Institute for Fruit Growing Pitești-Mărăcișeni, was measured. Fruits were harvested from the experimental field of the University of Agronomical Sciences and Veterinary Medicine (UASVM) Iassy, V. Adamachi Farm, at fruits maturation, when the last berry from the cluster turned its color to purple, approx. 60 days after flowering phenophase (Radu and Boboescu, 2007), on 26.06.2010 for Abanos and 01.07.2010 for Ronix variety.

Were determined several physico-chemical properties of the fruits: the average mass of a berry, moisture content, soluble solids ( $^{\circ}\text{Bx}$ ), titratable acidity, ascorbic acid, juice pH, reducing sugars (Schoorl method). Fruits were stored at  $-18\pm 2^{\circ}\text{C}$  until extraction (5 days), anthocyanin transformation being minimal for fruits kept in a frozen state (Mazza and Miniati, 1993).

A quantity of 5 g skin was treated initially with 50 mL extraction solution,  $\text{C}_2\text{H}_5\text{OH-HCl-H}_2\text{O}$  (96:1:3) and kept at room temperature ( $18\pm 2^{\circ}\text{C}$ ) overnight. Subsequently two more washes of plant material were made with 30 mL and 20 mL of solvent, resulting a final ratio between plant material and extract of 1:20. Before the third filtering was applied an ultrasound treatment on the sample containers, as a means of increasing the property transfer process and desorption. The three fractions were cumulated and stored extraction at  $4\pm 1^{\circ}\text{C}$ , in the dark.

Determination of total monomeric anthocyanins content (ACY) was performed by the pH differential method:

$$A \text{ (absorbance)} = (A_{520 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 0.68 - (A_{520 \text{ nm}} - A_{700 \text{ nm}}) \text{ pH } 3.56$$

Results were expressed as mg equivalent cyanidin-3-glucoside /100 g fruit:

$$\text{ACY (mg cy-3-gl, mg/L)} = (A \times \text{MW} \times \text{DF} \times 10^3) / (\varepsilon \times l);$$

where: ACY - total anthocyanin content (cyanidin-3-glucoside equivalents(cy-3-gl), mg/L), A - absorbance, MW - molecular weight (449.2 g/mol for cy-3- gl); DF - dilution factor; l -optical pathlength in cm (1 cm);  $\varepsilon$ - molar extinction coefficient for cy-3-gl(26 900L×mol<sup>-1</sup>× cm<sup>-1</sup>); 10<sup>3</sup>- factor for conversion from g to mg(J. AOAC Int., 2005).

To obtain the total phenolic compounds content (TPC) was used Folin-Ciocalteu photocolometric method (FC), by measuring absorbance at  $\lambda_{\text{max}}$  (765 nm) (Singleton and Rossi, 1965), results were expressed in grams of gallic acid equivalent (g GAE)/100g fresh weight (FW). Measurements were made using a UV-VIS spectrophotometer, Analytik Jena Specord 200, the results represent the average of three determinations, having calculated standard deviation ( $\pm$ ) and coefficient of variability of data, statistical analysis being performed with application ANOVA: Single factor from Microsoft Excel, Data Analysis Tools.

Using a Shimadzu LC 20 liquid chromatograph, with a Hypersil ODS C18 separation column (25 cm length) at 20 °C, was performed the separation of anthocyanins. Elution was achieved at a flow rate of 1.2 mL/min. As eluent A was used H<sub>2</sub>O:HCOOH:CH<sub>3</sub>CN (87:10:3) and as eluent B, H<sub>2</sub>O:HCOOH:CH<sub>3</sub>CN (40:10:50), increasing eluent B from 6% to 60%. Anthocyanin compounds were individualized with a diode array detector (DAD) Shimadzu at  $\lambda$  518 nm, and their identification was made according to Slimestad et al., 2002 and Oszmiański et al., 2009.

## RESULTS AND DISCUSSIONS

Immediately after harvest, were determined several physical and chemical properties of fresh fruits, presented in table 1.

Table 1

The physico-chemical properties of black currant fruits

| Variety       | M. fr.*<br>(g)   | M.*<br>(%)        | T. ac.*<br>(g m. a.) | Asc.ac.*<br>(mg %) | Rd. sg.*<br>(gl. %) | pH<br>(units)    | SDS*<br>(°Bx)     | TDS*<br>(%)       |
|---------------|------------------|-------------------|----------------------|--------------------|---------------------|------------------|-------------------|-------------------|
| <b>Abanos</b> | <b>1.24±0.05</b> | <b>81.01±1.04</b> | <b>2.68±0.01</b>     | 122.18±1.16        | 8.92±1.02           | 3.08±0.24        | 13.91±0.19        | 18.99±1.04        |
| <b>Ronix</b>  | 1.10±0.07        | 80.11±0.98        | 2.62±0.09            | <b>157.12±1.56</b> | <b>10.52±1.04</b>   | <b>3.10±0.32</b> | <b>14.92±1.02</b> | <b>19.89±0.98</b> |

**Legend:**\*M. fr. - average mass of fruit; M (%) - moisture; T. ac. (g m.a.) - titratable acidity (g malic acid/100g); Asc. ac. (mg %) - ascorbic acid (mg/100 g); Rd. sg. (gl. %) - reducing sugars (mg glucose/100 g fruits); SDS (°Bx) - soluble dry substance (°Brix); TDS (%) - total dry substance (%); CV<10; *p-value*>0,05.

Variety Abanos presented a content in moisture and titratable acidity higher than Ronix variety, containing a more significant quantity of ascorbic acid (157.12 mg/100 g), however values are lower than those presented in literature for this species (160-200 mg ascorbic acid/100 g).

After extraction of numerical data, obtained by analyzing the absorption spectra (fig. 1) were determined the total quantities of monomeric anthocyanins (ACY). ACY values at Ronix variety (table 2), increased up to a maximum of 319.97±1.89 mg g cy-3-O-gl equivalent/100g FW, compared to the variety Abanos, with only 286.41±1.19 mg cy-3-gl/100 g FW. Anthocyanins being the main phenolic compounds that give color to extract, differences between quantities

determined from the two species can be also observed through visual examination of extracts color (fig. 2).

Table 2

| ACY and TPC content of black currant fruit extracts |                           |                     |                 |
|---|---------------------------|---------------------|-----------------|
| Variety   | ACY<br>(mg cy-3-gl/100 g) | TPC<br>(g GAE/100g) | ACYofTPC<br>(%) |
| Abanos  | 286.41±1.19               | 1.99±1.31           | 33.49           |
| Ronix   | 319.97±1.89               | 2.24±1.05           | 33.31           |

CV<10; p-value>0.05

TPC content was, the same like for ACY, higher at Ronix variety (2.24 g GAE/100g FW), the percentage of anthocyanins from total phenolic compounds exceeding 33% in both varieties.

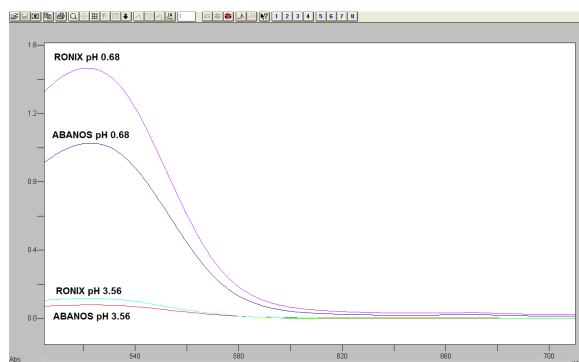


Fig. 1- Overlay of absorbance spectra at  $\lambda$  520 nm, pH 0.68 and 3.56



Fig. 2-Color of ethanolic extracts obtained from black currant fruits

It is well known that the anthocyanin decomposition is accelerated by the presence of ascorbic acid. Direct condensation between anthocyanins and ascorbic acid has been postulated as a mechanism for anthocyanin degradation (Marti et al., 2002). It may be noted that Ronix variety, containing higher amount of ascorbic acid, had the higher quantity of anthocyanins and phenolic compounds.

Based on the chromatograms obtained (fig. 3) were identified two main anthocyanidines, cyanidin and delphinidin, with the glycosidic forms: 3-O-glucoside and 3-O-rutinoside (fig. 5). The main anthocyanin present in the analyzed extracts, conform to peak area, was the delphinidin-3-O-rutinoside (dp-3-rut), with a participation percentage of 69.55% from the total anthocyanins area, at the Abanos variety and 49.63%, for Ronix variety. At the opposite pole, was identified cyanidin-3-O-glucoside (cy-3-gl), with a participation rate of 2.73% and 0.80%, at Abanos and respectively Ronix variety. Anthocyanin cyanidin-3-O-rutinoside (cy-3-rut), had a different behavior in this case, thus at the variety Abanos held a percentage of 19.02% of total area, while at the variety Ronix, the percentage was more than double 41.06%. This reflects in the color extracts, cyanidin (E163 a), according to *Codex Alimentarius*, gives deep red color to

solvent in which is dissolved, while delphinidin (E163 b) shows shades of blue, which can be observed also by visual examination of extracts color (fig. 2).

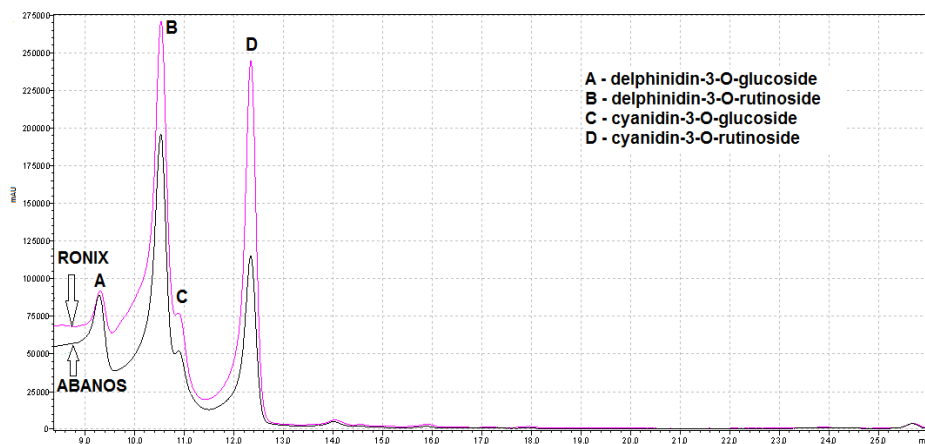


Fig. 3-Chromatograms of the two varieties of black currant analyzed, at 510 nm

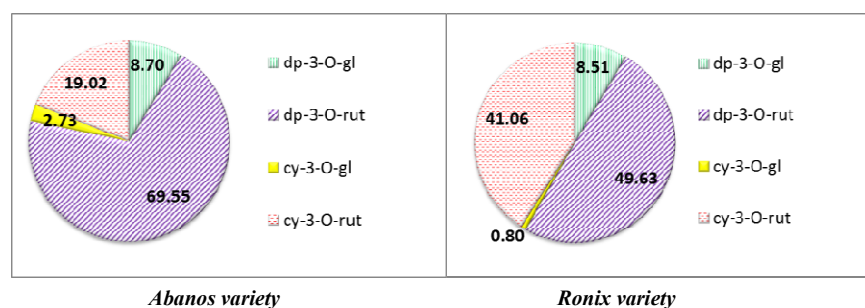


Fig. 4 -Area percentages (%) corresponding to the identified anthocyanins

Dp-3-gl was the most constant anthocyanin in the experiment, showing very similar values in the profile of both varieties, 8.70% at Abanos variety and 8.51% at Ronix variety (fig.4).

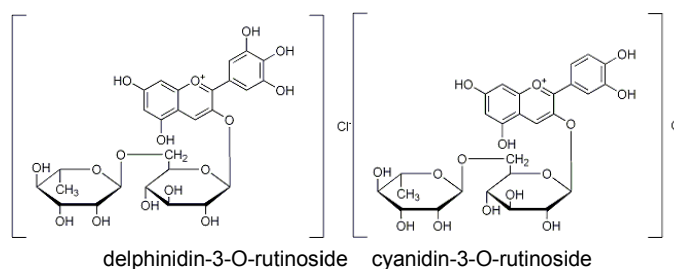


Fig. 5-Chemical structure of main anthocyanins identified in black currants

## CONCLUSIONS

1. Ascorbic acid content was determined to be lower than values offered by the literature for *Ribes nigrum L.* fruits, in both varieties analyzed. Variety with a higher content of ascorbic acid, also had the highest amount of anthocyanins.

2. Anthocyanin profile of this species contains four main anthocyanins, confirmed by chromatographic analysis, glucoside and rutoside forms of delphinidin and cyanidin, with dp-3-rut the most important anthocyanin.

3. Black currants, grown in the NE of Romania, belong to the group of horticultural products with an important content of phenolic compounds, in particularly anthocyanins, which confirms the huge bioactive potential of fruits, being recommended for the use in food, pharmaceutical and cosmetic industries.

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# STUDY REGARDING THE YEAR OF PRODUCTION'S INFLUENCE FOR THE APPLES MEANT FOR CONCENTRATED APPLE JUICE AT SC AGRANA JUICE SRL VASLUI

## STUDIU PRIVIND INFLUENȚA ANULUI DE PRODUCȚIE ASUPRA CALITĂȚII MERELOR–MATERIE PRIMĂ PENTRU OBTINEREA SUCULUI DE MERE CONCENTRAT ÎN CADRUL SC AGRANA JUICE SRL VASLUI

**NECHITA CHEREȘ Diana**<sup>1</sup>  
e-mail: nechitaisctv@yahoo.com

**Abstract.** *Research was carried out in both USAMV Iassy and SC Agrana Juice SRL Vaslui –company specializes in production of concentrated apple juice. This study aims to determine the quality of the raw material used by the firm knowing that for industrialization they used fruits that do not exhibit a quality satisfactory to be delivered in the market for consumption. Qualitative indicators of the raw material- fresh apples for industry , as such as specific values of titrating acidity or dry soluble substance content, are also needed in order to assure the suitability for processing. Climate conditions vary from one year to another and taking into account the technological features of apples at the harvest time, it may have a negative influence upon these qualitative indicators.*

**Key words:** quality, the raw material

**Rezumat.** *Cercetările au fost realizate atât în cadrul SC Agrana Juice SRL Vaslui-firmă specializată în fabricarea sucului concentrat de mere cât și în în cadrul USAMV Iași. Prezentul studiu urmărește determinarea calității materiei prime utilizate de firma vasluiană, având în vedere că pentru industrializare se folosesc fructe ce nu prezintă calitate satisfăcătoare pentru a fi livrate pe piață cu destinația consumului proaspăt. Totodată merele proaspete- materie primă pentru industrializare trebuie să corespundă unor parametri calitativi ce presupun anumite valori ale acidității titrabile și a conținutului în substanță uscată solubilă .Condițiile climatice ce variază anual, pot influența în mod nefavorabil acești parametri calitativi, astfel încât fructele recoltate în vederea industrializării nu corespund uneori din punct de vedere tehnologic.*

**Cuvinte cheie:** calitate, materie primă

### INTRODUCTION

Apples represent an important raw material for obtaining the concentrated juices due to the chemical composition and volume of raw-material.

Studies undertaken worldwide have the following aims:

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<sup>1</sup> Department for Agriculture Vaslui county, Romania

➤ testing and introduction in the technologies used to processing fruits

- the varieties genetically resistant to frost and disease, which are obtained in intensive plantations, with a small number of phytosanitary treatments (half of the treatments used for the standard varieties) (Körmedy, 1994) ;

- with a low degree of pollution, but high productivity (Snowdon, 1991) ;

➤ reducing the consumption of the raw material during the processing (Jamba, Carabulea , 2002).

In România, in 1999, ICCP Pitești has initiated such a research programme, too. In a first stage, were made observations and data were recorded regarding the the fenologia, yields and suitability for juice obtaining at 20 genotypes of apple.

The analysed parameters were: average weight of fruit (g), the yield in juice (% substance), the dry soluble content (<sup>0</sup>Bx.), total sugar (g/l), total acidity content (g/l), sugar/acidity ratio, the pH of the juice, the color, the taste, the brightness of the juice and the degree of oxidation in air.

Relating the sugar/acidity ratio it was found that Florina, Priam, Liberty, Jonathan varieties and also the Romanian varieties as such as Generos and Romus are most suitable. At the same time, the varieties of French origin as Florina, Priam and the Romanian varieties T119 and 120 T, have been noted due to the large yield of juice and low content of apple pomace.(Budan și colab., 2001)

The production activity at SC Agrana Juice SRL is for seasonal and runs from August-November along with harvesting the apples which represent the raw material. Finished products are concentrated apple juice and flavor of apples (<http://www.agranajuce.ro>). For the past 7 years, these products have been exported to 100%.

According to technical conditions laid down in the technical specification of the firm (ST-1/2008) the raw nmaterial used by the company have to exhibited a good phytosanitary state and balanced from the point of view of content in sugars and acids, too.

## **MATERIAL AND METHOD**

The raw material used by S.C. Agrana Juice SRL are fresh apples for industry, mainly the varieties more encountered such as Golden delicious, Jonathan and Starkrimson, collected mostly from Moldova to the right area, and Moldova Republic.

Qualitative determination for apples meant for apple juice has to be an objective exam and it supposed that a lot of physical-chemical determinations to be done. For this purpose, samples of raw material were taken and analysed during the period of study: 2007-2009. All those samples were taken at random from the raw material on the silo unloading, each sample being built in a trial about 1 kg apples for industry, in accordance with the norms in force.



**Fig.1** - Apple silo belongs to SC Agrana Juice SRL

*Table 1*

**Variants analysed and sampling date**

| <b>YEAR</b> | <b>SAMPLING DATE</b> | <b>VARIANT</b> |
|-------------|----------------------|----------------|
| 2007        | 12.09.2007           | V1             |
|             | 24.10.2007           | V2             |
| 2008        | 14.10.2008           | V1             |
|             | 23.10.2008           | V2             |
| 2009        | 02.10.2009           | V1             |
|             | 09.10.2009           | V2             |
|             | 02.11.2009           | V3             |

Qualitative determination for apples meant for apple juice were carried out at the Technology of horticultural products lab within USAMV Iassy and involved the determination of dry soluble substance content by refractometric method (SR 2213-5:2009); determination of titrating acidity (SR 8613-4:2009) determination of catalase activity through gas-meter method and determination of the pulp firmness through the iodine test.

**RESULTS AND DISCUSSIONS**

The titrating acidity of samples collected during the year-2007, expressed in malic acid g /100 g of product, varied between 0.27 g/100 g malic acid in case of Starkrimson variety and 0.52 g / malic acid /100 g of product by the Jonathan variety, while the Golden Delicious variety was obtained the mean value (average value) of 0.39 g malic acid/100 g of product.

In 2008 the titrating acidity average value for the variants under study was about 0.32 g malic acid /100 g of product with a minimum of 0.23 malic acid /100 g of product for Starkrimson variety and a maximum of 0.41 malic acid /100 g of product in case of the Jonathan variety; the Golden Delicious variety reached a maximum of 0.33 g malic acid /100 g of product, equal to the minimum value of the previous year.

In 2009, in spite of a reduction of the average value of titrating acidity for the analyzed samples compared with 2008, the maximum is reached at 0.41 g malic acid /100 g of product like the previous year 2008 and the Jonathan variety has been also presented the highest values. The lower value of titrating acidity for the variants under study - 0.33 g malic acid /100 g of product, was registered by the very same Starkrimson variety.

*Table 2*

**The titrating acidity average value for the variants under study (g malic acid /100 g of product)**

| SAMPLING DATE | VARIANT | VARIETIES        |             |             |
|---------------|---------|------------------|-------------|-------------|
|               |         | GOLDEN DELICIOUS | STARKRIMSON | JONATHAN    |
| 12.09.2007    | V1      | 0,44             | 0,30        | <b>0,52</b> |
| 24.10.2007    | V2      | 0,33             | 0,27        | 0,42        |
| 14.10.2008    | V1      | 0,33             | 0,27        | 0,41        |
| 23.10.2008    | V2      | 0,27             | 0,23        | 0,33        |
| 02.10.2009    | V1      | 0,27             | 0,20        | 0,41        |
| 09.10.2009    | V2      | 0,21             | 0,19        | 0,33        |
| 02.11.2009    | V3      | 0,19             | <b>0,16</b> | 0,29        |

The smaller quantity of soluble dry matter was registered in the year 2007, by the Golden Delicious variety -11.8 °Bx, while the maximum value - 9.1 °Bx, was reached by Starkrimson variety (tab.3).

*Table 3*

**Soluble dry matter content for the variants in the period under study(°Bx)**

| SAMPLING DATE | VARIANT | VARIETIES        |             |          |
|---------------|---------|------------------|-------------|----------|
|               |         | GOLDEN DELICIOUS | STARKRIMSON | JONATHAN |
| 12.09.2007    | V1      | 11,8             | 12,8        | 12,2     |
| 24.10.2007    | V2      | 13,2             | 14,6        | 14,0     |
| 14.10.2008    | V1      | <b>11,2</b>      | 13,4        | 12,6     |
| 23.10.2008    | V2      | 12,6             | 14,8        | 13,4     |
| 02.10.2009    | V1      | 11,6             | 15,8        | 14,6     |
| 09.10.2009    | V2      | 12,2             | 16,4        | 15,2     |
| 02.11.2009    | V3      | 13,6             | <b>16,8</b> | 15,6     |

In 2008 were not recorded significant differences regarding the dry soluble content compared to the previous year. The upper limit of the amount of dry soluble substance has been also recorded for the Starkrimson variety, while the minimum is also Golden Delicious variety.

The year 2009 is the year in which they recorded the highest values of soluble dry substance in the period under study, the average value of the parameter examined being 14.2 °Bx.

The maximum value is also reached by Starkrimson variety-16.8 °Bx, while the bottom limit of dry soluble substance content is noticed in the case of Golden Delicious variety, like previous years. Dry soluble substance content recorded by Jonathan variety varied between 7.2-10.4 °Bx.

Table 4

**Grades given in iodine test (average value in the period under study)**

| SAMPLING DATE | VARIANT | VARIETIES        |             |          |
|---------------|---------|------------------|-------------|----------|
|               |         | GOLDEN DELICIOUS | STARKRIMSON | JONATHAN |
| 12.09.2007    | V1      | 9                | 9           | 9        |
| 24.10.2007    | V2      | 10               | 10          | 10       |
| 14.10.2008    | V1      | 9                | 9           | 9        |
| 23.10.2008    | V2      | 10               | 10          | 10       |
| 02.10.2009    | V1      | 9                | 9           | 9        |
| 09.10.2009    | V2      | 9                | 10          | 10       |
| 02.11.2009    | V3      | 10               | 10          | 10       |

The first samples taken both in 2007 and 2008 respectively 2009, met the same score of 9 for all varieties analyzed, while later samples collected during the campaign reached the maximum 10 mark, for all three varieties which made the variants (tab 4).

Analyzing the samples by using the iodine test, they notice that that apples-raw material, regardless of their variety, were harvested at a high level of starch hydrolysis corresponding to the final stage of ripeness.

The main function of catalase is to decompose hydrogen peroxide, thus protecting the cell from toxic effects of hydrogen peroxide. According to the made determinations (tab.5), the catalasis presents the upper limit of activity with a maximum value - 5.8 cm<sup>3</sup> O<sub>2</sub> /g product recorded for the V2 variant, sampling in 2007 for Starkrimson variety and also presented a minimum value -2.2 cm<sup>3</sup> O<sub>2</sub> /g product ,in 2009, for Golden variety-V1 variant.

Table 5

**The catalasis activity (cm<sup>3</sup> O<sub>2</sub>/g product- determinated average values in the period under study)**

| SAMPLING DATE | VARIANT | VARIETIES        |             |          |
|---------------|---------|------------------|-------------|----------|
|               |         | GOLDEN DELICIOUS | STARKRIMSON | JONATHAN |
| 12.09.2007    | V1      | 3.6              | 4.8         | 4.2      |
| 24.10.2007    | V2      | 4.2              | <b>5.8</b>  | 5.6      |
| 14.10.2008    | V1      | 3.8              | 3.6         | 4.0      |
| 23.10.2008    | V2      | 3.6              | 4.9         | 4.3      |
| 02.10.2009    | V1      | <b>2.2</b>       | 2.8         | 2.6      |
| 09.10.2009    | V2      | 2.6              | 3.2         | 2.8      |
| 02.11.2009    | V3      | 3.6              | 4.9         | 4.3      |

The variants V2-2007 and V1- 2009 are, in fact, the variants which have presented the upper limit and the bottom limit of the analysed parameter.

The Starkrimson variety has recorded more significant catalasis activity compared to the Golden variety, while Jonathan variety presented the intermediate values, with a single exception-V1 in 2008, in which the differences between the three of variants are insignificant

## CONCLUSIONS

1. The titrating acidity of raw material, expressed in malic acid g/100 g of product, has recorded the minimum average value in 2009, less 0,10 g comparing with 2007 and 0,04 g comparing with 2008.

2. In the period under study, the dry soluble content, expressed in °Bx, has registered an average value of 14.2 °Bx. The upper limit of the amount of dry soluble substance has been recorded for all three varieties in 2009.

3. Apples –raw material, regardless of their variety, were harvested at a high level of starch hydrolysis corresponding to the final stage of ripeness.

4. The activity of catalase has been low during the whole period of study, the average values have ranged between 3.5-2.5 cm<sup>3</sup> O<sub>2</sub>/g product, which means that fruits have not reached the phase of physiological decline

5. The raw material used by the company has also degree of uniformity and quality parameters required to confere packet processing into juice.

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# STUDY REGARDING THE QUALITY OF CONCENTRATED APPLE JUICE OBTAINED AT SC AGRANA JUICE SRL OF VASLUI

## STUDIUL PRIVIND CALITATEA SUCULUI CONCENTRAT DE MERE OBTINUT LA SC AGRANA JUICE SRL VASLUI

**NECHITA CHEREȘ Diana<sup>1</sup>**  
e-mail: nechitaisctv@yahoo.com

**Abstract.** Research was carried out in both USAMV Iassy and SC Agrana Juice SRL of Vaslui –company specializes in production of concentrated apple juice. This study aim is to determine the quality of concentrated apple juice obtained at SC Agrana Juice SRL and the compliance with the to european market requirements .For this purpose, samples of concentrated apple juice were taken and analysed during period of study. Physical-chemical determinations were made in order to evaluate if the final product obtained at SC Agrana Juice SRL Vaslui meets the quality requirements imposed by standards in force.

**Key words:** quality, concentrated apple juice

**Rezumat.** Cercetările au fost realizate atât în cadrul SC Agrana Juice SRL Vaslui-firmă specializată în fabricarea sucului concentrat de mere cât și în în cadrul USAMV Iași. Prezentul studiu urmărește determinarea calității sucului concentrat de mere obținut de firma vasluiană și alinierea la cerințele pieței europene. În acest scop au fost prelevate probe din produsul finit obținut la SC Agrana Juice SRL. S-au efectuat analize fizico-chimice pentru aprecierea calității acestuia, pe baza standardelor în vigoare.

**Cuvinte cheie:** calitate, suc concentrat mere

### INTRODUCTION

Concentration of fruit juice is made for a dry matter content of 30-50% (65), the technical procedures used in concentration technology being: thermal concentration, crioconcentration (with change of phase) and the reverse osmosis (without change of phase) (Ashurst and Dennis, 1997).

Crioconcentration (Bes et al, 2006) is the concentration method with a phase change, that modifies in a more reduced proportion the chemical nature or organoleptic characteristics of the products transformed, but thermal method tends to be most spread technical procedure of concentration all over the world. It may be achieved in three stages of temperature (10-25 0 C, 40-100 0 C 0 c, 115-130) in two ways:

- normal vacuum- duplicate boilers;
- low vacuum, which may be continous or discontinous, in instalations-process called vacuum concentration; allows the reducing of the boiling point

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<sup>1</sup> Department for Agriculture Vaslui, Romania

temperature, of the duration of the process, and the loss of flavours and nutrients are minimal (Beceanu, 2011).

**The concentrated apple juice** is the product obtained from apple juice through the physical elimination of some quantity of water content. If the product is meant for direct consumption, the water quantity eliminated is at least 50%.

The concentrated apple juice is a viscous limp tasteless liquid lacking the caramel smell having the colour and consistency of bee honey and a dry substance content of about 65 °Bx. (OMAPDR no.768/2003).

By concentrating juices they obviously register low costs for manipulation and transport. At the same time, by concentration they also register a diminution of water activity leading to the increase of microbiological stability and extension of storage time in determined conditions. The so-called “commercially sterile juices concentrates” are meant for marketing (dilutable juices) (Ashurst, 2005). They also may be stored and used as a raw material for subsequent processing.

SC Agrana Juice SRL has used a performant concentration plant with quadruple effect also equipped with a distillation column designed to recover the characteristic flavour (which insures the best flavor recovering). Required evaporation heat is produced by thermal power plant of its own.

Under certain conditions - the vacuum of 10-100 MB and with the help of steam 40-105 °C- the capacity of the plant is about 450 t concentrated apple juice/24 h, bearing in mind that the maximum flow of the apple juice is 17000 L/h (SC Agrana Juice SRL - Instructions on technological process of obtaining concentrated apple juice).

## MATERIAL AND METHOD

Qualitative determination for the final product –concentrated apple juice - has to be an objective exam and it supposed that a lot of physical-chemical determinations to be done. For this purpose, samples of concentrated juices were taken and analysed in the period under study: 2007-2009. All those samples were taken at random from those 8 storage tanks of stainless steel, with a capacity of 60 cubic metres, which are owned by firm, each sample being about 1 kg concentrated apple juice (table 1).

Table 1

Variants analysed and sampling date

| YEAR | SAMPLING DATE | VARIANT |
|------|---------------|---------|
| 2007 | 12.09.2007    | V1      |
|      | 24.10.2007    | V2      |
| 2008 | 14.10.2008    | V1      |
|      | 23.10.2008    | V2      |
| 2009 | 02.10.2009    | V1      |
|      | 09.10.2009    | V2      |
|      | 02.11.2009    | V3      |

Qualitative determinations for the concentrated apple juice were carried out at the Technology of horticultural products lab within USAMV Iasi and have involved



determination of titrating acidity (SR 8613-4:2009); the determination of dry soluble substance content by refractometric method (SR 2213-5:2009) and determination of ash content. At the same time, chromatographic analysis by CIE Lab-76 method was carried out at Oenology lab from USAMV Iassy, in order to establish the chromatic characteristics of the final product.

The CIE Lab-76 method makes it easier to understand the correspondence between visual impression of colour and numeric expression of chromatic parameters. These chromatic characteristics allow us to better understand about the degree of opalescence and thus about the degree of oxidation of the product concerned. The colour components are calculated on the basis of absorbance spectra recorded in the field of UV-VIS using a S-200 Analytic Jena spectrophotometer coupled with an IBM computer. The absorbance spectra have been digitised and processed with the help of the "VINCOLOR" improved program. The results of measurements shall be expressed by following chromatics parameters:

- L – brightness or psychometric clarity;
- a – the coordinate of red-green complementary colours;
- b – the coordinate of yellow-blue complementary colours;
- C – chromaticity or colour saturation

## RESULTS AND DISCUSSIONS

The main acids found in concentrated apple juice (tab. 1) are the malic acid, tartaric acid, followed by the succinic acid, fumaric acid, citric acid, etc.-acids with lower concentration. The titrating acidity of apple juices is mostly due to the presence of malic acid and this is also noticeable in the case of analyzed samples.

*Table 2*

**Share of main acids identified in the concentrated apple juice for the period under study**

| Sampling date | Variants | Malic acid g/l | Tartric acid g/l | Succinic acid g/l | Fumaric acid mg/100g | Acidity g/l  |
|---------------|----------|----------------|------------------|-------------------|----------------------|--------------|
| 12.09.2007    | V1       | <b>4.90</b>    | 3.70             | 0.37              | <b>2.50</b>          | 9.75         |
| 24.10.2007    | V2       | 5.30           | 4.35             | <b>0.29</b>       | <b>0.60</b>          | 10.33        |
| 14.10.2008    | V1       | 6.00           | <b>2.56</b>      | 0.40              | 1.00                 | <b>9.05</b>  |
| 23.10.2008    | V2       | 5.80           | <b>4.60</b>      | 0.30              | 0.85                 | 11.20        |
| 02.10.2009    | V1       | 6.40           | 2.20             | 0.35              | 1.02                 | 9.53         |
| 09.10.2009    | V2       | <b>6.87</b>    | <b>4.60</b>      | <b>2.00</b>       | 1.65                 | <b>11.95</b> |
| 02.11.2009    | V3       | 6.10           | 3.60             | 0.37              | 0.40                 | <b>10.60</b> |

They were not recorded significant differences regarding the titrating acidity of concentrated juice samples expressed in malic acid g/100 g of product, from one year to the next - the average value of the parameter examined being 0.51 g malic acid /100g of product in 2007, 0.59 g malic acid /100g of product in 2008 and 0.65 g malic acid /100g of product in 2009. The minimum value of the 7 variants under study, was 0.47 g malic acid /100g of product, while the upper

limit of the parameter examined being 0.68 g malic acid /100g of product , in 2009.

*Tartric acid*- has been noticed in all 7 samples of concentrated apple juice with mean values ranging between 2.56-4,6 g/l, the contents of tartric acid being about 1.5 times smaller than content in malic acid.

*Succinic acid*- has been identified with less then 1 g/l values.

*Citric and fumaric acids* - the presence of small amount of these acids has been identified - they recorded 0,43 g/l as the highest value regarding the citric acid content and by µg order in case of fumaric acid ; the amounts of both these acids content identified as relatively devoid of matter.

Chemical determinations carried out on the seven variants of concentrated apple juice taken in the years 2007-2009, from different batches from S.C. Agrana Juice SRL Vaslui indicates the titrating acidity of compliance with STATE STANDARD No. 1073/70

Table 3

**Soluble dry matter content (°Bx) of the variants under study**

| SAMPLING DATE | VARIANT | SUS (°BX) |
|---------------|---------|-----------|
| 12.09.2007    | V1      | 69.7      |
| 24.10.2007    | V2      | 71.6      |
| 14.10.2008    | V1      | 70        |
| 23.10.2008    | V2      | 69.9      |
| 02.10.2009    | V1      | 69.8      |
| 09.10.2009    | V2      | 70.0      |
| 02.11.2009    | V3      | 70.2      |

In the case of concentrated juice, were not recorded significant differences between the variants analysed regarding the dry soluble content. Where there has been a near 70 S.U.S °Bx , the product is subjected to a new concentration in recirculation by Unipektin facility until the quantity of soluble dry substance considerably augments up to the desired value(until it has reached the desired quality parameter).

Thus, for the concentrated apple juice variants under study , the minimum value regarding the dry soluble content was about 69,7 °Bx, while upper limit of the amount af dry soluble substance was 70,2 °Bx. (tab 3). SC Agrana Juice SRL company comply with the rules imposed by the STAS 5956/71.

Table 4

**Mineral content (ash) of the variants under study(mg/g of product)**

| SAMPLING DATE | VARIANT | ASH CONTENT MG/G PRODUS |
|---------------|---------|-------------------------|
| 12.09.2007    | V1      | 3.47                    |
| 24.10.2007    | V2      | 3.64                    |
| 14.10.2008    | V1      | 3.56                    |
| 23.10.2008    | V2      | 4.29                    |
| 02.10.2009    | V1      | 3.50                    |
| 09.10.2009    | V2      | 4.04                    |
| 02.11.2009    | V3      | 4.41                    |

The ash content of samples collected in the period under study varied with values ranging between 3.47 - 4.41 mg/g of product. The bottom limit of ash content 3.47 mg/g of product was recorded in 2007, while highest value 4.41 mg/g of product was noticed in the last year of study (tab.4).

The mineral (ash) content increases as the technological process advances to obtain concentrated apple juice.

*The chromatic characteristics of concentrated apple juice*

The results of measurement regarding the chromatic characteristics of concentrated apple juice (tab 5) were expressed by following chromatics parameters.

Table 5

**Results obtained by C.I.E Lab-76 method for the variants analysed in the period 2007-2009**

| Sample           |         | Brightness L<br>0 (opaque<br>100 (transparent<br>colourless) | Colour coordinates        |                             | Colour<br>saturation |
|------------------|---------|--|---------------------------|-----------------------------|----------------------|
| SAMPLING<br>DATE | Variant |  | a<br>red (+)<br>green (-) | b<br>yellow (+)<br>blue (-) |                      |
| 12.09.2007       | V1      | 1,00   | -0,16                     | 0,25                        | 0,30                 |
| 24.10.2007       | V2      | <b>2,60</b>  | 0,11                      | <b>0,01</b>                 | <b>0,11</b>          |
| 14.10.2008       | V1      | 0,20   | 1,61                      | 3,16                        | 3,55                 |
| 23.10.2008       | V2      | 0,90   | 0,11                      | 1,08                        | 1,09                 |
| 02.10.2009       | V1      | <b>0.1</b>   | <b>8.01</b>               | <b>7.00</b>                 | <b>10.64</b>         |
| 09.10.2009       | V2      | 0.5  | 2.67                      | 1.71                        | 3.17                 |
| 02.11.2009       | V3      | 1.3  | 0.18                      | 0.08                        | 0.20                 |

**Brightness L**, characterizes the appearance of the product, its clarity. The brightness may have absolute values in the range 0 (for a sample Black opaque) to 100 (for transparent colourless samples).

The values obtained (tab. 5) are in compliance with the visual appearance of the concentrated apple juice. For the period under study, taken in the case of concentrated juice, brightness had values between 0.1 and 2.6, with an average of 0.95. The higher colouring intensity is the more the psychometric clarity of product is lower.

**C parameter**, or more simply chromaticity or colour saturation shows that the test sample may have a more or less pure colour, meaning is more or less mixed with white.

In relation with brightness L, chromaticity is in a negative correlation: the high brightness juices have a low chromaticity and vice versa- juices with high chromaticity, like the concentrated juice, are very low brightness. For instance, variants 1 of the year 2009 presented the lowest value of brightness 0.08.

For variants of concentrated apple juice analysed (fig. 5), the chromaticity C took values between 0,3 and 10.64, with an average value recorded of 2.73

**The a parameter** represents the coordinate of red-green complementary colours. This parameter often presents negative values for those samples in where green tonalities are prevailing over the red ones.

In fact, parameter *a* analysed for the concentrated juice indicated positive values (to red), with a single exception (V1-2007, slightly greenish)- for most of variants, the yellow-brownish tonalities predominate.

**The *b* parameter** represents the coordinate of yellow-blue complementary colours.

For variants of concentrated apple juice analysed (fig. 7), values of *b* parameter have ranged between 0.01-7.00, both the positive values and yellow tonalities have prevailed.

Average values have varied between a minimum located around 0.13 in 2007 and a maximum of 2.93, which indicates a brownish colour, higher values being appropriated for a more intense hue of yellow.

## CONCLUSIONS

1. The average value of titrating acidity had an ascending trend, reaching 0.60 malic acid g/100 g of product in the concentrated juice.

2. In terms of dry soluble content were not recorded differences between the variants analysed because due to evaporation of a large content of water during the four stages of concentration, the quantity of soluble dry substance considerably augments up to the desired value-70 °Bx. Otherwise the product will be recirculated through the concentration device.

3. The positive values recorded for both *a* and *b* parameters indicate yellow-brownish tonalities and moreover the brightness values and C parameter values and are in concordance with the visual aspect of the concentrated apple juice.

4. According to the tests run, SC Agrana Juice S.R.L. of Vaslui obtains a high quality product which complies with the requirements of the standards in force.

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# INFLUENCE OF FERTILIZATION ON NITROGEN AND POTASSIUM CONTENT IN LEAVES AND QUALITATIVE INDICES OF APPLE FRUITS

## INFLUENȚA FERTILIZĂRII ASUPRA CONȚINUTULUI DE AZOT ȘI POTASIU DIN FRUNZE PRECUM ȘI ASUPRA UNOR INDICI CALITATIVI LA FRUCTELE DE MĂR

**BOLOHAN Diana Elena<sup>1</sup>, VOLF Mariana<sup>1</sup>, ANGHEL Roxana<sup>1</sup>**  
e-mail: bolohandiana@yahoo.com

**Abstract.** Fruit quality is influenced by climate factors, biological factors and applied agrotechnics - fertilization is an important part of crop technology. Two indices that participate together in achieving the main fruits organoleptic characteristics are soluble solids ( $^{\circ}\text{Bx}$ ) and titratable acidity (g malic acid /100g acid product). Variants were fertilized with mineral fertilizer (NPK 15.15.15) and foliar fertilizer (Cropmax and Pentakeep-G), applied in various doses. Nitrogen (Nt) and potassium (Kt) content of leaves were positively influenced by the doses of fertilizer. It has noticed that the variants fertilized with a larger amount of potassium led to changes of fruits titratable acidity (TA) values. There were also variations of soluble solids content (SSC). The values of qualitative parameters are different due to the fertilizer type applied in different doses.

**Key words:** apple, titratable acidity, soluble solids, fertilization

**Rezumat.** Calitatea fructelor este influențată de factorii climatici, biologici dar și de agrotehnica aplicată – fertilizarea fiind o verigă importantă a tehnologiei de cultură. Doi indici ce participă împreună la realizarea principalelor caracteristici organoleptice ale fructelor sunt: substanța uscată solubilă ( $^{\circ}\text{Bx}$ ) și aciditatea titrabilă (g acid malic/100g produs). Variantele au fost fertilizate cu îngrășământ mineral (NPK 15.15.15) și îngrășământ foliar (Cropmax and Pentakeep-G) în diferite doze. Conținutul de azot (Nt) și potasiu (Kt) din frunze a fost influențat pozitiv de dozele de îngrășământ aplicate. S-a observat că la variantele fertilizate cu o cantitate mai mare de potasiu au dus la modificarea acidității titrabile (TA) din mere. De asemenea, s-au înregistrat de asemenea variații semnificative ale conținutului de substanță uscată solubilă (SSC). Valorile acestor parametri calitativi sunt diferite și datorită sortimentului de îngrășământ aplicat, în diferite doze.

**Cuvinte cheie:** măr, aciditate titrabilă, substanță uscată solubilă, fertilizare

### INTRODUCTION

Harvest maturity is determined by some qualitative indices such as flesh firmness, skin color, flesh color, sugar content, and dry substance, total acids content. It is known that fruit quality is positively influenced by right ratios of nutrients supply. Nutrients are involved in accumulation of sugar and water.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

Nitrogen contributes in particular to increase fruit size. High nitrogen content in soil determine fruit firmness decrease, reduces sugar content and vitamins; and increased organic acids (Nomedá et al., 2006).

Potassium increases fruit firmness, sugar content and coloration intensity. High concentrations of potassium in fruits increases fruit acidity, which change report has sugar/organic acid and ultimately change their taste in order to increase flavor. Potassium presence facilitates the water entering into cells, by increasing its protoplasm capacity retention.

In a study regarding nitrogen fertilization, Raese and Drake determined that the highest fruit quality was achieved with the lowest rate of nitrogen and fruit quality was negatively related to nitrogen. (Raese and Drake, 1997). William and Billingsley (1974) found that high amount of N applied to Golden Delicious apple trees increased the amount of green coloured fruit and decreased fruit quality. TA in fruits increased as a consequence of potassium fertilisation rate, especially for trees featuring a low supply condition (William and Billingsley, 1974, Nielsen et al., 1998).

## **MATERIAL AND METHODS**

Researches were carried out in Vasile Adamachi Farm, Iassy county, in 2010. This study was conducted over one crop Idared variety apple trees, in a 4x4m spacing. Fertilizing treatments were randomised within one block; groups of three trees; in three replications. In the experience were studied 9 variants of fertilization with mineral and foliar fertilizers, including the blank:

- V1 – blank;
- V2 – mineral fertilization - N60:P60:K60 (15:15:15);
- V3 - mineral fertilization - N90:P90:K90 (15:15:15);
- V4 - foliar fertilization - Pentakeep - G;
- V5 – foliar fertilization - Cropmax ;
- V6 - mineral fertilization N60P60K60 + Pentakeep-G;
- V7 - mineral fertilization N90P90K90 + Pentakeep-G;
- V8- mineral fertilization N60P60K60 + Cropmax ;
- V9– mineral fertilization N90P90K90 + Cropmax;

NPK 15.15.15 was applied 1/3 in autumn and 2/3 in early spring. Foliar fertilizers (Cropmax and Pentakeep-G) were applied three times, starting when the fruit was 5 mm in diameter, and every two weeks after.

Following the fertilization was determined in the leaves, total nitrogen content through the Kjeldahl method and total potassium content by flam-photometry method.

Fruits were harvested at commercial harvest from each fertilized group of trees.

Quality factors were determined immediatly:

- soluble solids content (SSC) in juice sample obtained from a cross sectional slice was determined with a hand refractometer, expressed as °Brix;
- titratable acidity (TA) from juice samples was determined by titrating with 0.1 N, NaOH, and was expressed as g malic acid/100g fruit.

## **RESULTS AND DISCUSSIONS**

All treatments applied in the orchard had positive influence on total nitrogen content and total potassium content in leaves. Treatments applied to soil (V2, V3) were equally to the Nt content, 2.22%; regarding potassium content

there was a slight increase directly proportional to the doses of NPK 15.15.15. Foliar treatments induced a small increase of accumulation of these macronutrients.

Table 1

| Fertilization variants |                         | Macronutrients      |                     |
|------------------------|-------------------------|---------------------|---------------------|
|                        |                         | Nt % leaves content | Kt % leaves content |
| V 1                    | control                 | 1.93                | 0.95                |
| V 2                    | N60:P60:K60             | 2.22                | 1.16                |
| V 3                    | N90:P90:K90             | 2.22                | 1.22                |
| V 4                    | Pentakeep – G           | 2.14                | 1.01                |
| V 5                    | Cropmax                 | 2.08                | 1.06                |
| V 6                    | N60P60K60 + Pentakeep-G | 2.13                | 1.04                |
| V 7                    | N90P90K90 + Pentakeep-G | 2.21                | 1.10                |
| V 8                    | N60P60K60 + CropmaX     | 2.26                | 1.02                |
| V 9                    | N90P90K90 + Cropmax     | 2.29                | 1.21                |

Regarding the combined application of mineral fertilizers and foliar (Table 1):

- V8 and V9 recorded the highest values of Nt (%), so we can say that application of mineral fertilizer and Cropmax had the greatest positive influence;
- Pentakeep-G fertilizer in combination with mineral fertilizer increased with 10% Nt leaf for V6 treatment and 14.5% Nt for V7 treatment than controls (V1), the increase was directly proportional to the dose of mineral fertilizer;
- small doses of mineral fertilizer do not influence too much the increase of Kt in leaves, high doses has a more significant influence;
- Cropmax in combination with mineral fertilizer had a greater efficacy compared to Pentakeep-G.

Table 2

| Fertilization variants |                         | Qualitative indicators |                             |                |
|------------------------|-------------------------|------------------------|-----------------------------|----------------|
|                        |                         | SSC (° Bx)             | TA (malic acid/100 g fruit) | Ratio Sugar/TA |
| V 1                    | control                 | 10.97                  | 0.319                       | 25.04          |
| V 2                    | N60:P60:K60             | 10.87                  | 0.343                       | 23.04          |
| V 3                    | N90:P90:K90             | 11.07                  | 0.405                       | 19.91          |
| V 4                    | Pentakeep - G           | 10.83                  | 0.392                       | 20.10          |
| V 5                    | Cropmax                 | 10.47                  | 0.380                       | 20.05          |
| V 6                    | N60P60K60 + Pentakeep-G | 11.00                  | 0.417                       | 19.21          |
| V 7                    | N90P90K90 + Pentakeep-G | 10.67                  | 0.392                       | 19.79          |
| V 8                    | N60P60K60 + Cropmax     | 10.93                  | 0.380                       | 20.94          |
| V 9                    | N90P90K90 + Cropmax     | 11.10                  | 0.441                       | 18.31          |

Following the fertilization there was a significant increase of the leaf total nitrogen content 7-18% Nt, while the soluble solids content decreased by a maximum of 4.6%. Except V3, V6 and V9, there was a slight increase of this production quality indicator. In contrast, foliar treatment with Cropmax (V5) had a negative influence, preventing the accumulation of sugars.

All variants of fertilization increased titratable acidity in fruit, at a rate of 7.5 - 38.2%. N90 P90 K90 in combination with foliar fertilizers applied led to the significant increases in apple fruit acidity. The maximum was recorded for V9 variant (*Table 2*).

There was a very strong correlation between the sugar/acid ratio and nitrogen and potassium leaves content. According to the literature, value of this ratio for Idared variety is 18.7 for a balanced taste, value retrieved in V9.

## CONCLUSIONS

1. Compared with the sample from control variant, all other fertilizers have increased the value of total potassium content in leaves, up to 1.22% Kt.
2. Soluble solids content was slightly decreased in most variants of fertilization.
3. V9 has led to the largest increases of the values for all parameters included in the study, both in the leaves and apple fruits.
4. High concentrations of nitrogen and potassium increased fruit acidity, which has change ratio sugar / organic acid, increasing values for this indicator.

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# INFLUENCE OF FERTILIZATION AND CLIMATE ON APPLE FRUITS RIPENING UNDER AGROECOPEDOLOGICAL CONDITIONS OF "V. ADAMACHI" FARM, IAȘI

## INFLUENȚA FERTILIZĂRII ȘI A UNOR INDICI CLIMATICI ASUPRA GRADULUI DE MATURARE A FRUCTELOR LA MĂR, ÎN CONDITIILE AGROECOPEDOLOGICE ALE FERMEI „V. ADAMACHI”, IAȘI

*BOLOHAN Diana Elena<sup>1</sup>, VOLF Mariana<sup>1</sup>, ANGHEL Roxana<sup>1</sup>*  
e-mail: bolohandiana@yahoo.com

***Abstract.** In the present study was monitored the level of apples maturity at harvest for two years consecutively. Experience was conducted in "V. Adamachi" Farm, Iasi, by application of mineral and foliar fertilizers in an apple trees orchard, Idared variety. The optimal time for fruit harvesting is determined by their destination. Assessing the level of starch hydrolysis through iodine test and fruit firmness, are two tests used to estimate the optimal timing of harvesting. Differences were observed between the fertilization variants through the level of fruit maturity during the two years of study. Lowest values were recorded for control variant and foliar application variants only. Apple fruits firmness at harvest was generally lower in 2011 compared to 2010, and so starch content, fact that rushed harvest time.*

**Key words:** fertilization, apple fruits, starch content, firmness

***Rezumat.** În lucrarea de față s-au făcut aprecieri asupra gradului de maturare a merelor în momentul recoltării, timp de doi ani consecutiv. Experiența desfășurată în cadrul fermei didactice „V. Adamachi”, Iași, a urmărit efectele aplicării de îngrășămintelor minerale și foliare într-o livadă de măr soiul Idared. Momentul optim de recoltare a fructelor se stabilește în funcție de destinația acestora. Aprecierea gradului de hidroliză al amidonului prin proba cu iod, precum și determinarea fermității fructelor, sunt două teste utilizate în estimarea momentului optim de recoltare. S-au observat diferențe ale gradului de maturare atât între variantele de fertilizare cât și între cei doi ani de studiu. Valorile cele mai scăzute s-au înregistrat la varianta martor și la variantele fertilizate exclusiv foliar. Fermitatea fructelor cât și conținutul în amidon la recoltare, în anul 2011, au înregistrat valori mai ridicate, comparativ cu 2010, fapt ce a grăbit recoltarea acestora.*

**Cuvinte cheie:** fertilizare, măr, conținutul în amidon, fermitate

### INTRODUCTION

Idared variety is one of the most popular and commercially important apple cultivars in Romania. Idared fruits are harvested in the end of september, beginning of october, and can be stored for 5 - 7 months in order to be placed on

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

the market in spring time. To ensure maximum storability, fruits should be harvest when mature, but not fully ripped, otherwise physiological processes are underway fact which complicates long term storage (Ingle et al., 2000). Starch index for should be 6 or 7 according to the “European code for assessing the degree of starch hydrolysis”. Starch estimates at harvest were not consistently affected by nitrogen, but tended to be lower in fruits from trees receiving the low rate of nitrogen (136g N/ tree) in august. The highest fruit firmness occurred in fruit from trees receiving 136g N/tree in august (Drake, 2002). Knowing fruit firmness is very important; it enables us to determine harvest time, packing and transport, as well as quality and shelf life of apples (Beceanu, 2002).

## **MATERIAL AND METHODS**

Researches were carried out in Vasile Adamachi Farm, Iassy county, over two years. This study was conducted over one crop Idared variety apple trees, in a 4x4m spacing. Fertilizing treatments were randomised within one block; groups of three trees; in three replications. In the experience were studied 9 variants of fertilization with ground and foliar fertilizers, including the control:

- V1 – control;
- V2 – mineral fertilization - N60:P60:K60;
- V3 - mineral fertilization - N90:P90:K90;
- V4 - foliar fertilization - Pentakeep - G;
- V5 – foliar fertilization - Cropmax ;
- V6 - mineral fertilization N60P60K60 + Pentakeep-G;
- V7 - mineral fertilization N90P90K90 + Pentakeep-G;
- V8- mineral fertilization N60P60K60 + Cropmax ;
- V9– mineral fertilization N90P90K90 + Cropmax;

NPK 15.15.15 was applied 1/3 in autumn and 2/3 in early spring. Foliar fertilizers (Cropmax and Pentakeep-G) were applied three times, starting when the fruit was 5 mm in diameter, and every two weeks after.

The starch index was determined by using a 0.1N potassium iodine solution. Firmness [UP] is a measure of texture and it was measured with a penetrometer Stanhope Seta. These two indicators were determined immediately after harvesting apple fruit samples.

## **RESULTS AND DISCUSSIONS**

Sum of temperatures during the vegetation period in the two years of study, was approximately equal and recorded slight positive deviations from normal. Regarding the rainfall, in 2011 were recorded 353.2 mm during the growing season, as opposed to 2010 when rainfall recorded for the same period were in half.

The literature recommends a period about 165-170 days from blooming to harvesting for Idared variety, because it is suitable for long-term storage ( 5-7 months in cold storage). Due to slightly higher temperatures and more abundant rainfall in 2011, there was a more accelerated ripening of the fruits for Idared apple variety, compared to previous year.

It is noticed that in 2010, as well as in 2011, trees have blossomed in the same time, but harvest time was different; there was a gap of about two weeks between 2010 and 2011 (table 1).

Table 1

**Blooming and harvest time, Idared variety**

| Year | Blooming time | Harvest time  | Number of days |
|------|---------------|---------------|----------------|
| 2010 | 25 aprilie    | 10 octombrie  | 168            |
| 2011 | 15 aprilie    | 25 septembrie | 153            |

Compared with the control sample, we observed that, in 2010 a better firmness of apple fruits was obtained in V2 and V8 variants. A lower firmness was achieved for fertilization variants V4 and V9. Over the other variants of fertilization there isn't a significant deviation from the control (table 2).

In 2011 an improved firmness was observed in V2 and V5 compared to the control variant. A lower firmness was recorded in V4, V6 and V9. Firmness of fruits harvested in 2011 showed lower values than those from the previous year, values that shows a better firmness.

The hydrolysed starch for apple fruits harvested in 2011 was higher than that of fruits harvested in the previous year; so the amount of unhydrolyzed starch was lower. Compared with the sample control, we find that nearly all fertilization variants rushed starch hydrolysis resulting higher values for starch index.

Table 2

**Influența fertilizării asupra gradului de maturare al fructelor in 2011**

| Fertilization variants |                         | Starch index (0-10) |      | Firmness [UP 1mm] |      |
|------------------------|-------------------------|---------------------|------|-------------------|------|
|                        |                         | 2010                | 2011 | 2010              | 2011 |
| V1                     | control                 | 5.7                 | 7.7  | 29.1              | 27.1 |
| V2                     | N60:P60:K60             | 6.7                 | 7.3  | 28.8              | 25.5 |
| V3                     | N90:P90:K90             | 5.7                 | 6.3  | 29.8              | 27.0 |
| V4                     | Pentakeep - G           | 6.3                 | 5.3  | 30.6              | 34.1 |
| V5                     | Cropmax                 | 6.3                 | 8.3  | 29.0              | 26.3 |
| V6                     | N60P60K60 + Pentakeep-G | 6.7                 | 7.0  | 29.8              | 30.9 |
| V7                     | N90P90K90 + Pentakeep-G | 6.3                 | 7.3  | 29.2              | 27.1 |
| V8                     | N60P60K60 + Cropmax     | 5.7                 | 8.3  | 28.7              | 27.1 |
| V9                     | N90P90K90 + Cropmax     | 5.7                 | 8.7  | 32.5              | 32.5 |

Fruits harvested in 2011 showed various degrees of hydrolysed starch content, comparing control with variants of fertilization. It highlights the fertilization variants with Cropmax that favored the starch hydrolysis speed in apple fruits.

A correlation between fruit firmness and degree of starch hydrolysis in apple fruits shows for 2010 low fruit firmness for a low degree of starch hydrolysis, while in 2011 was registered an inversely increase of fruit firmness with a higher degree of starch hydrolysis. This is due to differences of precipitation recorded in the studied years, 2011 registered heavy rainfalls compared to 2010, fact that ensured better hydration of fruits and thereby increased fruit firmness.

## CONCLUSIONS

1. Compared with the control sample, nearly all fertilizations variants rushed starch hydrolysis, it is highlighted that fertilization variants with Cropmax led to higher values for starch index.

2. Apple fruits firmness values registered in the two-year study shows that in 2011 the fruits were harvested at a ripeness level more advanced compared to 2010, although the number of days from bloom to harvest was reduced.

3. A correlation between fruit firmness and starch hydrolysis in apple fruit for studied variants of fertilization reveals: in 2010, fruit firmness was lower at a low starch hydrolysis, while in 2011 fruit firmness was higher for a more pronounced starch hydrolysis, fact explained through the heavy rainfall in the second year of study.

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# RESEARCHES ON THE INFLUENCE OF PERENNIAL LEGUMES IN INCREASING THE EFFICIENCY OF TEMPORARY MEADOW

## CERCETĂRI PRIVIND INFLUENȚA LEGUMINOASELOR PERENE ÎN SPORIREA RANDAMENTULUI PAJIȘTILOR TEMPORARE

IANCU D.<sup>1</sup>

e-mail: stiancu50@yahoo.com

**Abstract.** At the Experimental Center for Meadow Culture, Preajba, Gorj County, was located a bi factorial experience of 4 x 4 type, in 3 repetition (4 mixes and 4 doses of nitrogen). In the mixture formed only from 3 grasses (in equal parts), productions were 1,69-5,57 D.M. t/ha. In the mixture composed of grasses (60%) and *Trifolium pratense* (40%) were made 6,63 2.65d.s. If with grasses (60%) has been added *Lotus corniculatus* (40%) the production was 2.57-6,16 t/ ha D.M. When in the mixture has occurred *Trifolium repens* (40%) with grasses (60%) were observed productions of 3.20- 6,30 t/ha D.M.

**Key words:** cocksfoot, birds foot, fertilization, fescue, white and red clover, timothy.

**Rezumat.** La Centrul Experimental pentru Cultura Pajiștilor, Preajba, județul Gorj, s-a amplasat o experiență bifactorială de tipul 4x4, în 3 repetiții (4 amestecuri și 4 doze de azot). În amestecul format numai din 3 graminee (în părți egale), producțiile au fost de 1,69-5,57 t/ha s.u. În amestecul alcătuit din graminee (60%) și *Trifolium pratense* (40%) s-au realizat 2,65-6,63 t/ha s.u. Dacă alături de graminee (60 %) s-a adăugat *Lotus corniculatus* (40%) producția a fost de 2,57-6,16 t/ha s.u. Când în amestec a intervenit *Trifolium repens* (40 %) alături de graminee (60 %) s-au înregistrat producții de 3,06-6,30 t/ha s.u.

**Cuvinte cheie:** golomăț, ghizdei, fertilizare, păiuș, trifoi alb și roșu, timoftică.

### INTRODUCTION

In Dolj county the permanent grasslands have an large extension, as being one of the most important categories of agricultural land use. Large areas of permanent grassland are in a advanced stage of degradation, realizing low yields and mediocre or poor quality.

One of the most important steps in the process of improvement the degraded permanent grasslands in northern Oltenia is the establishment of temporary grasslands (Ionescu, 2003).

The research has revealed high productive potential of temporary grassland compared to permanent grassland improved by surface methods. The laws and

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<sup>1</sup> University of Craiova, Romania

regulations in normal years in terms of rainfall, the temporary meadows can easily realized 6-8 t/ha D.M. (Osiceanu and Ionescu, 2009; Motca et al., 1994).

In terms of phasing the annual production, temporary meadows performs much better, giving, under the grassland system, 2-3 scythe a year and used by grazing, 3-4 cycles. The floristic composition of temporary grassland is superior to permanent grassland. The temporary grassland legumes may have consistent participation, while in the permanent participation these species have sometimes a symbiotic participation (Pavel and Lulea, 1976).

The feed obtained from temporary grassland is superior in terms of quality, characterized by a high content of protein, minerals and vitamins and lower cellulose. Considering all these aspects is necessary for areas occupied by temporary grass to grow, replacing permanent grassland, heavily degraded area where surface measures prove ineffective.

## MATERIAL AND METHODS

The research were conducted at the Experimental Center for Meadows Culture Preajba, the Gorj county, where was placed a bi factorial experience, by the method of subdivided parcels in three repetitions (Iancu S., 2009).

Experimental factors were as follows:

**The A factor** – the mixture of species:  $a_1$  - *Dactylis glomerata* 33% + *Festuca pratensis* 33% + *Phleum pratense* 34%;  $a_2$  - *Dactylis glomerata* 20% + *Festuca pratensis* 20% + *Phleum pratense* 20% + *Trifolium pratense* 40%;  $a_3$  - *Dactylis glomerata* 20% + *Festuca pratensis* 20% + *Phleum pratense* 20% + *Lotus corniculatus* 40%;  $a_4$  - *Dactylis glomerata* 20% + *Festuca pratensis* 20% + *Phleum pratense* 20% + *Trifolium repens* 40%.

**The B factor** – the nitrogen dose:  $b_1= 0$ ;  $b_2= 50$  kg/ha;  $b_3= 100$ kg/ha;  $b_4= 150$  kg/ha

The quantities referred to graduations  $b_2$  and  $b_3$  were managed entirely in the spring. The dose of 150 kg / ha was applied in two fractions: 100 kg / ha in the spring and another 50 kg / ha after the first mowing. Together with nitrogen, in spring, were applied 50 kg / ha  $P_2O_5$  and 50 kg / ha  $K_2O$ .

Complex 15-15-15 and ammonium nitrate were used as fertilizer, making the calculations required to meet experimental protocol. Was harvested under a system of grassland at earing grasses and legumes flowering.

The statistical processing was done using ANOVA program and the interpretation with DL 5 %, DL 1% și DL 0,1 %.

## RESULTS AND DISCUSSION

On average in the two years of experimentation (2010-2011), were obtained the following important experimental results:

➤ The unilateral influence of legume species in the mixture on the production of temporary meadows.

Depending on the mixture composition, the yields ranged from 3,98 t/ha D.M. (*Dactylis glomertata* 33 % + *Festuca pratensis* 33 % + *Phleum pratense* 34 %) and 5,02 t/ha D.M. (same grass, but in proportion of 20% each + *Trifolium pratense* 40 %).

The percentage increases were between 18-26% and the production between 0.71 and 1.04 t / ha D.M., all were statistic uninsured (table 1).

Table 1

**The influence of legumes species separate from the mixture on the production of temporary pastures**

| Nr. crt. | The mixture  | The production t/ha s.u. | %   | The Diference | The signifi-cance |
|----------|--|--------------------------|-----|---------------|-------------------|
| 1        | a <sub>1</sub> D.g. 33% + F.p. 33% + Ph.p. 34%                                 | 3,98                     | 100 | -             | Control           |
| 2        | a <sub>2</sub> D.g. 20% + F.p. 20% + Ph.p. 20% + <i>Trifolium pratense</i> 40% | 5,02                     | 126 | 1,04          | —                 |
| 3        | a <sub>3</sub> D.g. 20% + F.p. 20% + Ph.p. 20% + <i>Lotus corniculatus</i> 40% | 4,69                     | 118 | 0,71          | —                 |
| 4        | a <sub>4</sub> D.g. 20% + F.p. 20% + Ph.p. 20% + <i>Trifolium repens</i> 40 %  | 4,84                     | 122 | 4,86          | —                 |

DL 5% = 1,19 t/ha D.M.; DL 1 % = 1,81 t/ha D.M.; DL 0,1 % = 2,91 t/ha D.M.

➤ The unilateral influence of nitrogen dose on the production of temporary pastures.

At the unfertilized witness, the production was 2.49 t / ha D.M. By increasing the nitrogen dose from 50 to 150 kg / ha, production gradually increased from 4.43 to 6.17 t / ha D.M. The percentage increases ranged from 78% and 148% (table 2).

Table 2

**The unilateral influence of nitrogen dose on the production of temporary meadows.**

| Nr. crt. | The N dose (kg/ha) on a 50 P <sub>2</sub> O <sub>5</sub> , 50 K <sub>2</sub> O background | The production t/ha s.u. | %   | The diference | The segnification |
|----------|---|--------------------------|-----|---------------|-------------------|
| 1        | b <sub>1</sub> 0  | 2,49                     | 100 | -             | Control           |
| 2        | b <sub>2</sub> 50   | 4,43                     | 178 | 1,94          | ***               |
| 3        | b <sub>3</sub> 100  | 5,44                     | 218 | 2,95          | ***               |
| 4        | b <sub>4</sub> 150  | 6,17                     | 248 | 3,68          | ***               |

DL 5 % = 0,54 t/ha D.M.; DL 1 % = 0,74 t/ha D.M.; DL 0,1 % = 0,99 t/ha D.M.

Production increases ranged between 1.94 and 3.68 t / ha DM, all highly significant.

- The interaction influence of the grasses and legumes mixture with nitrogen dose on production.

The special influence of the nitrogen doses and especially the higher doses of 100-150 kg / ha, is due to the specific rain falls in spring, in the growing season, characterized both by significant rain quantity and the uniform distribution. During a drought years or with unevenly distributed rainfall, such doses are not indicated, because fertilizers cannot be exploited by plants.

The combined influence of two experimented factors (the mixture and nitrogen dose), have differentiated the production from 1.69 t / ha D.U. up to 6.63 t / ha D.M. and the lowest production was obtained from the mixture consisting only of grasses, fertilization, and the highest of grass mixture with *Trifolium pratense*, fertilized with 150 kg / ha nitrogen.

All three nitrogen doses tested (N50, N100 and N150) have very significant production increases, except for the a4b2 combination.

Close to maximum yields were obtained using mixtures of grasses with *Trifolium repens*, all after fertilization with 150 kg / ha N (6.30 t/ha D.M.). Mixture consisting on grasses species only gave this variant of fertilization, a slightly smaller quantity (5.57 t/ha D.M.). This production increase was significant. The mineral fertilization led to production increases ranging from 1.34 t/ha to 3.98 t/ha. The application of chemical fertilizers made percentage gains between 43.8% to 150.2%, to the unfertilized witness (table 3).

In all four mixtures, increasing the nitrogen dose caused progressive increases in dry matter from unfertilized witness.

Note that the highest relative yields and hence, the greatest differences between fertilized and control variants were obtained from the mixture of grasses only. This mixture gave the lowest production at the no fertilizer conditions, but has very well capitalized the nitrogen applied, demonstrating the need for strict use of chemical fertilizers in such mixtures.

In conclusion, averaged over the two years of research, the mixtures of grasses with legumes gave higher production from the mixture of grasses only. The quantitative differences between the mixtures were low, the first priority being to one based on *Trifolium pratense*.

Between the doses of nitrogen used were noted the 100 and 150 kg / ha, which resulted in all cases in very significant quantitative increases.



Table 3

The combined influence of the legume from the mixture with the nitrogen dose on the temporary grassland production

| Nr. crt. | The mixture  | The N dose (kg/ha) | The production t/ha D.M. | %   | The difference | The signification |
|----------|--|--------------------|--------------------------|-----|----------------|-------------------|
| 1        | Graminee<br>100 %<br>a <sub>1</sub>  | b <sub>1</sub> 0   | 1,69                     | 100 | -              | Martor            |
| 2        |  | b <sub>2</sub> 50  | 3,86                     | 228 | 2,17           | ***               |
| 3        |  | b <sub>3</sub> 100 | 4,82                     | 285 | 3,13           | ***               |
| 4        |  | b <sub>4</sub> 150 | 5,57                     | 330 | 3,88           | ***               |
| 5        | Graminee 60%<br>+ <i>Trifolium</i><br><i>pratense</i> 40%<br>a <sub>2</sub>    | b <sub>1</sub> 0   | 2,65                     | 100 | -              | Martor            |
| 6        |  | b <sub>2</sub> 50  | 4,79                     | 181 | 2,14           | ***               |
| 7        |  | b <sub>3</sub> 100 | 6,02                     | 227 | 3,37           | ***               |
| 8        |  | b <sub>4</sub> 150 | 6,63                     | 250 | 3,98           | ***               |
| 9        | Graminee 60%<br>+ <i>Lotus</i><br><i>corniculatus</i><br>40%<br>a <sub>3</sub> | b <sub>1</sub> 0   | 2,57                     | 100 | -              | Martor            |
| 10       |  | b <sub>2</sub> 50  | 4,68                     | 182 | 2,11           | ***               |
| 11       |  | b <sub>3</sub> 100 | 5,33                     | 207 | 2,76           | ***               |
| 12       |  | b <sub>4</sub> 150 | 6,16                     | 240 | 3,59           | ***               |
| 13       | Graminee 60%<br>+ <i>Trifolium</i><br><i>repens</i> 40 %<br>a <sub>4</sub>     | b <sub>1</sub> 0   | 3,06                     | 100 | -              | Martor            |
| 14       |  | b <sub>2</sub> 50  | 4,40                     | 144 | 1,34           | *                 |
| 15       |  | b <sub>3</sub> 100 | 5,59                     | 183 | 2,53           | ***               |
| 16       |  | b <sub>4</sub> 150 | 6,30                     | 206 | 3,24           | ***               |

DL 5 % = 1,08 t/ha D.M.; DL 1 % = 1,47 t/ha D.M.; DL 0,1 % = 1,97 t/ha D.M.

## CONCLUSIONS

1. The best mixture for temporary meadows from the Subcarpathian area of Oltenia was composed of *Dactylis glomerata* (20%), *Festuca pratensis* (20%), *Phleum pratense* (20%) and *Trifolium pratense* (40%), with an average production of 5,41 t/ha D.M.

2. Compared to the mixture composed only of grasses, he realized a production increase of 26%.

The mineral fertilization with N<sub>50</sub>, N<sub>100</sub> and N<sub>150</sub> brought production increases of 1.94 to 3.68 t / ha D.M., very significant.

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# THE EFFECT OF CHEMICAL FERTILIZERS AND REMANING EFFECT OF ORGANIC FERTILIZERS UPON THE PRODUCTION AT WINTER WHEAT IN SUGAR BEET – WHEAT – CORN ROTATION

## EFFECTUL ÎNGRĂȘĂMINTELOR CHIMICE ȘI REMANENȚA CELOR ORGANICE ASUPRA PRODUCȚIEI GRÂULUI DE TOAMNĂ, ÎN ROTAȚIA SFECLĂ PENTRU ZAHĂR – GRÂU – PORUMB

*ANDRIEȘ Iuliana*<sup>1</sup>, *CIURUȘNIUC Ana-Maria*<sup>1</sup>, *COTEANU A.Gh.*<sup>1</sup>  
e-mail: iulianaandries@yahoo.com.au

**Abstract.** *In bifactorial experience placed after the method of subdivided plots at Ezăreni station, Iași has watched the effect of nitrogen fertilizers ( $N_0$ ,  $N_{40}$ ,  $N_{80}$ ,  $N_{120}$ ,  $N_{160}$ ) applied on the next agrofunds:  $P_{64}K_{64}$ ; manure 30 t/ha applied on pre-plant (sugar beet) and chaff 5 t/ha + the rest from sugar beet applied to winter wheat. It found that the greatest production of 7940 kg/ha was obtained in chemically fertilized variant followed by organic fertilized with 7055 kg/ha. The fertilization with chemical fertilizers, on average, determined in  $N_0$  variant a production of 6228 kg/ha and 8441 kg/ha in the variant fertilized with  $N_{160}$ , production increase being 35,53%. The interaction of factors highlighted variant fertilized with  $P_{64}K_{64} \times N_{160}$ , who obtained a maximum production of 8788 kg/ha, with an increase of 42,75% compared to variant control.*

**Key words:** wheat, fertilizer, wheat production

**Rezumat.** *Într-o experiență bifactorială, organizată în parcele subdivizate la ferma Ezăreni-Iași, s-a urmărit efectul îngrășămintelor cu azot ( $N_0$ ,  $N_{40}$ ,  $N_{80}$ ,  $N_{120}$ ,  $N_{160}$ ) aplicate pe grofundurile  $P_{64}K_{64}$ , gunoi de grajd 30 t/ha aplicat la planta premergătoare (sfeclă pentru zahăr) și paie tocate 5 t/ha + coletele de sfeclă aplicate la grâul de toamnă. S-a constatat că cea mai mare producție, de 7940 kg/ha, s-a obținut în varianta fertilizată chimic, urmată cu 7055 kg/ha de variantele fertilizate organic. Fertilizarea cu îngrășămintă chimice, în medie, a determinat o producție de 6228 kg/ha în varianta  $N_0$  și 8441 kg/ha în varianta fertilizată cu  $N_{160}$ , sporul de producție fiind de 35,53%. Interacțiunea dintre factori a scos în evidență varianta fertilizată cu  $P_{64}K_{64} \times N_{160}$ , care a realizat o producție maximă de 8788 kg/ha, cu un spor de 42,75% față de varianta martor.*

**Cuvinte cheie:** grâu, fertilizare, producția de grâu

### INTRODUCTION

From the research carried out so far both in Romania and foreign show that nitrogen has a significant influence on winter wheat yield (Hera et al., 1984, Mihăilă

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

et al., 1992, 1980). It was also shown that application of nitrogen and phosphorus fertilizers increase the productivity indices (Hera et al., 1971, Toncea, 1985).

The most important factor determining the growth and of winter wheat caryopses is fertilization with nitrogen (Dorneanu, 1976, Hera, 1984).

The fertilization applied in autumn and spring in divided doses increased the coefficient of use of fertilizers with 8 – 9%. The cumulative effect of nitrogen and phosphorus has a major influence in increasing production of wheat (Burlacu Gh. et al., 2007).

In a long experience at Ezareni station, Iasi, there was realized higher productions of nitrogen fertilizers variants overlap with retention of manure applied to preceding wheat pre – plant: sugar beet (Mogârzan et al., 1998).

When applying nitrogen fertilizers may be an appropriate strategy to ensure the plants requirements for nitrogen. Generally, the nitrogen fertilizers applied to the seedbed preparation are positively affects the wheat plant development and implementation later increases the amount of protein caryopses (Fowler and Brydon, 1989).

The purpose of this paper is to quantify the effect of chemical fertilizers and remaining organic fertilizers over caryopses production for winter wheat.

## MATERIAL AND METHODS

The experience was establish in autumn 2010 on Ezareni station, Iasi, by the method in three repetitions subdivided parcels.

The experimental factors were:

A factor – Organic and chemical fertilizer phosphorus and potassium with three graduations:

a<sub>1</sub> – manure 30 t/ha applied to pre – plant (sugar beet) + P<sub>64</sub>K<sub>64</sub>;

a<sub>2</sub> – chaff 5 t/ha applied to pre – plant + the rest from sugar beet applied to wheat + P<sub>64</sub>K<sub>64</sub>;

a<sub>3</sub> – P<sub>64</sub>K<sub>64</sub>.

B factor – Chemical fertilizer with nitrogen, phosphorus and potassium:

b<sub>1</sub> – N<sub>0</sub>P<sub>0</sub>K<sub>0</sub>; b<sub>2</sub> – N<sub>40</sub>P<sub>64</sub>K<sub>64</sub>; b<sub>3</sub> – N<sub>80</sub>P<sub>64</sub>K<sub>64</sub>; b<sub>4</sub> – N<sub>120</sub>P<sub>64</sub>K<sub>64</sub>; b<sub>5</sub> – N<sub>160</sub>P<sub>64</sub>K<sub>64</sub>

In autumn plowing were incorporated 64 kg/ha a.i. phosphorus and potassium.

In C agrofounded, the nitrogen fertilizers were applied to split, the first half (40 kg N/ha) in the second and third variants and 80 kg N/ha in fourth and fifth variants, the second half of 40 kg N/ha was applied in late spring in third, fourth and fifth variants and the third half was applied in heading stage in fifth variant.

The experience was mounted on a cambic chernozem soil with medium fertility – good, the content in phosphorus and potassium is moderate – normal with pH values mild acid to neutral reaction and with clayey-sandy texture.

We used the Glosa variety created by ICCPT Fundulea.

## RESULTS AND DISCUSSIONS

In this experience we have analyzed the influence of both chemical and organic fertilizers remaining on caryopses production in crop year 2010 - 2011.

We have studied the influence of A factor on winter wheat production (tab. 1) and we have observed that the chemical fertilizers with phosphorus and potassium have made the biggest production of 7940 kg/ha, significantly, with 12,53% higher than version control which has obtained a production of 7055 kg/ha.

Winter wheat productivity was positively influenced by nitrogen doses (tab. 2) the differences of production being very significant. The most carryopses production of 8441 kg/ha was performed on variant fertilized with 160 kg N/ha followed by N<sub>120</sub>, N<sub>80</sub> and N<sub>40</sub> variants with the production of 8039 kg/ha, 7787 kg/ha, respectively 6836 kg/ha.

The productions increased with enlarge doses of nitrogen, the percentage of growth was 9,76% in the N<sub>40</sub> version, 25,03% in the N<sub>80</sub> version, 29,07% in N<sub>120</sub> version and 35,53% in the N<sub>160</sub> version.

*Table 1*

**The remanent effect of organic fertilizers on wheat production in crop year 2010 – 2011**

| <b>A factor<br/>The remanent organic<br/>fertilizers + chemical fertilizers</b> | <b>Average<br/>Kg/ha</b> | <b>%</b> | <b>Differences<br/>Kg/ha</b> | <b>Significance</b> |
|---|--------------------------|----------|------------------------------|---------------------|
| a <sub>1</sub> Manure 30 t/ha + P <sub>64</sub> K <sub>64</sub>                 | 7055                     | 100,00   | Mt.                          |                     |
| a <sub>2</sub> Chaff 5 t/ha + P <sub>64</sub> K <sub>64</sub>                   | 7404                     | 104,94   | 349,3                        |                     |
| a <sub>3</sub> Chemical fertilizer P <sub>64</sub> K <sub>64</sub>              | 7940                     | 112,54   | 884,4                        | *                   |
| DI 5%   | 588,1 kg/ha              |          |                              |                     |
| DI 1%   | 975,2 kg/ha              |          |                              |                     |
| DI 0,1%   | 1821,4 kg/ha             |          |                              |                     |

The figure 1 shows the correlation between the nitrogen doses applied and the obtained production and it's observed that between these two components is a significant correlation ( $R^2 = 0,9833^*$ ).

Analyzing the effect of chemical fertilizers interation and retition of organic fertilizers on production (tab. 3) we observed that the agrofound manure 30 t/ha + P<sub>64</sub>K<sub>64</sub> variant fertilized with 160 kg N/ha realized a production of 8124 kg/ha, very significant with 31,96% higher than the control variant which has obtained a yield of 6156 kg/ha. Compared with the control variant, N<sub>120</sub> realized a growth rate of 18,98% being significant. N<sub>80</sub> and N<sub>40</sub> variants have obtained higher productions than the control variant, with 14,89% and respectively 7,17%, but statistically uninsured.

On the chaff 5 t/ha + P<sub>64</sub>K<sub>64</sub> agrofound, productions was realized with significant differences in interaction with N<sub>80</sub> (7930 kg/ha), N<sub>120</sub> (9113 kg/ha) and N<sub>160</sub> (8412 kg/ha). In variant fertilized with 40 kg N/ha was obtained a yield of 6622 kg/ha, with 7,56% more than version control, but not statistically assured and the unfertilized variant acheved a small production with 211 kg/ha compared to control variant.

Table 2

The effect of nitrogen fertilizer on wheat production in crop year 2010–2011

| B factor<br>Nitrogen doses | Average<br>Kg/ha | %      | Differences<br>Kg/ha | Significance |
|----------------------------|------------------|--------|----------------------|--------------|
| N <sub>0</sub>             | 6228             | 100,00 | Mt.                  | -            |
| N <sub>40</sub>            | 6836             | 109,76 | 607,4                | *            |
| N <sub>80</sub>            | 7787             | 125,03 | 1559,4               | ***          |
| N <sub>120</sub>           | 8039             | 129,07 | 1811,4               | ***          |
| N <sub>160</sub>           | 8441             | 135,53 | 2212,9               | ***          |
| DI 5%                      | 554,0 kg/ha      |        |                      |              |
| DI 1%                      | 753,0 kg/ha      |        |                      |              |
| DI 0,1%                    | 1008,4 kg/ha     |        |                      |              |

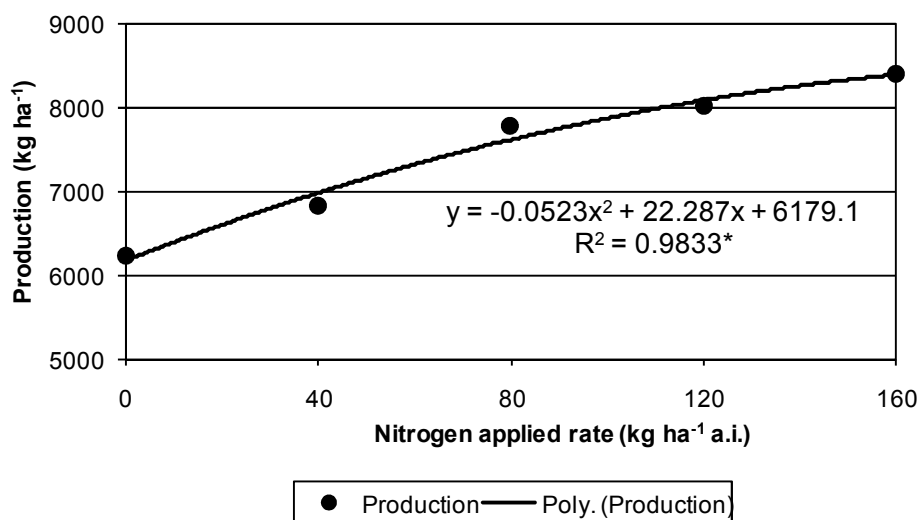


Fig. 1 - The correlation between the nitrogen doses applied and obtained production

All variants on agrofound that have received only chemical fertilizers have achieved higher production version control. Therefore, the variant fertilized with nitrogen had a production increase 6,93%, but wasn't statistically assured while the variant fertilized with 40 kg N/ha achieved a production of 7288 kg/ha, the differences being significant. The variants wich were applied 80, 120 and 160 kg N/ha obtained production of 8630 kg/ha, 8681 kg/ha and 8788 kg/ha, compared to the control variant differences are very significant.

Based on the analyzes performed it's observed that one kilogram a.i. of fertilizer to caryopses increased the production of 2,626 kg to 10,591 kg.

The figure 2 shows the correlation between the nitrogen doses applied on the three agrofonduri and obtained productions, in this case all three

nitrogen dose applied on agrofound is correlated with obtained production, the standard deviation is statistically assured (significant).

Table 3

The effect of interaction of chemical fertilizers and retention of organic fertilizers on wheat production in crop year 2010 – 2011

| A factor<br>Organic<br>fertilizers+<br>PK                  | B factor<br>Nitrogen<br>doses | Average<br>Kg/ha | %      | Differences<br>Kg/ha | Significances | caryopses<br>kg to 1 kg<br>fertilizer<br>a.i. |  |
|--|-------------------------------|------------------|--------|----------------------|---------------|---|--|
| Manure<br>30 t/ha<br>+<br>P <sub>64</sub> K <sub>64</sub>  | N <sub>0</sub>                | 6156             | 100,0  | Mt.                  | -             | -   |  |
|  | N <sub>40</sub>               | 6598             | 107,17 | 441,3                |               | 2,626   |  |
|  | N <sub>80</sub>               | 7073             | 114,89 | 916,6                |               | 4,403   |  |
|  | N <sub>120</sub>              | 7325             | 118,98 | 1168,3               | *             | 4,710   |  |
|  | N <sub>160</sub>              | 8124             | 131,96 | 1967,6               | ***           | 6,831   |  |
| Chaff<br>5 t/ha<br>+<br>P <sub>64</sub> K <sub>64</sub>    | N <sub>0</sub>                | 5945             | 96,57  | -211,0               |               | -   |  |
|  | N <sub>40</sub>               | 6622             | 107,56 | 465,3                |               | 2,769   |  |
|  | N <sub>80</sub>               | 7930             | 128,81 | 1774,0               | ***           | 8,528   |  |
|  | N <sub>120</sub>              | 8113             | 131,79 | 1957,0               | ***           | 7,891   |  |
|  | N <sub>160</sub>              | 8412             | 136,64 | 2255,3               | ***           | 7,830   |  |
| Chemical<br>fertilizers<br>P <sub>64</sub> K <sub>64</sub> | N <sub>0</sub>                | 6583             | 106,93 | 426,3                |               | -   |  |
|  | N <sub>40</sub>               | 7288             | 118,38 | 1131,0               | *             | 6,732   |  |
|  | N <sub>80</sub>               | 8360             | 135,80 | 2203,0               | ***           | 10,591  |  |
|  | N <sub>120</sub>              | 8681             | 141,01 | 2524,3               | ***           | 10,178  |  |
|  | N <sub>160</sub>              | 8788             | 142,75 | 2631,3               | ***           | 9,136   |  |
| DI 5%  |                               | 959,5 kg/ha      |        |                      |               |   |  |
| DI 1%  |                               | 1304,2 kg/ha     |        |                      |               |   |  |
| DI 0,1%  |                               | 1746,7 kg/ha     |        |                      |               |   |  |

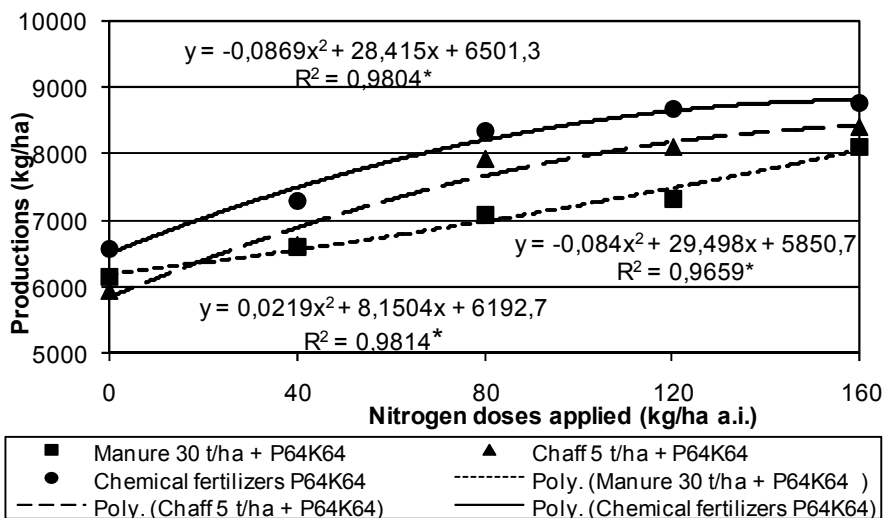


Fig. 2 - The correlation between nitrogen doses applied and obtained productions to the three adrofound

## CONCLUSIONS

1. Analyzing the remaining effect of organic and chemical fertilizers it is observed that the application of chemical fertilizers has obtained the highest yield of 7940 kg/ha wheat.
2. In variants that remaining manure was followed three years and chaff after two years, yields were reduced by 7055 kg/ha and 7040 kg/ha.
3. The productions of carryops increased with enlarge nitrogen doses applied, the highest of 8441 kg/ha being at N<sub>160</sub>.
4. The interaction of factors highlighted version P<sub>64</sub>K<sub>64</sub> x N<sub>160</sub> with a production of 8788 kg/ha, the differences from the control variant being very significant.
5. The correlations between nitrogen doses applied and the productions obtained are significant, the standard deviation being statistically assured.

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# THE EFFECT OF CONCENTRATION OF SOME BIOSTIMULATORS UPON THE CARYOPSES OF THREE WHEAT CULTIVARS – A RESEARCH STUDY CARRIED OUT AT EZARENI STATION, IAȘI

## EFFECTUL CONCENTRAȚIEI UNOR BIOSTIMULATORI ASUPRA PRODUCȚIEI DE CARIOPSE LA TREI SOIURI DE GRÂU, ÎN CONDIȚIILE FERMEI EZĂRENI – IAȘI

*ANDRIEȘ Iuliana*<sup>1</sup>, *CIURUȘNIUC Ana-Maria*<sup>1</sup>, *COTEANU A.Gh.*<sup>1</sup>  
e-mail: iulianaandries@yahoo.com.au

**Abstract.** *In climatic conditions of the agricultural year 2010-2011 at Ezareni station, Iași, on a cambic chernozem soil was followed the effect of BCO – 4DMA, BCO 4 K and BCO 4K + Zn acetate biostimulators in concentration of 12,5 ppm, 25 ppm and 50 ppm upon the cariops production of three wheat cultivars - Arieșan, Crina and Boema. BCO 4K + Zn acetate biostimulator determined the highest production of 8213 kg/ha with 15,77% higher than the control variant – BCO – 4DMA; compared with control variant which was treated with water, increase production was 31,57%; between concentrations of biostimulators wasn't significant differences; Boema variety has ranked on the first place with a production of 7997,8 kg/ha. Interaction between factors highlighted variant BCO 4K +Zn acetate x 50 ppm x Boema with 9007 kg/ha, increase production being very significant.*

**Key words:** biostimulators, concentration, wheat, production.

**Rezumat.** *În condițiile climatice din anul agricol 2010-2011, la ferma Ezăreni – Iași, pe un sol cernoziom cambic s-a urmărit efectul biostimulatorilor BCO – 4DMA, BCO 4 K și BCO 4K + acetat de Zn, în concentrații de 12,5 ppm, 25 ppm și 50 ppm asupra producției de cariopse la trei soiuri de grâu – Arieșan, Crina și Boema. Biostimulatorul BCO 4K + acetat de Zn a determinat producția maximă de 8213 kg/ha, cu 15,77% mai mare decât în varianta martor - BCO – 4DMA; față de varianta martor tratată cu apă, sporul de producție a fost de 31,57%; între concentrațiile de biostimulatori nu s-au constatat diferențe semnificative; soiul Boema s-a situat pe locul întâi cu o producție de 7997,8 kg/ha; interacțiunea dintre factori a evidențiat varianta BCO 4K + acetat de Zn x 50 ppm x soiul Boema, sporul de producție fiind foarte semnificativ.*

**Cuvinte cheie:** biostimulatori, concentrație, grâu, producție.

### INTRODUCTION

The research made in the last years showed that in plants life an important role plays some organic compounds that regulate various physiological processes.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

Called today growth stimulators, plant hormones or phytohormones act on stimulating or inhibiting the processes of their action (Ghițău, 2011).

Growth stimulators at low concentration accelerates the processes of growth and the high concentration can stop this process making herbicides (Iovu, 1964).

Corneliu Oniscu and his collaborators (1972, 1975, 1978 and 1979) have synthesized a series of derivatives from class of fenoxialchil sulfamoil carboxylic acids which represents a new class of stimulators generally called BCO.

In sugar beet, carrots, vines and roses crops were tested two substances (BCO 2 and BCO 4) from the class of BCO biostimulators with auxinic action outstanding. It was obtained good results, allowing the product to be approved as BCO 4 biostimulator sugar beet crops.

Ghițău Carmen and collaborators (2010) have investigated in laboratory conditions the effect of some biostimulators applications in different concentration on the germination process (energy and germination capacity), root length and coleoptil of winter wheat variety Boema. The highest percentage of germination was recorded in the following biostimulators: BCO 4K with 91,12% germination, BCO 2K + Zinc acetate with 94,50% germination BCO 4 DMA with 95,5% germination, BCO 2 DMA + Zinc acetate with 93,87% germination.

On wheat growth, stimulators acting on the root system, brotherhood, plant height, photosynthetic capacity, duration synthesis, renaissance and also can influence the density, resistance to stress and MMB (Ghițău, 2011).

## MATERIAL AND METHODS

The experience has been established at the Ezareni station, Iasi in subdivided plots with three repetitions.

The experimental factors are:

A factor – The biostimulators:

a<sub>1</sub> → BCO – 4DMA;

a<sub>2</sub> → BCO 4K;

a<sub>3</sub> → BCO 4K + acetat de Zn.

B factor – Biostimulator concentrations:

b<sub>1</sub> → 50 ppm;

b<sub>2</sub> → 25 ppm;

b<sub>3</sub> → 12,5 ppm.

C factor – Varieties of wheat:

c<sub>1</sub> → Arieșan;

c<sub>2</sub> → Crina;

c<sub>3</sub> → Boema.

The biostimulators applied were obtained from prof. dr. Corneliu Oniscu, „Gheorghe Asachi” Technical University of Iasi.

These biostimulators are from the class of fenoxialchil sulfamoil carboxylic acids, which has a low toxicity and can be also biodegradable.

The climate conditions of the agricultural year 2010 – 2011 have been favorable for wheat plant growth. The multiannual average of rainfall at the weather station AgroExpert – Miroslava was 517,8 mm. The average annual of rainfall is

providing 80% more than 380 – 480 mm and 50% of the annual amount of precipitation exceeds 500 mm .

## RESULTS AND DISCUSSIONS

The biostimulators applied has positively influenced wheat productions. Therefore, the highest productions of 8213 kg/ha was recorded at BCO 4K + Zinc acetate biostimulator, with a difference of 1119 kg/ha, very significant compared to control BCO – 4DMA biostimulator (tab. 1).

Table 1

The influence of biostimulators on wheat yield in crop year 2010-2011

| Biostimulators        | Production Kg/ha | % of variant control | Differences Kg/ha | Significance | Observations |
|-----------------------|------------------|----------------------|-------------------|--------------|--------------|
| BCO – 4DMA            | 7094             | 100,0                | Mt.               | -            | PLACE III    |
| BCO 4K                | 7978             | 112,46               | 884               | **           | PLACE II     |
| BCO 4K + Zinc acetate | 8213             | 115,77               | 1119              | ***          | PLACE I      |

DL 5% 340,4 kg/ha  
 DL 1 % 564,4 kg/ha  
 DL 0,1% 905,2 kg/ha

When the biostimulators variant were compared with wather trated extraroot production increases were 31,57% in BCO 4K + Zinc acetate, 27,81% in BCO 4K and 13,64% in BCO 4 DMA (tab. 2).

Table 2

Production caused by the biostimulators compared with the variant trated with water in crop year 2010-2011

| Biostimulators        | Production Kg/ha | % of variant control | Differences Kg/ha | Significnce | Observations |
|-----------------------|------------------|----------------------|-------------------|-------------|--------------|
| BCO – 4DMA            | 7094             | 113,64               | 852               | ***         | PLACE III    |
| BCO 4K                | 7978             | 127,81               | 1736              | ***         | PLACE II     |
| BCO 4K + acetat de Zn | 8213             | 131,57               | 1971              | ***         | PLACE I      |
| Treated with water    | 6242             | 100,0                | Mt.               | -           | PLACE IV     |

DL 5% 340,4 kg/ha  
 DL 1% 464,4 kg/ha  
 DL 0,1% 590,2 kg/ha

The concentrations of growth regulators has not significantly differentiated the productions (tab. 3), with a difference of 245,1 kg/ha, but uninsured statistic was obtained at a concentration of 50 ppm.

On table 4, where wheat yields are influenced by variety, we observed that Crina and Ariesan varieties were not different between them, with similar production. The highest production of 7997,8 kg/ha was obtained by Boema variety. Crina variety made production less than Boema, with 326,3 kg/ha

being distinctly less significant and Ariesan variety achieved a lower production with 380,4 kg/ha from Boema, being very significant in less.

From the interaction of three factors studied, has observed the highest production of 9007 kg/ha obtained by the variant BCO – 4K + Zinc acetate x 50 ppm x Boema variety with a difference of 1461 kg/ha, being very significant to the control variant BCO – 4DMA x 12,5 ppm x Boema variety (tab. 5).

*Table 3*

**The influence of biostimulator concentrations on wheat yield in crop year 2010-2011**

| Concentration ppm | Production Kg/ha | % of variant control | Differences Kg/ha | Significance | Observations |
|-------------------|------------------|----------------------|-------------------|--------------|--------------|
| 50                | 7949,6           | 103,18               | 245,10            | -            | PLACE I      |
| 25                | 7631,7           | 99,05                | - 72,80           | -            | PLACE III    |
| 12,5              | 7704,5           | 100,0                | Mt.               | -            | PLACE II     |

DL 5% 185,0 kg/ha

DL 1% 259,7 kg/ha

DL 0,1% 366,6 kg/ha

*Table 4*

**The influence of variety on wheat yield in crop year 2010-2011**

| Variety | Production Kg/ha | % of variant control | Differences Kg/ha | Significance | Observations |
|---------|------------------|----------------------|-------------------|--------------|--------------|
| Boema   | 7997,8           | 100,0                | Mt.               | -            | PLACE I      |
| Crina   | 7671,5           | 95,92                | -326,3            | 00           | PLACE II     |
| Ariesan | 7617,4           | 95,24                | -380,4            | 000          | PLACE III    |

DL 5% 196,3 kg/ha

DL 1% 263,1 kg/ha

DL 0,1% 347,1 kg/ha

Analyzing the biostimulator interactions it has observed these:

The highest yield of 7546 kg/ha was obtained by Boema variety in the variant treated with BCO - 4 DMA biostimulator in concentration of 12,5 ppm and the lowest production of 6580 kg/ha at Crina variety with a concentration of 12,5 ppm;

BCO – 4K biostimulator determined the highest production of 8536 kg/ha in interaction with 12,5 ppm and Crina variety, while the lower production of 6946 kg/ha was obtained in interaction with 25 ppm and Crina variety;

BCO – 4K + Zinc acetate realized the highest production of 9007 kg/ha at 50 ppm at Boema variety interaction and the lower production of 7253 kg/ha at 12,5 ppm and Ariesan variety interaction.

Table 5

The influence of interaction between biostimulators x concentration x varieties on wheat yield in crop year 2010-2011

| Biostimulators         | Concentrations ppm | Variety | Production Kg/ha | % of variant control | Differences Kg/ha | Significance |
|------------------------|--------------------|---------|------------------|----------------------|-------------------|--------------|
| BCO – 4 DMA            | 50                 | Boema   | 7347             | 97,36                | -199              |              |
|                        |                    | Crina   | 7417             | 98,28                | -129              |              |
|                        |                    | Arieşan | 6943             | 92,00                | -603              | 0            |
|                        | 25                 | Boema   | 7133             | 94,52                | -413              | 0            |
|                        |                    | Crina   | 7030             | 93,16                | -516              | 0            |
|                        |                    | Arieşan | 7130             | 94,48                | -416              | 0            |
|                        | 12,5               | Boema   | 7546             | 100,0                | Mt.               |              |
|                        |                    | Crina   | 6580             | 87,19                | -966              | 00           |
|                        |                    | Arieşan | 6720             | 89,05                | -826              | 00           |
| BCO 4 K                | 50                 | Boema   | 8447             | 111,94               | 901               | **           |
|                        |                    | Crina   | 8110             | 107,47               | 564               | *            |
|                        |                    | Arieşan | 7282             | 96,50                | -264              |              |
|                        | 25                 | Boema   | 7895             | 104,62               | 349               |              |
|                        |                    | Crina   | 6946             | 92,04                | -600              | 0            |
|                        |                    | Arieşan | 8377             | 111,02               | 931               | **           |
|                        | 12,5               | Boema   | 7996             | 105,96               | 450               | *            |
|                        |                    | Crina   | 8536             | 113,11               | 990               | **           |
|                        |                    | Arieşan | 8220             | 108,93               | 674               | *            |
| BCO 4 K + Zinc acetate | 50                 | Boema   | 9007             | 119,36               | 1461              | ***          |
|                        |                    | Crina   | 8371             | 110,93               | 825               | **           |
|                        |                    | Arieşan | 8623             | 114,27               | 1077              | **           |
|                        | 25                 | Boema   | 8380             | 111,05               | 834               | **           |
|                        |                    | Crina   | 7794             | 103,28               | 248               |              |
|                        |                    | Arieşan | 8000             | 106,01               | 454               | *            |
|                        | 12,5               | Boema   | 8230             | 109,06               | 684               | *            |
|                        |                    | Crina   | 8260             | 109,46               | 714               | **           |
|                        |                    | Arieşan | 7253             | 96,11                | 293               |              |
| DL 5%                  |                    |         | 412,9 kg/ha      |                      |                   |              |
| DL 1%                  |                    |         | 703,5 kg/ha      |                      |                   |              |
| DL 0,1%                |                    |         | 1155,5 kg/ha     |                      |                   |              |

## CONCLUSIONS

1. When the factors were analyzed individually the biostimulators and wheat varieties positively influenced the caryopses production, and concentrations of 12,5 ppm, 25 ppm and 50 ppm did not lead significant production differences.

2. Compared with version control the biostimulators BCO – 4 DMA, BCO – 4 K and BCO – 4 K + Zinc acetate realized increases of 12,46% and 15,77%, and when the biostimulators were compared with variant treated with water extraroot there were high increase in all biostimulators production of 31,57% at BCO – 4 K + Zinc acetate, of 27,81% at BCO – 4 K and 13,63% at BCO – 4 DMA .

3. Biostimulators concentration realized production of 7949,6 kg/ha at 50 ppm, of 7631,7 kg/ha at 25 ppm and 7704,5 kg/ha at 12,5 ppm.

4. The highest carryopse production of 7997,8 kg/ha was obtained by Boema variety and the other two variety Crina and Ariesan determined production of 7671,5 kg/ha and respectively 7617,4 kg/ha.

5. Interaction between the factors analyzed showed the variant BCO – 4 K + Zinc acetate x 50 ppm x Boema with a production of 9007 kg/ha, with 19,36% higher than version control (BCO – 4K x 12,5 ppm x Boema).

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# CONTRIBUTIONS REGARDING THE MORPHOLOGICAL CHARACTERS OF *MONARDA CITRIODORA* CERV. EX LAG. IN FIELD CONDITIONS

## CONTRIBUȚII PRIVIND INFLUENȚA CONDIȚIILOR PEDO- CLIMATICE ASUPRA UNOR CARACTERE MORFOLOGICE LA SPECIA *MONARDA CITRIODORA* CERV. EX LAG.

*CIURUȘNIUC Ana-Maria*<sup>1</sup>, *ROBU T.*<sup>1</sup>,  
*SAGHIN GH.*<sup>2</sup>, *ANDRIEȘ Iuliana*<sup>1</sup>, *COTEANU A.Gh*<sup>1</sup>.  
e-mail: ciurusniucanamaria@yahoo.com

**Abstract.** *This paper brings new contributions to the knowledge of morphological characters belonging to the species *Monarda citriodora* Cerv. ex Lag. (family *Lamiaceae*) cultivated in the climatic conditions in three areas of Moldova: Iasi, Vaslui and Pojorâta (Suceava). Research focused on species growing *Monarda citriodora* Cerv. ex Lag. in different climatic conditions, followed by observations and measurements of plants, as established by seed crops as well as those established by seedling. It should be noted that the differences were found between plants grown in three locations and the establishment of methods of culture.*

**Key words:** *Monarda citriodora* Cerv. ex Lag, morphological characters

**Rezumat:** *Lucrarea aduce noi contribuții la cunoașterea unor caractere morfologice care aparțin speciei *Monardacitriodora* Cerv. ex Lag. (familia *Lamiaceae*) cultivată în condițiile pedo-climatice din trei zone ale Moldovei: Iași, Vaslui și Pojorâta (Suceava). Cercetările au vizat cultivarea speciei *Monarda citriodora* Cerv. ex Lag. în condiții pedo-climatice diferite, urmate de observații și măsurători ale plantelor, atât la culturile înființate prin semințe cât și la cele înființate prin răsad. De remarcat este faptul că s-au constatat diferențieri atât între plantele cultivate în cele trei locații, cât și între metodele de înființare ale culturii.*

**Cuvinte cheie:** *Monarda citriodora* Cerv. ex Lag., caractere morfologice

### INTRODUCTION

*Monarda* L., also known popular as bergamot, decorative mint, Indian mint, bee balm, OswegoTea, is part of the family *Lamiaceae*, botanical family known for many species of medicinal plants, aromatic and decorative, that it contains. Genus species, originating in North America, have been successfully used in medicinally purposes since the seventeenth century by numerous Indian tribes to treat digestive affections, having many effects like febrifuge, diaphoretic,

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

<sup>2</sup> Development Research Station of Agricultural Suceava, Romania

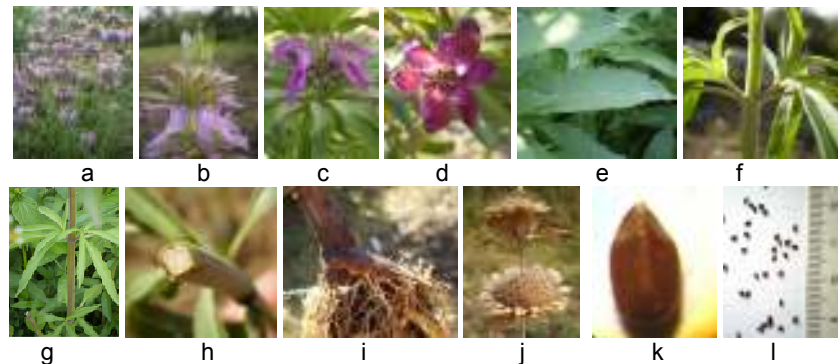
antirheumatic, carminative, sedative, tonic.

In specialty literature the number of genus *Monarda* L. varies from 12 (Guşuleac, 1961) to 30 species (Selaru, 2007), being herbaceous plants, mostly perennial, but also annual.

Bentham (1876), Briquet (1897), Wunderlich (1967) Harley et al. (2004) placed the genus *Monarda* L in the family *Lamiaceae.*, *Nepetoideae* subfamily, tribe *Mentheae*, subtribe *Menthinae* (Ryding, 2009, 2010, Moon et al., 2009).

*Monarda citriodora* Cerv. ex Lag. is an annual species, with heights of 25-90 cm well-developed root system, swivel type, branched. Stems are branched, erect, square pubescent. The leaves are opposite, petiolate, lanceolate or oblong-lanceolate, pubescent, with serrate edges (fig. 1).

Flowers pink-purple, are grouped in whorls 2-7 capitulum formed terminals, accompanied by colorful bracts, tubular calyx, 5 lobes, two lipped corolla, long tube, glabrous narrow interior, straight or slightly curved upper lip, lower short, the three lobes (P. Grisvard, Chaudun V., 1964). Previous stamens are ascending hind rudimentary or absent, anthers linear. The ovary is deep, with four lodges. The style is bifida peak (Guşuleac M., 1961) (fig. 1), the fruits are glossy ovoid nukas.



**Fig. 1-** *Monarda citriodora* Cerv. ex Lag. : a- inflorescences; b, c- flowers; d-bracts; e, f- leaves; g, h- stem squared; i- root; j, k, l- seeds

### **MATERIALS AND METHODS**

The experiments were conducted in three areas of the climatic Moldova: Plant growing on experimental field of discipline Phytotechnique of the University of Agricultural Sciences and Veterinary Medicine of Iasi, the experimental field of Văleni Station Vaslui and Suceava-Agricultural Research and Development center Pojorâta. The experiments were established in spring 2011, located in plots subdivided in three repetitions, being differentiated by the establishment of culture: sown directly in the field and greenhouse seedling obtained Veterinary Medicine Teaching Science Station.

Observations and biometric measurements were performed on a total of 30 plants, from May 2011 until August 2011, aiming at growing plants in all stages (fig. 2).

Biological material used in the establishment of culture is the *Monarda citriodora* Cerv. ex Lag.



## RESULTS AND DISCUSSION

In tables 1 and 2 are presented data on sowing date, sunrise, planting date, flowering date, plant height and flowering so early to full flowering of the three locations. Note that in cultures established plants by seeds, Vaslui area, slope heights presented in full flowering phenophase lower than those in cultures established by seedling. In contrast, plants in full flowering phenophase of culture established by seeds of Pojorâta had an average height of 75.3 cm, compared to the culture created by the seedling, which measured on average 68.2 cm.

Table 1

**Characters of plants *Monarda citriodora* Cerv. ex Lag in cultures established by seed.**

| Location | Sowing date | Date sunrise | Appearance I pairs of leaves | Date of flowering | Height at full flowering - cm |
|----------|-------------|--------------|------------------------------|-------------------|-------------------------------|
| Iași     | 6.05        | 16.05        | 30.05                        | 20.08             | 61,71                         |
| Vaslui   | 14.05       | 23.05        | 2.06                         | 15.08             | 41,8                          |
| Pojorâta | 27.05       | 14.06        | 23.06                        | 15.09             | 75,3                          |

Table 2

**Characters of plants *Monarda citriodora* Cerv. ex Lag in cultures established by seedling**

| Location | Planting date | Height before blooming - cm | Date of flowering | Height at full flowering - cm |
|----------|---------------|-----------------------------|-------------------|-------------------------------|
| Iași     | 13.05         | 40,7                        | 6.06              | 67,87                         |
| Vaslui   | 20.05         | 38,7                        | 10.06             | 61,71                         |
| Pojorâta | 27.05         | 46,75                       | 18.06             | 68,2                          |



**Fig. 2 - Aspects of the experimental field, a- the seed culture, b, c, d-seedling crop established by, e-seedling of *M. citriodora* Cerv. ex Lag**

Table 3 presents the characterization climate of 2011 in areas where they were created experiences: Iași, Vaslui and Pojorâta. Multi-annual average temperatures in 2011 in Vaslui. County was 10.55°C, 10.34° C in Iași and 5.36° C in the Pojorâta.

Table 3

## The characterization of the climate of 2011

| Specification                  | Iași            |                  | Vaslui          |                  | Pojorâta        |                  |
|--------------------------------|-----------------|------------------|-----------------|------------------|-----------------|------------------|
|                                | Temperatures °C | Precipitation mm | Temperatures °C | Precipitation mm | Temperatures °C | Precipitation mm |
| Jan                            | -2              | 18.8             | -2.5            | 23.4             | -7              | 52.6             |
| Feb                            | -2.4            | 8.8              | -2.4            | 8.2              | -3.3            | 37.0             |
| Mar                            | 3.7             | 21.0             | 3.7             | 22.2             | -1.1            | 41.3             |
| Apr                            | 10.3            | 81.4             | 10.3            | 47.0             | 5.2             | 45.6             |
| May                            | 16.5            | 41.0             | 17              | 45.6             | 10.8            | 145.6            |
| Jun                            | 20.2            | 113.8            | 21.1            | 108.4            | 14.9            | 143              |
| Jul                            | 22.6            | 66.0             | 23.2            | 86.4             | 16.1            | 242.2            |
| Aug                            | 21.5            | 28.2             | 24.7            | 77.2             | 16.5            | 169.3            |
| Sept                           | 18.8            | 22.8             | 15.7            | 71.2             | 9.9             | 85.8             |
| Oct                            | 9.2             | 42.6             | 11.5            | 29.7             | 2.9             | 29.8             |
| Nov                            | 3.0             | 1.4              | 6.4             | 10.2             | 4.0             | 53.7             |
| Dec                            | 2.7             | 9.8              | -2.1            | 58.4             | -4.5            | 58.2             |
| Average annual/ Monthly amount | 10.34           | 455.6            | 10.55           | 587.9            | 5.36            | 1104             |

In table 4 the values of biometric measurements made on plants species *Monarda citriodora* Cerv. ex Lag., cultivated in the climatic three areas of Moldova, established by seedling.

Table 4

***Monarda citriodora* Cerv. ex Lag. species biometric measurements the full flowering phenophase in the three areas of culture, established by seedling**

| Nr. crt | Specification (avarage)         | Vaslui | Iași  | Pojorâta |
|---------|---------------------------------|--------|-------|----------|
| 1       | Height (cm)                     | 61,71  | 67,87 | 68,2     |
| 2       | No. of branches                 | 14,2   | 15,42 | 16       |
| 3       | Leaf length (cm)                | 5,21   | 5,58  | 6        |
| 4       | Leaf width (cm)                 | 1,16   | 1,16  | 1,28     |
| 5       | No. of whorls of inflorescences | 4,5    | 4,5   | 4,5      |
| 6       | Inflorescences diameter (cm)    | 2,96   | 3,5   | 3,7      |

Following comments made in the three areas of crop plants species *M. citriodora* Cerv. ex Lag., on the character height, the lowest average of 61.71 inches was reported in the culture of Vaslui, and highest, 68.2 cm, the plants cultivated in Pojorâta.

The average number of branches of the stem varies little, from 14.2 ramifications of plants grown in Vaslui, from 15.42 to plants cultivated in Iasi branch to 16 branches from plants Pojorâta. *M. citriodora* Cerv. ex Lag. leaf length presented values of 5.21 cm for plants in Vaslui, up to 6 cm from the plants grown at Pojorâta. On leaf width, plants grown in Vaslui and Iași have registered the same value of 1.16 cm, whereas leaves of plants in culture Pojorâta had widths of 1.28 cm. Number of whorls of inflorescences was monarda average of 4.5 in all

three are as of culture, while inflorescence diameter ranged from an average value of 2.96 cm from the plants grown in Vaslui, from 3.7 cm to those grown in Pojorâta.

*Table 5*

***Monarda citriodora* Cerv. ex Lag. species biometric measurements the full flowering phenophase in the three areas of culture, founded by seeds**

| Nr. crt | Specification (avarage)         | Vaslui | Iași  | Pojorâta |
|---------|---------------------------------|--------|-------|----------|
| 1       | Height (cm)                     | 41,8   | 61,71 | 75,3     |
| 2       | No. of branches                 | 7,42   | 7,42  | 14,85    |
| 3       | Leaf lenght (cm)                | 4,68   | 4,86  | 5,96     |
| 4       | Leaf width (cm)                 | 1,12   | 1,21  | 1,33     |
| 5       | No. of whorls of inflorescences | 2,25   | 2,66  | 3,88     |
| 6       | Inflorescence diameter (cm)     | 2,78   | 2,86  | 3,94     |

In table 5, biometric measurements made on plants of *Monarda citriodora* Cerv. ex Lag. grown in three, established by direct sowing in the field (tab. 1) had values different from those established by the seedlings, culture and between locations. Thus, the average height of plants at full flowering phenophase, measured in Vaslui only 41.8 cm, whereas those of Pojorâta recorded an average height of 75.3 cm.

The average number of stem branches ranged from 7.42 branch to plants grown in Vaslui and Iași, until 14.85 on the ramifications of the strain grown in Pojorâta. On leaf length, plants grown in Vaslui and Iași have measured an average of 4.68 cm and 4.86 cm, while those grown in the mountains, Pojorâta, have an average length of 5.96 cm.

Average width of leaves showed values between 1.12 cm from the plants grown in Vaslui, 1.21 cm in those grown in Iasi and 1.33 cm from the plants grown in the Pojorâta.

The culture plants established in Vaslui, inflorescences showed the average diameter of 2.78 cm, 2.86 cm cultivated in Iasi and in those of Pojorâta 3.94 cm. On the average number of whorls/flowering plants of culture established in Vaslui area included an average of 2.25 whorls/inflorescence, those of culture in Iasi, 2.66 whorls/inflorescence and plants grown in Pojorâta an average of 3, 88 whorls/inflorescence.

## CONCLUSIONS

1. *Monarda citriodora* Cerv.ex Lag. species grown in the three areas of Moldova (Iași, Vaslui and Pojorâta) have different values of biometric measurements made during the growing season, indicating that both the climatic conditions of the three areas of culture, and how to establish the culture influence the morphology of plants.

2. The lowest average height (41.8 cm) reported the culture of Vaslui, and

the greatest height (75.3 cm) that measured a Pojorâta area plants were recorded in the culture established by seed.

3. The average number of stem branches ranged from 14.2 (Vaslui) to 16 branches/strain (Pojorâta) to set the seedling crop plants, and from 7.42 (Vaslui and Iași) to 14.85 branch/stem (Pojorâta) to set the seed crop plants.

4. The average length of the leaf of the highest values measured in cultures established by seedling plants (6 cm-Pojorâta) and the lowest average value (4.68 cm-Vaslui) to set the seed crop plants.

5. Lowest average number of whorls/inflorescence was reported at plants established by seed culture Vaslui area (2.66 whorls/inflorescence), and the highest number of whorls/blossom from the culture established by seedling plants in all three locations (4.5 vertical/inflorescence).

6. Biometric measurements made at plants in cultures established by seeds (number of branch/stem, leaf length, leaf width, number of whorls/inflorescence, diameter of inflorescences) had lower values than biometric measurements of plants in the seedling crop established.

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**THE EFFECT OF PLANT FERTILISATION AND DENSITY  
ON SOME OF THE PHYSICAL PROPERTIES  
OF ACHENES ON A FEW SUNFLOWER  
(*HELIANTHUS ANNUUS L.*) HYBRIDS**

**EFECTUL FERTILIZĂRII ȘI DESIMII PLANTELOR ASUPRA UNOR  
ÎNSUȘIRI FIZICE ALE ACHENELOR LA CÂȚIVA HIBRIZI DE  
FLOAREA-SOARELUI (*HELIANTHUS ANNUUS L.*)**

**COTEANU A. Gh.<sup>1</sup>, ANDRIEȘ Iuliana<sup>1</sup>, CIURUȘNIUC Ana-Maria<sup>1</sup>**  
e-mail: andrei\_gheorghe@yahoo.com

*Abstract.* Considering a cambic chernozem, in the ecological conditions of the agricultural year 2009-2010, at the Ezăreni – Iași farm, two physical sunflower parameters of quality were taken under observation, under the influence of fertilisation, plant density and cultivated hybrids. The value of 60,07g was the highest for the interaction of two factors, the  $N_{32}P_{24}K_{48}$  fertilisation and the 40,000 pl/ha density. The highest mass per storage volume was: 41,84 kg/hectolitre for the  $N_{120}P_{60}K_{120}$  fertilisation, 40,5 kg/ hectolitre for the 40000 plants/hectare density, 41,2 kg/ hectolitre for the Performer hybrid. The interaction between  $N_{120}P_{60}K_{120} \times 40000$  pl/ha  $\times$  Performer resulted into the highest mass per storage volume of 42 kg/hectolitre.

**Key words:** hybrids, fertilisation, Thousand Grain Weight, sunflower

*Rezumat.* În condițiile ecologice ale anului agricol 2009-2010, pe un cernoziom cambic, de la ferma Ezăreni – Iași s-au urmărit doi indicatori fizici de calitate la floarea-soarelui, sub influența fertilizării, desimii plantelor și hibrizilor cultivați. Masa a 1000 de boabe (MMB) a fost de 60,07 g, cea mai mare, la interacțiunea a doi factori, fertilizarea cu  $N_{32}P_{24}K_{48}$ , și desimea de 40000 pl/ha. Masa hectolitrică cea mai mare a fost: la fertilizarea cu  $N_{120}P_{60}K_{120}$ , de 41,84 kg/hl; la desimea de 40000 pl/ha, cu 40,5 kg/hl; la hibridul Performer, cu 41,2 kg/hl. Interacțiunea  $N_{120}P_{60}K_{120} \times 40000$  pl/ha  $\times$  Performer a realizat cea mai mare masă hectolitrică, de 42 kg/hl.

**Cuvinte cheie:** hibridi, fertilizare, floarea – soarelui, masa a 1000 de boabe

## INTRODUCTION

Research regarding the effect of fertiliser doses over the productivity of the fitosystem of sunflower and of the quality parameters was done both in the country and abroad. Hakoomat A. and the collaborators (2005) that have kept a check on the influence of applying azoth doses on the production of achenes and sunflower oil concluded that azoth plays a very important role in the formation of physical parameters of quality for sunflower.

Vrânceanu A. (2000), in the paper called „Hybrid Sunflower ”discusses the effect of fertilizers on sunflower, claiming that phosphorus fertilizers can be

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

applied individually, substantially increasing production in comparison to the fertiliser based on azoth, without contributing to diminishing the content of oil from the seeds.

Research done in Romania and abroad on the productivity of sunflower fitosystem has also had in view the effect of the genetic material and of nutrition space, factors which have a very important role, influencing the value of production. Olewe V.I.O. (2005) present the fact that the density of plants on the surface unit influences production and productivity elements.

## MATERIALS AND METHODS

During 2010 spring, on a cambic chernozem soil type within Ezăreni-Iasi farm a trifactorial experience was established by the method of subdivided parcels in four repetitions.

The three factors investigated were:

Factor - fertilization, five graduations:

a1 -  $N_0P_0K_0$  a2 -  $N_{32}P_{24}K_{48}$  a3 -  $N_{64}P_{36}K_{72}$  a4 -  $N_{96}P_{48}K_{96}$  a5 -  $N_{120}P_{60}K_{120}$

Factor B - density seeding with three graduations:

b1 - 40,000 pl / ha b2 - 55,000 pl / ha b3 - 70,000 pl / ha

Factor C - Hybrid sunflower - four graduations:

c1 - Favorite (Romanian) c2 - Performer (Romanian)

c3 - PR63A90 (Pioneer) c4 - PR64A83 (Pioneer)

Corn was used as a pre-plant, which allowed an autumn plowing performed under optimal conditions, releasing the land earlier, at a depth of 25-30 cm.

## RESULTS AND DISCUSSION

The 2009-2010 crop year presented good conditions reported in the annual average precipitation recorded at Miroslava meteorological station, the values being above the average. The amount of the annual rainfall exceeded the annual average over 39.4 mm. During the growing season of sunflower, the rainfall was lower with 9.4mm than the annual average.

Moreover, the agricultural year 2009-2010 proved to be a year with temperatures higher than the annual average, from time to time, during the summer months, heat periods being also recorded. The yearly average temperature of 9.6°C was exceeded by 1.5 C, which shows a global warming trend. During the sunflower growing, temperatures exceeded the annual average by 2.36°C.

During the performed laboratory tests to determine the specific hectoliter mass, it was observed that the highest value of this timer occurred in  $N_{120}P_{60}K_{120}$  fertilization with 41.84 kg / hl (tab. 1); at the density of 40.000 pl / ha a value of 40.5 kg / h of the hectoliter mass was recorded (tab. 2); the Performer hybrid recorded 41.2 kg / hl, the largest mass per storage volume (tab. 3).

Mass per storage volume increased with the increasing of the fertilizer dose, reaching a maximum of 41.84 kg/hl, with 2.31 kg/hl in addition to the stander-by, in case of fertilization variant  $N_{120}P_{60}K_{120}$ .

Table 1

Influence of fertilization on the hectoliter mass in 2010

| Fertilization                                     | MH (Kg / hl) | % percent compared to the stander-by | Difference (kg / hl) | Significance |
|---|--------------|--------------------------------------|----------------------|--------------|
| N <sub>0</sub> P <sub>0</sub> K <sub>0</sub>      | 39,53        | 100,0                                | Mt.                  | -            |
| N <sub>32</sub> P <sub>24</sub> K <sub>48</sub>   | 39,98        | 101,1                                | 0,45                 | Xxx          |
| N <sub>64</sub> P <sub>36</sub> K <sub>72</sub>   | 40,40        | 102,2                                | 0,87                 | Xxx          |
| N <sub>96</sub> P <sub>48</sub> K <sub>96</sub>   | 40,62        | 102,7                                | 1,09                 | Xxx          |
| N <sub>120</sub> P <sub>60</sub> K <sub>120</sub> | 41,84        | 105,8                                | 2,31                 | Xxx          |
| DL 5%   | 0,1 kg / hl  |                                      |                      |              |
| DL 1%   | 0,15 kg / hl |                                      |                      |              |
| DL 0,1%   | 0,2 kg / hl  |                                      |                      |              |

Table 2

The density influence on the hectoliter mass in 2010

| Density pl / ha | MH (Kg / hl) | % percent compared to the stander-by | Difference (kg / hl) | Significance |
|-----------------|--------------|--------------------------------------|----------------------|--------------|
| 40,000          | 40,5         | 100                                  | Mt.                  |              |
| 55,000          | 40,2         | 99,2                                 | -0,3                 | ooo          |
| 70,000          | 40,0         | 98,7                                 | -0,5                 | ooo          |
| DL 5%           | 0.10 kg/hl   |                                      |                      |              |
| DL 1%           | 0.15 kg/hl   |                                      |                      |              |
| DL 0,1%         | 0.20 kg/hl   |                                      |                      |              |

Statistically, the mass per storage volume recorded differences in the density of minus 55 to 70 000 plants / ha.

Table 3

The hybrid influence on hectoliter mass in 2010

| Hybrids   | MH (Kg / hl) | % percent compared to the stander-by | Difference kg / hl compared to the stander-by | Significance |
|-----------|--------------|--------------------------------------|---|--------------|
| Favorite  | 39,9         | 100,0                                | Mt.   |              |
| Performer | 41,2         | 103,2                                | 1,3   | xxx          |
| PR63A90   | 38,9         | 97,4                                 | -1,0  | ooo          |
| PR64A83   | 41,1         | 103,0                                | 1 to 2  | xxx          |
| DL 5%     | 0,10 kg / hl |                                      |   |              |
| DL 1%     | 0,15 kg / hl |                                      |   |              |
| DL 0,1%   | 0,20 kg / hl |                                      |   |              |

Among the sunflower hybrids there were some differences in terms of mass per storage volume. The largest mass per storage volume was recorded in Performer hybrid, with 41.2 kg / hl, followed by PR64A83, with 41.1 kg / hl and the lowest hybrid PR63A90 with 38.9 kg / hl.

The interaction of several factors may have different reactions of the mass of 1000 grains, thus the evolution of the mass of 1000 grains was followed after the interaction between fertilization and density. (tab. 4).

Regarding the effects that the density x fertilizer interaction has on the mass of 1000 grains, significant variations of the MMB are being observed, both based on the used variants of fertilization and the density of plants / ha.

Table 4

## The effect of the fertilization interaction x densities on the MMB in 2010

| Fertilization                                     | Densities<br>pl / ha | MMB<br>(g) | % percent<br>compared to<br>the stander-<br>by | Difference<br>(g) | Significan<br>ce |
|---|----------------------|------------|--|-------------------|------------------|
| N <sub>0</sub> P <sub>0</sub> K <sub>0</sub>      | 40,000               | 56,97      | 100,00   | Mt.               | -                |
|   | 55,000               | 57,65      | 101,19   | 0,68              |                  |
|   | 70,000               | 57,12      | 100,26   | 0,15              |                  |
| N <sub>32</sub> P <sub>24</sub> K <sub>48</sub>   | 40,000               | 60,07      | 105,44   | 3,10              | xxx              |
|   | 55,000               | 58,67      | 102,98   | 1,70              | xxx              |
|   | 70,000               | 57,70      | 101,28   | 0,73              |                  |
| N <sub>64</sub> P <sub>36</sub> K <sub>72</sub>   | 40,000               | 59,75      | 104,87   | 1,04              | x                |
|   | 55,000               | 59,17      | 103,86   | 2,19              | xxx              |
|   | 70,000               | 58,47      | 102,63   | 1,50              | xxx              |
| N <sub>96</sub> P <sub>48</sub> K <sub>96</sub>   | 40,000               | 59,07      | 103,68   | 2,35              | xxx              |
|   | 55,000               | 58,45      | 102,60   | 2,10              | xxx              |
|   | 70,000               | 58,42      | 102,54   | 1,45              | xx               |
| N <sub>120</sub> P <sub>60</sub> K <sub>120</sub> | 40,000               | 59,75      | 104,87   | 2,78              | xxx              |
|   | 55,000               | 58,02      | 101,84   | 1,05              | x                |
|   | 70,000               | 56,90      | 99,87  | -0,07             |                  |
| DL 5%   | 0.8 g                |            |  |                   |                  |
| DL 1%   | 1.1 g                |            |  |                   |                  |
| DL 0,1%   | 1.5 g                |            |  |                   |                  |

Thus, in case of fertilization variant N<sub>32</sub> P<sub>24</sub> K<sub>48</sub> x 40000 pl/ha the highest 1000 grain weight of 60.07 g was obtained.

### CONCLUSIONS

1. Physical analyzes on sunflower achenes showed an increase of a thousand grain weight and a hectoliter mass from the stander-by version; the fertilization with N<sub>32</sub>P<sub>24</sub>K<sub>48</sub> at the density of 40,000 pl/ha caused the greatest mass of 1,000 grains of 60,07 g;

2. Mass per storage volume was positively influenced by fertilization and hybrid density, the greatest mass per storage volume occurred in N<sub>120</sub> P<sub>60</sub> K<sub>120</sub> fertilization with 41.84 kg/ha; at the density of 40,000 pl/ha 40.5 kg/ha were registered; the Performer hybrid recorded 41.2 kg/hl, the largest mass per storage volume.

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# THE NUTRITION AREA AND FERTILISATION EFFECT ON THE PRODUCTION OF ACHENES ON SOME OF THE SUNFLOWER HYBRIDS

## EFFECTUL FERTILIZĂRII ȘI SPAȚIULUI DE NUTRIȚIE ASUPRA PRODUCȚIEI DE ACHENE LA CÂȚIVA HIBRIZI DE FLOAREA-SOARELUI

COTEANU A. Gh.<sup>1</sup>, ANDRIEȘ Iuliana<sup>1</sup>, CIURUȘNIUC Ana-Maria<sup>1</sup>  
e-mail: andrei\_gheorghe@yahoo.com

**Abstract.** In a trifactorial experiment such as fertilisation x plant density x sunflower hybrids, the achenes production was taken under observation during the 2009-2010 agricultural year, on a cambic chernozem, in sunflower friendly ecological conditions. The maximum plant height was that of 175,3 cm on the  $N_{120}P_{60}K_{120}$  fertilisation, 174,6 cm for the density of 70000 plants/hectare and 171,8 cm for the PR63A90 hybrid. Taking into consideration the studied factors in the analysis of achenes production, the following were found : the  $N_{96}P_{48}K_{96}$  fertilisation has determined the highest production of 3762,43 kg/hectare; the density of 70000 plants/hectares led to the highest production of 3745,8 kg/hectare; the PR64A83 hybrid achieved the maximum production of 3609 kg/hectare.

**Key words:** hybrids, fertilisation, sunflower, production

**Rezumat.** Într-o experiență trifactorială de tipul fertilizare x desimea plantelor x hibrizi de floarea-soarelui s-a urmărit producția de achene în anul agricol 2009-2010, pe un sol cernoziom cambic, în condiții ecologice favorabile florii-soarelui. Înălțimea plantelor maximă, de 175,3 cm s-a realizat la aceeași doză de fertilizare ( $N_{120}P_{60}K_{120}$ ) la desimea de 70000 pl/ha (174,6 cm) și la hibridul PR63A90 (171,8 cm). Analizând producția de achene pe factorii cercetați s-au constatat următoarele: fertilizarea cu  $N_{96}P_{48}K_{96}$  a determinat cea mai mare producție, de 3762,43 kg/ha; la desimea de 70000 pl/ha s-a obținut producția maximă de 3745,8 kg/ha; hibridul PR64A83 a realizat producția maximă de 3609 kg/ha.

**Cuvinte cheie:** hibrizi, fertilizare, floarea – soarelui, producție

## INTRODUCTION

Fertilization is an important component in the technology of plant, representing the completion of the soil deficit in plant mineral elements necessary for it to develop properly. In terms of fertilization, we may argue that the sunflower has a low reactivity to the effect of fertilizers taking into account the achieved production growth.

Some of the many studies on sunflower culture refer to the influence of nitrogen fertilizers and the configuration and activity of the photosynthetic

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

device. Thus, Gimenez C., (1994), Mundstock C.M., (1994), Trapani N., (1996) conducted a research to clarify the mystery of the plant response to nitrogen fertilizers.

Plamer S.J., (1996) states that after the tests, the expansion and architecture of plants can be controlled with certain doses of nitrogen, resulting in the fact that nitrogen has a tremendous influence on the length and width of leaves of sunflower.

In what concerns the nitrogen effect on the production of achenes in sunflower Ibrahim M.E. and the collaborators (2007), following investigations have found that sunflower efficiently use nitrogen fertilizers applied to the plant seed, registering increases in the production.

Axinte M. and the collaborators (2007) present the fact that the productivity of sunflower achenes is influenced simultaneously by the plants density on surface area.

This paper aims at quantifying the effect of fertilizers and sowing densities on the production of sunflower achenes and also on vegetative growth.

## MATERIALS AND METHODS

During 2010 spring, on a cambic chernozem soil type within Ezăreni farm at the University of Agricultural Sciences and Veterinary Medicine - Iasi, a trifactorial experience was established by the method of subdivided parcels.

The three investigated factors were:

Factor A – fertilization – five graduations:

a1 –  $N_0P_0K_0$

a2 –  $N_{32}P_{24}K_{48}$

a3 –  $N_{64}P_{36}K_{72}$

a4 –  $N_{96}P_{48}K_{96}$

a5 –  $N_{120}P_{60}K_{120}$

Factor B - Plant density - three graduations:

b1 - 40,000 pl / ha

b2 - 55,000 pl / ha

b3 - 70,000 pl / ha

Factor C - Hybrid sunflower - four graduations:

c1 - Favorite

c2 - Performer

c3 - PR63A90

c4 - PR64A83

The seedbed preparation consisted of the basic work of the soil, the plowing being done at a depth of 30 cm; in spring two crossings were made with the combinator, at which time compound fertilizers of type 0:12: 24 and half the dose of ammonium nitrate under the experimental protocol have been applied, the second half being applied at the first mechanical weeding.

To achieve the specified densities, the combined SPC 8 type drills, equipped with three types of distribution disks and respecting all the technological norms was used.

## RESULTS AND DISCUSSIONS

During the agricultural year 2009-2010, at AGROEXPERT - Miroslava meteorological station the rainfall values that were ranked above the annual average were recorded. Average rainfall recorded at Miroslava meteorological station in the crop year 2009-2010 compared to the annual average. In terms of temperature, the crop year 2009-2010 was a year with higher temperatures than the annual average, hot temperatures being also recorded during some months of summer.

The number of leaves per plant was determined during the full bloom, showing a variation according to density, degree of fertility and of course to the used hybrid (table 1).

*Table 1*

**The influence of the investigated factors on the number of leaves and plant height in 2012**

| Specification                                     | No. of leaves       |                              |                | Plant height |                              |                |
|---|---------------------|------------------------------|----------------|--------------|------------------------------|----------------|
|   | No. leaves on plant | % Compared to the stander-by | The difference | Cm           | % Compared to the stander-by | The difference |
| <b>Influence of fertilization</b>                 |                     |                              |                |              |                              |                |
| N <sub>0</sub> P <sub>0</sub> K <sub>0</sub>      | 15,60               | 100,00                       | Mt.            | 171,3        | 100,00                       | Mt.            |
| N <sub>32</sub> P <sub>24</sub> K <sub>48</sub>   | 16,59               | 106,34                       | 0,98           | 172,8        | 100,8                        | 1,5            |
| N <sub>64</sub> P <sub>36</sub> K <sub>72</sub>   | 16,71               | 107,11                       | 1,1            | 173,4        | 101,2                        | 2,1            |
| N <sub>96</sub> P <sub>48</sub> K <sub>96</sub>   | 17,40               | 111,47                       | 1,79           | 174,3        | 101,7                        | 3,0            |
| N <sub>120</sub> P <sub>60</sub> K <sub>120</sub> | 18,26               | 116,97                       | 2,65           | 175,3        | 102,3                        | 4,0            |
| <b>Influence of plant density</b>                 |                     |                              |                |              |                              |                |
| 40000 pl/ha                                       | 16,73               | 100,00                       | Mt.            | 172,5        | 100,0                        | Mt.            |
| 55000 pl/ha                                       | 17,32               | 103,52                       | 0,59           | 173,3        | 100,4                        | 0,8            |
| 70000 pl/ha                                       | 16,70               | 99,82                        | -0,03          | 174,6        | 101,2                        | 2,1            |
| <b>Influence of hybrids</b>                       |                     |                              |                |              |                              |                |
| Favorite  | 16,79               | 100,00                       | Mt.            | 174,4        | 100,0                        | Mt.            |
| Performer   | 17,78               | 105,89                       | 0,99           | 176,0        | 100,9                        | 1,6            |
| PR63A90   | 16,50               | 98,27                        | -0,29          | 171,8        | 98,5                         | -2,6           |
| PR64A83   | 16,58               | 98,74                        | -0,21          | 171,4        | 98,2                         | -3,0           |

Fertilization showed an important role in increasing the vegetative mass; after modifying the doses of fertilizers, a directly proportional growth was observed, but while increasing the seeding density, an increase in plant height was also observed, the density of 70,000 pl / ha showing an increase in height of 1.2% compared with the stander-by.

Moreover, the genetic material influences the number of leaves per plant and its height. After analyzing the data on the number of leaves per plant it could be seen that the Performer hybrid achieved the highest increase in the number of leaves per plant, with a difference from the stander-by of 5.89% reaching a height of 176 cm, with 1.6 cm more than the stander-by.

In case of the sunflower achenes production, the influences of factors were separately pursued as well as the interaction between them. Fertilization has always played an important role in all crops, but sunflower requires a richer fertilization, being a huge consumer of nutrients. In Table 2 we present the influence of fertilization on the production of sunflower achenes.

Table 2

**Influence of fertilization on production of achenes in 2010**

| Fertilization                                     | Product kg / ha | Percent compared to the stander-by% | Difference (kg / ha) | Significance | kg of Achenes to 1 kg of fertilizer |
|---|-----------------|-------------------------------------|----------------------|--------------|-------------------------------------|
| N <sub>0</sub> P <sub>0</sub> K <sub>0</sub>      | 2588,25         | 100,00                              | Mt.                  | -            |                                     |
| N <sub>32</sub> P <sub>24</sub> K <sub>48</sub>   | 3166,50         | 122,34                              | 578,25               | xx           | 5,56                                |
| N <sub>64</sub> P <sub>36</sub> K <sub>72</sub>   | 3568,89         | 137,88                              | 980,64               | xxx          | 5,70                                |
| N <sub>96</sub> P <sub>48</sub> K <sub>96</sub>   | 3762,43         | 145,36                              | 1174,18              | xxx          | 4,89                                |
| N <sub>120</sub> P <sub>60</sub> K <sub>120</sub> | 3716,60         | 143,59                              | 1128,35              | xxx          | 3,76                                |
| DL 5%   | 308,2 kg/ha     |                                     |                      |              |                                     |
| DL 1%   | 409,3 kg/ha     |                                     |                      |              |                                     |
| DL 0,1%   | 532,8 kg/ha     |                                     |                      |              |                                     |

The best results of fertilization occurred in case of N<sub>96</sub> P<sub>48</sub> K<sub>96</sub> dose, an increase of 45.36% compared to the stander-by (1174.18 kg/ha) being registered, the difference compared to the stander-by being very significant. Once the dose was increased by applying a variant of fertilization of N<sub>120</sub> P<sub>60</sub> K<sub>120</sub>, the production decreased up to 45.83 kg/ha (in percentage of 1.77%) compared with its predecessor.

The plant density per unit area influenced the production as shown in table 3.

Table 3

**The effect of density on production in 2010**

| Density pl / ha | Production kg / ha | Percent compared to the stander-by % | Difference (kg / ha) | Significance |
|-----------------|--------------------|--------------------------------------|----------------------|--------------|
| 40,000          | 2938.39            | 100                                  | Mt.                  | -            |
| 55,000          | 3397.42            | 115.62                               | 459.03               | xx           |
| 70,000          | 3745.80            | 127.47                               | 807.41               | xxx          |
| DL 5%           | 157,3 kg/ha        |                                      |                      |              |
| DL 1%           | 361,2 kg/ha        |                                      |                      |              |
| DL 0,1%         | 2,529 kg/ha        |                                      |                      |              |

With reference to our results summarized in the table above, we notice that the most beneficial effect on production has a density of 70,000 pl/ha, this being recorded during the experiments, the best production compared to the stander-by with an addition of 27, 47% from the stander-by version, the difference being very significant. At the density of 55,000 pl/ha, the difference from the stander-by was significantly distinct.

The production is also influenced by the genetic heritage of the hybrid used in the crop, the data being presented in Table 4.

Table 4

**Hybrid effect on production in 2010**

| Hybrids   | Production kg / ha | Percent compared to the stander-by% | Difference (kg / ha) | Significance |
|-----------|--------------------|-------------------------------------|----------------------|--------------|
| Favorite  | 3049.80            | 100                                 | Mt.                  | -            |
| Performer | 3261.73            | 106.94                              | 211.93               | x            |
| PR63A90   | 3521.58            | 115.46                              | 471.78               | xxx          |
| PR64A83   | 3609.04            | 118.33                              | 559.24               | xxx          |
| DL 5%     | 153,7 kg/ha        |                                     |                      |              |
| DL 1%     | 244,5 kg/ha        |                                     |                      |              |
| DL 0,1%   | 361,5 kg/ha        |                                     |                      |              |

Note that the Performer hybrid production grew by 211.93 kg / ha compared to the stander by. The PR64A83 hybrid registered a notable difference from the stander-by, of 559.24 kg / ha, exceeding with 18.33% production of the Favorite hybrid taken as the stander-by.

**CONCLUSIONS**

1. During fertilization with N<sub>96</sub> P<sub>48</sub> K<sub>96</sub>, 3762.43 kg/ha have been obtained, with 45.36% more than in N<sub>0</sub> P<sub>0</sub> K<sub>0</sub> stander-by version (2588.25 kg/ha);
2. The density of 70,000 pl / ha resulted in the highest yield of achenes of 3716.60 kg / ha with 27.47% higher than the stander-by density, 40000 pl/ha;
3. The most productive hybrid was PR64A83, with a production of 3,609.04 kg/ha, with 18.33% higher than the Favorite hybrid stander-by.

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# COORDINATE TRANSFORMATIONS IN ORDER TO INTEGRATE LOCAL MAP INFORMATION IN THE NEW GEOCENTRIC EUROPEAN SYSTEM

## TRANSFORMARI DE COORDONATE IN SCOPUL INTEGRARII INFORMATIEI CARTOGRAFICE LOCALE IN NOUL SISTEM GEOCENTRIC EUROPEAN

**MIHALACHE (FICIUC) Raluca Mariana<sup>1</sup>, POPIA (ILICA) Mădălina<sup>1</sup>**  
e-mail: rmihalacheficiuc@yahoo.com

**Abstract.** *The concept of "geodetic datum" was recently assimilated by geodetic specialized Romanian literature, and through it, we can operate in the more complex space of coordinate systems along with the reference surface specifications to which they relate. The necessary correspondence between local and global-geocentric data constitutes a current problem which is resolved under the accuracy terms, claimed in general by the terrestrial measurement works and in particular by the cadastral survey works. Thereby its aims the validation of currently using existing space data in the Local-Iasi area and the integration in the European new coordinate system, adopted in 2009, of the local information, for the urban real-estate works. The conducted study is linked to the final transition, of our country, to the national geodesic satellite network and the achievement of a new digital city plan.*

**Key words:** Datum, Data, coordinate, survey

**Rezumat.** *Noțiunea de „datum geodezic” a fost asimilată de curând și în literatura de specialitate geodezică din țara noastră, iar prin intermediul ei, putem opera în spațiul tot mai complex al multitudinilor de sisteme de coordonate, împreună cu specificațiile suprafețelor de referință la care acestea se raportează. Corespondența necesară între datumurile geodezice locale și cele global – geocentrice constituie o problemă de actualitate, care se cere rezolvată în termenii de precizie pe care îi necesită lucrările de măsurători terestre, în general și cele de cadastru, în mod special. Prin aceasta, se urmărește, validarea utilizării datelor spațiale existente în prezent în spațiul Local-Iasi, pe zona de studiu și integrarea informației locale în noul sistem european de coordonate adoptate în anul 2009, pentru lucrările de cadastru imobiliar-edilidar. Studiul efectuat este legat de trecerea definitivă la rețeaua geodezică națională satelitară a țării noastre și de realizare a unui nou plan digital al municipiului Iaș.*

**Cuvinte cheie:** Datum, date, coordonate, cadastru

### INTRODUCTION

Romania's admission as a member with full rights into the European Union, event of historic importance, involves the adoption and/or elaboration of technical standards for the elaboration of digital cartographic outputs, which must

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<sup>1</sup> "Gheorghe Asachi" Technical University of Iasi, Romania

fulfill the international standards concerning the codification, symbolism, manner of reference of data, storage of spatial information, creation and administration of a national GIS (Geographic Information System). This framework will enable the exchange between the European Community and international community and the creation of an infrastructure of National Spatial Data which will be compatible with the infrastructure for spatial information from Europe. These desideratum are possible and achievable by the adoption and fulfillment of the International Standard ISO 19111, adopted as a pan-European standard with the purpose of a precise and correct identification of the system of reference and of coordinates for each. The global positioning system (GPS) is frequently used in establishing geodetic networks because GPS provides location and time information with a high accuracy anywhere on the Earth.

Regarding the transition from local-national to European datum the solution implementations phase was successfully resolved (creating the permanent GNSS reference station network and coordinates transformations "Transdat" soft-A.N.C.P.I.). This procedure should be extended to other local reference systems such as those created for big cities and municipalities. This local system served to create some small dimension local geodetic networks, which afterwards, formed the base on surveying the terrain details.

For further application of 2D coordinate transformation models it is necessary to know the minimum number of points, according to the used model, which have plane rectangular coordinate in both local and global reference system. Data required for the coordinates in the European system (ETRS 89) have been obtained through GPS measurements campaigns conducted in 2005 with the creation of geospatial network of Iasi and then in 2010 with the network extending for the entire metropolitan area of Iasi [10].

The calculation algorithms present the following steps:

1. Parameters calculation of the WGS-84 coordinates (epoch 2005) 3D transformation in ETRS-89 coordinates, based on three shared points, by applying the transformation model with 7 parameters, like „Bursa-Wolf” [1].

2. WGS-84(epoch 2005) coordinates transformation of 7 shared points from GPS thickening network in ETRS-89 coordinates, expressed in Cartesian ellipsoidal system.

3. Ellipsoidal geodetic coordinate's conversion of the 7 shared GPS thickening points in Stereo-70/GRS-80 cartographic projection system (Stereo-2010).

4. 2D transformation parameters calculation of specified 7 points from the „Local-Iasi” into Stereo-70/GRS-80 system, with conformal linear method (table 2).

5. 2D transformation parameters calculation of those 7 points from the „Local-Iasi” into Stereo-70/GRS-80 system, with affine method (table 3).

7. Coordinates transformation of those 7 shared points from „Local-Iasi” in Stereo-70/GRS-80 system (Stereo 2010), using trans-calculus parameters previously calculated (table 1, table 3).



## CONFORMAL LINEAR TRANSFORMATION IN 2D SPACE

This transformation keeps only the topographic conditions and use the simplified hypothesis of one system translation and rotation in the same space from the other [5].

The correction equations is written in linearized form :

$$x_i * a - y_i * b + \Delta x - X_i = v_{x_i}$$

$$y_i * a + x_i * b + \Delta y - Y_i = v_{y_i}$$

and in matrix form:

$$B_{2n,4} X_{4,1} + L_{2n,1} = V_{2n,1} \text{ where:}$$

where  $n$  is the number of double points with known coordinates in both systems.

The unknowns of the system are the  $(\Delta x, \Delta y)$  translation and  $(a, b)$  rotation parameters, unknown coefficients  $(x_i, y_i)$  are the coordinates of the double points in the "source" system and the  $(X_i, Y_i)$  free terms are the coordinates of the same points in "the target" system.

Introducing the minimum condition  $([vv] \rightarrow \min)$ [6] and canceling the function partial derivatives, related to unknowns of the system, is obtained by the normal equations system, with four equations with four unknowns  $(a, b, \Delta x, \Delta y)$ :

$$N_{4,4} X_{4,1} + B_{4,2n}^T L_{2n,1} = O_{4,1} \text{ where } N_{4,4} = B_{4,2n}^T B_{2n,4}$$

The unknowns matrix is calculated with the matrix inverse method:

$$X_{4,1} = -(N_{4,4})^{-1} B_{4,2n}^T L_{2n,1}$$

The precision determinations of each trans-calculus parameter from the unknown matrix is expressed with a mean square error:

$$s_j = s_0 \sqrt{Q_{jj}}, j = 1, 4 \text{ in which : } s_0 = \sqrt{\frac{v_{2n,1}^T v_{2n,1}}{2n-4}}$$

In the end, based on trans-calculus parameters, these formulas will determine the new points coordinates:

$$\begin{pmatrix} X_i \\ Y_i \end{pmatrix} = \begin{pmatrix} \Delta x \\ \Delta y \end{pmatrix} + \begin{pmatrix} a & -b \\ b & a \end{pmatrix} * \begin{pmatrix} x_i \\ y_i \end{pmatrix}$$

In table 1.1 are the coordinates of the seven GPS thickening points, in "Tasi-local" and Stereo-70/GRS-80 coordinates systems. The coordinates from the local system have been obtained with specific method of thickening the local geodetic network (backward multiple intersection method), rigorously compensated, applying the least squares principle, indirect measurements process.

## AFFINE TRANSFORMATION IN 2D SPACE

In two dimensional affine transformation different corrections are introduced for each of the two coordinates axes directions [5].

For the affine transformations are considered known seven GPS thickening points coordinates in both system, "Iasi-local" and Stereo-70/GRS-80, from the table 1. The transformation results are in the table 1.3

Table 1

Coordinates of the GPS thickening points in "Iasi-local" and Stereo-70/GRS-80 system

| Point | Plane rectangular coordinates "Iasi-local" system |           | Plane rectangular coordinates „Stereo-70/GRS-80" system |            |
|-------|---|-----------|---|------------|
|       | X(m)  | Y(m)      | X(m)  | Y(m)       |
| I 130 | 10596.933   | 9027.482  | 633186.851  | 695299.976 |
| I 131 | 10419.701   | 9297.834  | 633017.575  | 695675.456 |
| I 132 | 9764.476  | 9515.597  | 632368.889  | 695812.583 |
| I 133 | 9375.654  | 9726.531  | 631986.453  | 696034.953 |
| I 137 | 9751.542  | 10637.455 | 632389.108  | 696934.600 |
| I 138 | 9422.152  | 10859.701 | 632066.365  | 697166.475 |
| I 150 | 9506.601  | 8753.605  | 632088.538  | 965058.326 |

Table 2

Conformal linear transformation („Transdatum"soft [2])

| Trans-calculus parameters from the unknowns matrix |                      |                     | Point | Plane rectangular coordinates „Stereo-70/GRS-80" system |            |
|--|----------------------|---------------------|-------|---|------------|
| Parameter  | Value                | Determination error |       | X(m)  | Y(m)       |
|  |                      |                     | I 130 | 633186.797  | 695299.952 |
| $\Delta x$   | 622325.27870319 m    | 0.22 m,             | I 131 | 633017.585  | 695575.478 |
| $\Delta y$   | 686587.22275504 m    | 0.22 m.             | I 132 | 632368.919  | 695812.561 |
| a  | 0.9998058950 rad.    | 0.00 rad.           | I 133 | 631986.403  | 696034.938 |
| b  | -0.029536821000 rad. | 0.00 rad.           | I 137 | 632389.124  | 696934.583 |
| Standard trans-calculus error for a point          |                      |                     | I 138 | 632066.362  | 697166.515 |
|  |                      |                     | I 150 | 632088.588  | 695058.333 |
|  |                      |                     |       | 0.036m  |            |

Table 3

Affine transformation („Transdatum"soft [2])

| Trans-calculus parameters from the unknowns matrix |                     |                     | Plane rectangular coordinates „Stereo-70/GRS-80" system |            |            |
|--|---------------------|---------------------|---|------------|------------|
| Parameter  | Value               | Determination error | Point   | X(m)       | Y(m)       |
| $\Delta x$   | 622324.898668895 m. | 0.485 m.            |   |            |            |
| $\Delta y$   | 686587.286455268 m. | 0.485m.             | I 130   | 633186.813 | 695299.958 |
| a  | 0.999834584 rad.    | 0.00 rad.           | I 131   | 633017.598 | 695575.490 |
| b  | 0.029546926 rad.    | 0.00 rad.           | I 132   | 632368.916 | 695812.562 |
| c  | -0.029535567 rad.   | 0.00 rad.           | I 133   | 631986.390 | 696034.937 |
| d  | 0.999798047 rad.    | 0.00 rad.           | I 137   | 632389.131 | 696934.575 |
| Standard trans-calculus error for a point          |                     |                     | I 138   | 632066.362 | 697166.505 |
|  |                     |                     | I 150   | 632088.569 | 695058.340 |
|  |                     |                     |   | 0.038m     |            |

# THE TRANSFORMATIONS COORDINATES POSSIBILITIES BETWEEN GEODETIC DATUM USING ANNS

## 1. Back propagation artificial neural network

➤ Neural networks have emerged as a field of study within AI and engineering via the collaborative efforts of engineers, physicists, mathematicians, computer scientists, and neuroscientists

➤ A neural network is first and foremost a graph, with patterns represented in terms of numerical values attached to the nodes of the graph and transformations between patterns achieved via simple message-passing algorithms. Certain of the nodes in the graph are generally distinguished as being *input* nodes or *output* nodes, and the graph as a whole can be viewed as a representation of a multivariate function linking inputs to outputs. Numerical values (*weights*) are attached to the links of the graph, parameterizing the input/output function and allowing it to be adjusted via a *learning algorithm* [5].

➤ Neural networks have been trained to perform complex functions in various fields of application including pattern recognition, identification, classification, speech, vision, and control systems. Back-propagation (BP) was created by generalizing the Widrow-Hoff learning rule to multiple-layer networks and nonlinear differentiable transfer functions. Input vectors and the corresponding target vectors are used to train a network until it can approximate a function, associate input vectors with specific output vectors, or classify input vectors in an appropriate way as defined by you [7].

The multilayer perceptron (MLP) model was selected for this study because MLPs have ability to learn, operate fast, require small training sets and can be implemented simply among several kinds of ANN models. MLP consists of one input layer with  $N$  inputs, one hidden layer with  $q$  units and one output layer with  $n$  outputs. The output of the model ( $y$ ) with a single output neuron can be represented by:

$$y = f \left( \sum_{j=1}^q W_j f \left( \sum_{l=1}^N w_{j,l} x_l \right) \right)$$

where  $W$  is the weight between the hidden layer and the output layer,  $w$  is the weight between the input layer and the hidden layer,  $x$  is the input parameter. A sigmoid function is used as activation function for hidden and out layers that is defined by:  $f(z) = 1 / (1 + e^{-z})$

where  $z$  denotes the input information of the neuron.

Each neuron within network collects information by means of all its input connections fulfills a predefined mathematical operation and offers an output value. Neurons are linked by weighted connections, storing the information. By adjusting the weights, the neuronal network is able to learn.

The network contains I input neurons, J hidden neurons and K output neurons. The weights of the input layer and the hidden one, respectively the hidden layer and the output one are noted with  $w = \{w_{ij}\}$ , respectively  $v = \{v_{jk}\}$ .

Functions of activation the neurons in the hidden layer and in the output one are noted with  $g(\cdot)$ ,  $h(\cdot)$  respectively. Driving such a network is made by using a set of driving data which make use of M desired in - out pairs, under the following form:

$$x^{(m)} = \{x_1^{(m)}, x_2^{(m)}, \dots, x_I^{(m)}\} \quad \div \quad d^{(m)} = \{d_1^{(m)}, d_2^{(m)}, \dots, d_K^{(m)}\},$$

$m=1, \dots, M$

Consequently, for an approximation as correct as possible of the desired outputs  $d^{(m)}$ , through the real outputs  $o^{(m)}$ , it is to be applied an adjusting grid weights method using as a target function a valuation of the approximation errors with the total square deviation.:

$$APT = \sum_{m=1}^M \|d^{(m)} - o^{(m)}\|^2 = \sum_{m=1}^M \sum_{k=1}^K (d_k^{(m)} - o_k^{(m)})^2$$

➤ **BPANN** model was selected because it has been more widely applied in engineering among all other ANN applications. BPANN has a feed-forward and supervised learning structure which consists one input layer, one or more hidden layers and one output layer, as shown in Figure 1[8].

For determining the performance of the neural network, the mean square error (MSE) can be used that is defined by:

$$MSE = \sum_1^N (y_{known} - y_{neuronal})^2 / N^2$$

where  $N$  is the number of the inputs,  $y_{known}$  denotes the known (target) output value and  $y_{neuronal}$  denotes the network output value.

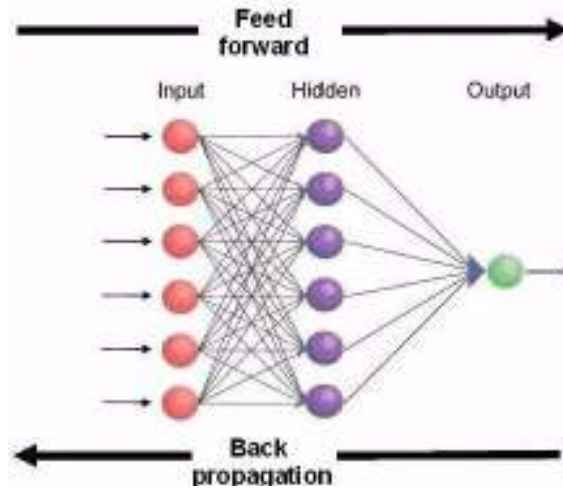


Fig.1 - The general structure of a BPANN [9]

## CONCLUSIONS

1. The existing graphical information in the “Local-Iasi” system can be spatially integrated in the new European geodetic datum, through the coordinates transformation with a precision appropriate with real estate cadaster rules.

2. In order to determine the 2D transformation parameters, three coordinate transformation models (linear, affine and second degree polynomial), with similar results falling within the tolerance of 5 cm for the position of a point in the plan. The best result of  $\pm 3,6$  cm was obtained for the linear transformation but it is possible that an extension of shared points increase in number the precision on the other two transformation methods, which are related to a complex distribution of the relative position error of those two work systems.

3. An artificial neural network can be applied to cadastral coordinate transformation.

4. In order to study the 2D coordinate transformation, algorithms of applying a back-propagation artificial neural network (BPANN) is proposed.

5. For testing the accuracies of the proposed algorithms, some points must be used to train the artificial neural network, and others to evaluate the performance of the proposed algorithms. The points used to train the artificial neural network will be defined as reference points, while the other points which will be used to evaluate the performance of the proposed algorithms will be defined as check points.

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# GEOREFERENTIATION OF THE GRAPHIC FUND FOR TOPOGRAPHIC PLANS DRAWN UP ON GEODESIC TRAPEZIA AT GENERAL CADASTRAL WORKS

## GEOREFERENȚIEREA FONDULUI GRAFIC AL PLANURILOR TOPOGRAFICE ÎNTOCMITE PE TRAPEZE GEODEZICE, ÎN LUCRĂRILE DE CADASTRU GENERAL

MOCA V.<sup>1</sup>, ONIGA Ersilia Valeria<sup>2</sup>, CÂRDEI Mihaela<sup>2</sup>  
e-mail: valmoca@uaiasi.ro

**Abstract.** For a precise integration of raster data into the national projection system, the affine transformation method was used; this method provides a satisfactory precision in this case. For the same time for the accurate georeferentiation of raster data the graphic fund of the basic cadastral plan was used. It was created at a scale of 1:5 000 using the Stereographic projection system – 1970. The cadastral sheet (geodesic trapezium) included the incorporated and unincorporated area from the administrative unit of the Bilca commune – Suceava County. The evaluation of the georeferentiation accuracy was analyzed using the mean squared error that was established according to the differences of the vectorial coordinates of the control points and respectively, the corresponding points from the topographic surface of the field.

**Key words:** georeferentiation, affine transformation, control points, mean squared error

**Rezumat.** În vederea integrării precise a datelor raster în sistemul de proiecție național, s-a folosit metoda transformării affine care asigură o precizie satisfăcătoare. Pentru georeferențierea precisă a datelor raster s-a folosit fondul grafic al planului cadastral de bază, întocmit la scara 1:5 000, în sistemul proiecției Stereografice-1970. Foaia de plan (trapezul geodezic) a cuprins zona intravilanului și a extravilanului din unitatea administrativ-teritorială a comunei Bilca, din județul Suceava. Evaluarea preciziei de georeferențiere a fost analizată prin eroarea medie pătratică, care a fost determinată în funcție de diferențele dintre coordonatele vectoriale ale punctelor de control și, respectiv, ale punctelor corespondente de pe suprafața topografică a terenului.

**Cuvinte cheie:** georeferențiere, transformarea afină, puncte de control, eroarea medie pătratică.

### INTRODUCTION

The basic elements of the general cadastre –the parcel, the construction and the owner – are identified and recorded in the cadastral documents after performing the proper cadastral measurements in each territorial – administrative unit of Romania (Moca, et al., 2011).

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

<sup>2</sup>“Gheorghe Asachi” Technical University of Iasi, Romania

The basic topographic plan made between 1951 and 1990 was created using the photogrammetric technology. The plan was made at scales of 1:2 000, 1:5 000 and 1:10 000 and it included almost 85% of Romania's territory. Between 2001 and 2010, in order to create the digital and analog format of the orthophotoplan aerophotogrammetric elevations were performed, the resolution of the cartographic representation was at a scale of 1:10 000.

The global positioning technologies based on satellite navigation systems started being used in Romania after 1990. The A class GPS National Geodetic Network that was created until the beginning of 2008 included, according to the official data: 28 permanent stations uniformly distributed that were determined using the three spatial coordinates (3D), the precision level being of less than  $\pm 1$  cm (Andrei, C. O., 2010). Referring to the positioning methods used, the **kinematic method** was most appreciated. It relies on the use of two frequencies GNSS receivers and a short observation period thus providing centimetric accuracy (Hofmann – Wellenhof et al., 2008).

The kinematic method of absolute positioning is also known as **Real Time Kinematic** (RTK) as it allows the determination of spatial coordinates (3D), in real time. In the case of Romania's territory, the **Romanian Position Determination System** (ROMPOS, 2008) was adopted. This method permanently provides corrections for point positioning from the support geodetic network as well as for the elevation points of topographic details.

Starting with 2009, Romania adopted the **European Terrestrial Reference System 1989 (RO\_ETRS 89)** along with a series of standard software applications, the most important of which being the **TransDatRO** programme.

## MATERIAL AND METHOD

For the integration of raster data in the Stereographic projection system – 1970 the **affine transformation method** was used. This method is currently used in the georeferentiation process of scanned topographic plans and maps (Băduț, M., 2004).

The affine transformation operation includes translation, rotation and the scale factor but the deformations are different on the two axes of coordinates (X, Y).

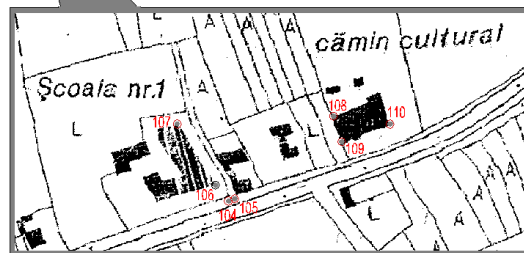
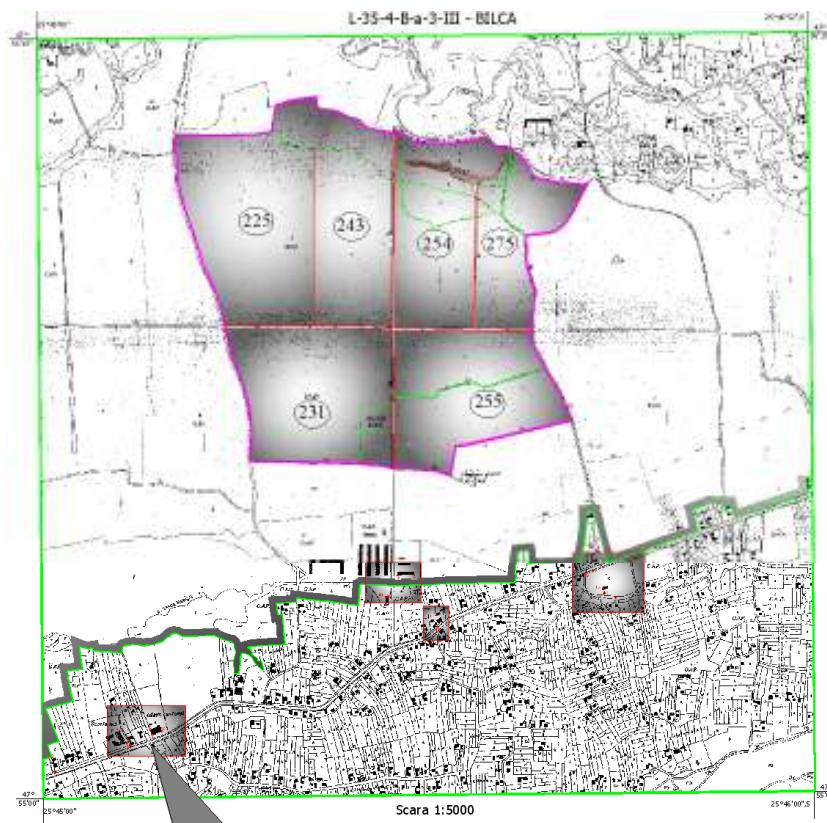
The most often used transformation formulas are those of the affine transformation, like:

$$X_c = AX + BY + C; \quad Y_c = DX + EY + F$$

For the accurate georeferentiation of raster data the graphic fund of the basic cadastral plan was used, at a scale of 1:5 000, with the nomenclature L-35-4-B-a-3-III that included the limits of cadastral sectors and cadastral parcels that existed in 1978, the year the Bilca territory was aerophotographed (Figure 1).

The correlation/rectification of the raster image in CAD software was performed according to the 24 control points, of which: 4 points are represented by the geodetic trapezium's corners (1, 2, 3 and 4) and 20 intersection points of the axes of the kilometeric grid with the side of 0.5 km (1.1, 1.2, 1.3, 1.4, ..., 5.1, 5.2, 5.3, 5.4). The distribution and the selection of the control points of the mathematical transformation of the raster image took into consideration the uniform distribution principle of the images on the graphic support of the cadastral plan, thus assuring the accuracy.





**LEGEND:** ○ 106 and 107 – ground control points, the School building Bilca;  
 ○ 108, 109 and 110 – ground control points, Community centre's building Bilca;  
 225, 231, 243, 254, 255, 275 – agricultural physical blocks from the unincorporated Bilca area

**Fig. 1 - GEOREFERENTIATION OF THE BASIC CADASTRAL PLAN, SCALE 1:5 000 AND THE POSITION OF GROUND CONTROL POINTS**

## RESULTS AND DISCUSSIONS

The accuracy of the georeferentiation process of the basic cadastral plan was evaluated by pointing out and verifying the differences between the values of the plane stereographic coordinates of the 24 control points.

Depending of the values of these differences, that are presented in Table 1, between the calculated coordinates ( $x_r$  and  $y_r$ ) and the measured coordinates ( $x_i$  and  $y_i$ ), the following parameters of transformation precision were determined.

**RMS error (RMSE)** is the distance between the input (map or reference) location of a **Ground Central Point (GCP)** and the transformed location for the same GCP. It is a difference between the desired output coordinate for a GCP and the actual output coordinate for the same point is transformed with the geometric transformation.

**Root Mean Square Error (RMSE)** is calculated with a distance equation:

$$RMS_{error} = \sqrt{(x_r - x_i)^2 + (y_r - y_i)^2}$$

$$RMS_{error} 1 = \sqrt{(-1.419)^2 + (0.691)^2} = \sqrt{2.014 + 0.477} = 1.578 \text{ m}$$

where:  $x_i$  and  $y_i$  are the input source coordinates;

$x_r$  and  $y_r$  are the retransformed coordinates.

The values determined for the **24 control points** ranged between a minimum value of **0.038 m** and a maximum value of **3.178 m** (Table 1).

For the residuals, the following calculations are made to determine the total RMS error, the X RMS error, and the Y RMS error:

$$R_x = \sqrt{\frac{1}{n} \sum_{i=1}^n XR_i^2} = \sqrt{\frac{1}{24}(14.397)} = 0.775 \text{ m};$$

$$R_y = \sqrt{\frac{1}{n} \sum_{i=1}^n YR_i^2} = \sqrt{\frac{1}{24}(25.723)} = 1.035 \text{ m};$$

$$T = \sqrt{R_x^2 + R_y^2} = \sqrt{(0.775)^2 + (1.035)^2} = 1.293 \text{ m};$$

$$T = \sqrt{\frac{1}{n} \sum_{i=1}^n XR_i^2 + YR_i^2} = \sqrt{\frac{1}{24}(14.397 + 25.723)} = 1.293 \text{ m}$$

where:  $R_x$  – total X RMS error;  $n$  – the number of GCPs and  $i$  – GCP number;

$R_y$  – total Y RMS error;  $XR_i$  – the X residual for GCPi;

$T$  – total RMS error;  $YR_i$  – the Y residual for GCPi.

**The total root mean square error** calculated for the 24 control points (GCPs) was evaluated using the value of **1.293 m**, that confirms a relatively accurate georeferentiation of raster images.

**A normalized value representing each point's RMS error** in relation to the total RMS error:  $E_i = R_i / T$  where:  $E_i$  – error contribution of GCPi;  $R_i$  – the RMS error for GCPi;  $T$  – total RMS error.

In the case study of the georeferentiation process, the standard errors of each analyzed point ranged between a minimum value of **0.029 m** and a maximum value of **2.458 m**.

Table 1

**Stereographic plane coordinates – 1970, calculated and measured,  
of the trapezium's corners and of the points of the kilometric network**

| Point no.                           | Calculated stereographic coordinates (m) |            | Measured stereographic coordinates (m) |            | Coordinated differences (m) |                 |              |
|-------------------------------------|--|------------|--|------------|-----------------------------|-----------------|--------------|
|                                     | Xr < 70 >                                | Yr < 70 >  | Xi < 70 >                              | Yi < 70 >  | $(X_r - X_i)^2$             | $(Y_r - Y_i)^2$ | RMSE         |
|                                     |  |            |  |            | $XR_i^2$                    | $YR_i^2$        | $R_i$        |
| <b>L-35-4-B-a-3-III Trapezium</b>   |  |            |  |            |                             |                 |              |
| 1                                   | 715629.342                               | 556039.690 | 715630.761                             | 556038.999 | 2.014                       | 0.477           | 1.578        |
| 2                                   | 715653.385                               | 558376.010 | 715653.573                             | 558373.914 | 0.035                       | 4.393           | 2.104        |
| 3                                   | 713312.343                               | 556058.641 | 713314.272                             | 556061.166 | 3.721                       | 6.376           | 3.178        |
| 4                                   | 713336.653                               | 558397.586 | 713337.088                             | 558397.005 | 0.189                       | 0.338           | 0.726        |
| 11                                  | 713499.020                               | 556499.700 | 713500.000                             | 556500.000 | 0.961                       | 0.090           | 1.025        |
| 12                                  | 713499.334                               | 557000.318 | 713500.000                             | 557000.000 | 0.444                       | 0.101           | 0.738        |
| 13                                  | 713499.649                               | 557501.142 | 713500.000                             | 557500.000 | 0.123                       | 1.303           | 1.194        |
| 14                                  | 713499.963                               | 558000.863 | 713500.000                             | 558000.000 | 0.001                       | 0.744           | 0.863        |
| 21                                  | 713999.969                               | 556500.022 | 714000.000                             | 556500.000 | 0.001                       | 0.000           | 0.038        |
| 22                                  | 714000.244                               | 557000.480 | 714000.000                             | 557000.000 | 0.059                       | 0.230           | 0.538        |
| 23                                  | 714000.518                               | 557501.118 | 714000.000                             | 557500.000 | 0.268                       | 1.249           | 1.232        |
| 24                                  | 714000.793                               | 558000.890 | 714000.000                             | 558000.000 | 0.628                       | 0.791           | 1.191        |
| 31                                  | 714500.331                               | 556500.344 | 714500.000                             | 556500.000 | 0.109                       | 0.118           | 0.477        |
| 32                                  | 714500.438                               | 557000.641 | 714500.000                             | 557000.000 | 0.191                       | 0.410           | 0.776        |
| 33                                  | 714500.544                               | 557501.094 | 714500.000                             | 557500.000 | 0.296                       | 1.196           | 1.221        |
| 34                                  | 714500.651                               | 558000.916 | 714500.000                             | 558000.000 | 0.423                       | 0.838           | 1.123        |
| 41                                  | 715000.711                               | 556500.666 | 715000.000                             | 556500.000 | 0.505                       | 0.443           | 0.974        |
| 42                                  | 715000.764                               | 557000.802 | 715000.000                             | 557000.000 | 0.583                       | 0.643           | 1.107        |
| 43                                  | 715000.817                               | 557501.069 | 715000.000                             | 557500.000 | 0.667                       | 1.142           | 1.345        |
| 44                                  | 715000.869                               | 558000.943 | 715000.000                             | 558000.000 | 0.754                       | 0.888           | 1.282        |
| 51                                  | 715500.738                               | 556500.988 | 715500.000                             | 556500.000 | 0.544                       | 0.975           | 1.233        |
| 52                                  | 715500.765                               | 557000.963 | 715500.000                             | 557000.000 | 0.585                       | 0.927           | 1.229        |
| 53                                  | 715500.791                               | 557501.045 | 715500.000                             | 557500.000 | 0.625                       | 1.091           | 1.310        |
| 54                                  | 715500.818                               | 558000.979 | 715500.000                             | 558000.000 | 0.668                       | 0.958           | 1.275        |
| <b>Total Root Mean Square Error</b> |  |            |  |            | <b>0.775</b>                | <b>1.035</b>    | <b>1.293</b> |

The digital conversion of the existent analog cartographic material was made with a graphic error of +/- **0.2 mm** that corresponds to the precision of the numerical scale. **The maximum graphic error** admitted in reporting the points from the geodetic and elevation network, for the cadastral plan, **scale 1:5 000** was of +/- **1.00 m**.

In the case of the performed study as ground control points were used a series of punctiform elements represented by buildings' corners, means of communication, enclosures and others. The field measurements were performed using the GNSS South S82T receiver and the real time kinematic positioning method that is also known as RTK (*Real Time Kinematic*).

The calculation of the surfaces of the six agricultural physical blocks (225, 231, 243, 254, 255, 275) required the use of the plane Stereographical coordinates – 1970 (X, Y) of the points from the geometric contour (figure 1).

**The resulting surfaces of the six agricultural physical blocks** were determined using the previously mentioned methods are included in table 2.

Table 2

**Surfaces calculated on digital support and GPS measured of the physical agricultural blocks from Bilca territory**

| Agricultural physical block's code | Agricultural physical blocks' surface  |  |                                    | Surface differences |                 |
|------------------------------------|--|--|------------------------------------|---------------------|-----------------|
|                                    | Vectorized cadastral plan 1:5 000/1978 | Digital on the orthophotoplan 1:10000/2006 | Measured in the field, GPS in 2011 | $\pm (S_i - S)$     | $\pm (S_j - S)$ |
|                                    | $S_i$ (ha)                             | $S_j$ (ha)                                 | $S$ (ha)                           | ha                  | ha              |
| 225                                | 22.7634                                | 22.9631                                    | 22.9875                            | -0.2241             | -0.0244         |
| 231                                | 18.6214                                | 18.7384                                    | 18.7485                            | -0.1271             | -0.0101         |
| 243                                | 14.4787                                | 14.4989                                    | 14.5134                            | -0.0347             | -0.0145         |
| 254                                | 14.3317                                | 14.6294                                    | 14.6582                            | -0.3265             | -0.0288         |
| 255                                | 17.1101                                | 17.0118                                    | 16.9222                            | +0.1879             | +0.0896         |
| 275                                | 10.6842                                | 10.4438                                    | 10.4546                            | +0.2296             | -0.0108         |
| Roads                              | 1.3149                                 | 1.3309                                     | 1.3001                             | +0.0148             | +0.0308         |
| <b>TOTAL</b>                       | <b>99.3044</b>                         | <b>99.6163</b>                             | <b>99.5845</b>                     | <b>-0.2801</b>      | <b>+0.0318</b>  |

After compensating the surfaces of the vectorized / digital physical blocks on the total surface of the cadastral sector of **99.5845 ha**, they were constrained on the **control area of 540.9816 ha**. This surface of the geodetic trapezium, nomenclature L-35-4-B-a-3-III, represents the undistorted measure from the projection plan.

### CONCLUSIONS

1. **The accuracy of the georeferentiation process** of the cadastral plan was made evident depending of the differences between the coordinates of the control points determined from the raster image and the real / measured ones in the field with GPS receivers of geodetic precision.

2. **The discrepancy** between the vectorized / digital surfaces and the measured surfaces was compensated first of all on the values of the physical blocks measured in the field after which they were constrained to the **control area of 540.9816 ha** of the trapezium.

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# 3D REPRESENTATION OF THE TOPOGRAPHICAL SURFACES FOR THE REHABILITATION AND DEVELOPMENT OF THE STOCK PONDS

## REPREZENTAREA 3D A SUPRAFETELOR TOPOGRAFICE PENTRU REABILITAREA ȘI REAMENAJAREA IAZURILOR PISCICOLE

*ONIGA Valeria Ersilia*<sup>1</sup>, *CRENGANIȘ (BARGAN) Loredana*<sup>1</sup>  
e-mail: ersylya83@yahoo.com

**Abstract.** *This article presents the methodology for creating the terrain 3D digital model based on topographic surveys and also for using it to carry out some preliminary calculations of the design process, to reduce the damage caused by floods and land arrangement occupied by water. The 3D digital terrain model allows users to perform a series of operations such as viewing, analyzing, measuring, querying and others.*

**Key words:** Delauney triangulation, 3D model, volumes, surfaces

**Rezumat.** *Articolul prezintă metodologia de creare a modelului digital 3D al terenului pe baza ridicărilor topografice, precum și utilizarea acestuia în vederea efectuării unor calcule preliminare procesului de proiectare. pentru reducerea pagubelor produse de inundații și amenajării terenurilor ocupate de ape. Modelul 3D digital al terenului permite utilizatorilor să efectueze o serie de operații, cum ar fi: vizualizarea, analizarea, măsurarea, interogarea și altele.*

**Cuvinte cheie:** triangulația Delauney, model 3D, volume, suprafețe

### INTRODUCERE

Starting with few years ago, more and more designers and architects around the world have approached this relatively new field of three-dimensional modeling preferring 3D design systems instead of traditional 2D representation (Cosmin, 2007). The 3D model can be easily manipulated, viewed, analysed and modified according to various requirements and demands. Designing on a 3D model is faster than a traditional design in 2D space. The advantage is that by viewing the model, it eliminates the difficult task of three-dimensional thinking, as compared to classical design plans.

The first parts of the paper approach a brief review of the triangulation method with linear interpolation used by the TopoLT program to represent the topographic surfaces in 3D (Raković et. al, 2004). In this paper were treated some aspects related to the data collection based on land surveying methods and to the areas and volumes calculations used to represent the characteristic curves of the Ibaneasa III lake, situated in Ungureni commune territory, Botosani county, both through practical methods and using the TopoLT software.

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<sup>1</sup> "Gheorghe Asachi" Technical University of Iasi, Romania

## MATERIAL ANF METHODS

### 1. Accumulation's characteristics and hydrology

The stoke pond Ibaneasa III is situated in the Prut river's hydrographic basin, having the land code XIII-1.15.11 and is part of a chain of stoke ponds on the upstream of Ibaneasa river (Prisaca, Slobozia, Vorniceni, Ibaneasa I and II). The Ibaneasa III lake was inaugurated in 1958, having at the maximum retention level a 17.38 ha water surface, with a total volume of 0.214 m<sup>3</sup>, of which an utilization volume of 0.145 m<sup>3</sup>. The dam has a spillway with a surge front with a wide threshold. The spillway has 30.50 m in length and 1.00 m in height. On the front overflow there are metallic guides for mounting the piscicultural grillages and walkway passages. According to STAS 4273/83, the stoke ponds fit to the fourth class of importance. Aquaculture activities are carried out on 18.43 ha. The water volume of the lake at the maximum retention level (104.80 m) is 270,000 m<sup>3</sup> according to an average depth Hmed. = 1.70 m.

### 2. The Delauney triangulation principle

There are several methods, more simple or more complex, to generate 3D terrain models using various tools (Zăvoianu et al., 2002). Modeling functions or geometrical models are used in order to create the digital terrain model (Grid, contour lines and TIN-Triangulated Irregular network). The TIN model reduces redundant data from grid model especially in regions where the land is bump. TIN model accuracy is higher than any other models of terrain surface representation because, this model uses 3D triangles to represent the 3D surface (Savu, 2010). TIN network elements can be generated by Delaunay triangulation method which creates a family of triangles for all the points characterised by the X, Y and Z coordinates. The triangles have the surface greater than zero, share a vertex or edge and the circle circumcircle of each triangle does not contain another vertex.

## RESULTS AND DISCUSSIONS

### 1. Surfaces and water volumes calculation, using practical formulas and graphical representation of the characteristic curves

To achieve the necessary calculations, an accumulation levelling site plan was drawn using a topographic survey, with AutoCad software.

The water surfaces of the lake, corresponding to different rates of the dam water level, were determined by planymetry process on the site contour plan of the stoke pond Ibăneasa III, in which case, the water surface was considered horizontally (Giurma et al, 2006). Such areas were measured on the contour curves with 5 m equidistance, from 102.00 m elevation above sea level (thalweg elevation), the area at this elevation being considered null, up to 106.00 m above sea level (dam crown elevation). The water volume variation in an accumulation is given by the functional dependence  $V = f(H)$ .

For this case study area has been used the average surfaces of the horizontal sections method. For this purpose the volume of water in the lake was divided into nine distinct sections separated by horizontal sections at different water levels (Giurma I. et al, 2006), resulting a total of eight partial volumes (table 1), calculated with the following formula:

$$\Delta V_{i-1,i} = \frac{S_{i-1} + S_i}{2} \cdot \Delta H \quad [m^3]$$

where:  $S_{i-1}$  - the area closed on the lower level curve,  
 $S_i$  - the area closed on the upper level curve,  
 $\Delta H$  - difference of altitude between two successive curves (0.50 m).  
The cumulative volumes were then calculated (table 1), using the relation:

$$V_i = V_{i-1} + \Delta V_{i-1,i} \quad [m^3]$$

Table 1

| Ibaneasa III accumulation characteristic curves |                                     |                   |                   |                                   |                                   |
|---|-------------------------------------|-------------------|-------------------|-----------------------------------|-----------------------------------|
| Elevation [m]                                   | Equidistance between the curves [m] | Surfaces          |                   | Volumes                           |                                   |
|   |                                     | Partials          | Averages          | Partials                          | Cumulatives                       |
|   |                                     | [m <sup>2</sup> ] | [m <sup>2</sup> ] | [10 <sup>3</sup> m <sup>3</sup> ] | [10 <sup>3</sup> m <sup>3</sup> ] |
| 102.0   |                                     | 0.00              |                   |                                   | 0.00                              |
| 102.5   | 0.50                                | 4609              | 2305              | 1.15                              | 1.15                              |
| 103.0   | 0.50                                | 55940             | 30275             | 15.14                             | 15.14                             |
| 103.5   | 0.50                                | 123730            | 89835             | 44.92                             | 60.05                             |
| 104.0   | 0.50                                | 162850            | 143290            | 71.65                             | 131.70                            |
| 104.5   | 0.50                                | 174050            | 168450            | 84.23                             | 215.92                            |
| 105.0   | 0.50                                | 192840            | 183445            | 91.72                             | 307.65                            |
| 105.5   | 0.50                                | 211350            | 202095            | 101.05                            | 408.69                            |
| 106.0   | 0.50                                | 226450            | 218900            | 109.45                            | 518.14                            |

The pairs of values (S, H) and (V, H) were graphically represented, in an rectangular axis system, achieving the lake surfaces and water volumes variation curves (figure 1).

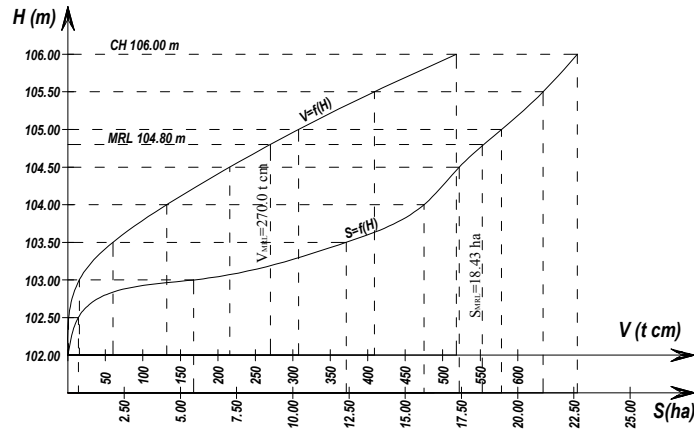


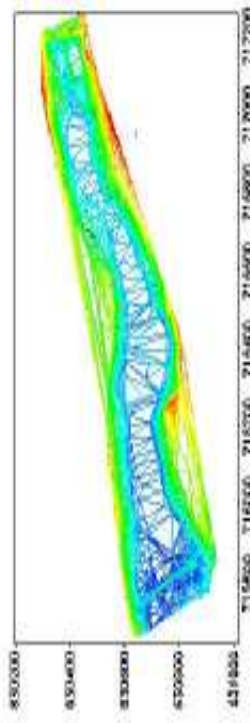
Fig. 1 – Ibaneasa III accumulation characteristic curves: S = f (H)- variation curve of the

lake water surface,  $V = f(H)$ - variation curve of the lake water volumes

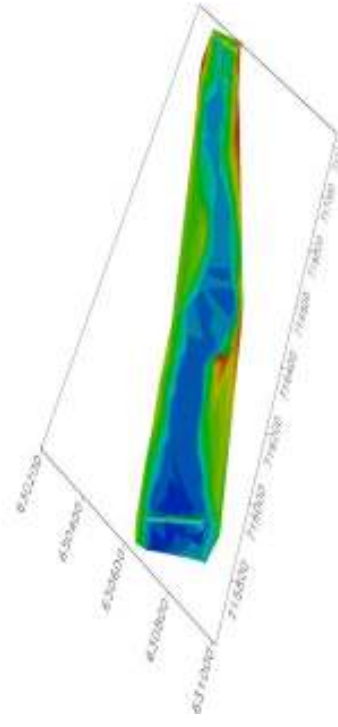
## 2. Surfaces and water volumes calculation using the TopoLT software

TopoLT is software that works under the AutoCAD or IntelliCAD platform. The TopoLT software constitutes a help for those who carry out topographical or cadastral plans in a digital format (Balaneanu and Oprea, 2007).

The first step was to create the 3D terrain model of Ibaneasa III acumulation (as Delaunay triangulation network) in a gradual colors ranging from a minimum (thalweg elevation) up to a maximum (crown height), using the M3D command. In order to create the 3D terrain model was used the X, Y, and Z coordinates inventory, as well as space lines and polylines, which represent a forced slope change. The 3D terrain model can be viewed by running the VM3D command from CAD software (fig. 3).



**Fig. 2** – The triangles network for the 3D terrain model representation



**Fig. 3** – The Ibaneasa III acumulation 3D model, created by TopoLT software

The water volumes are calculated as a sum of 3D objects (block prism), which are obtained by spatial intersections between the 3D model and the constant elevation plan or by intersections between two 3D models using the **Vol** command. The calculation method is purely geometrical, approximation methods are not used, so the 3D models to be used should describe the surface as accurate as possible.



For this case study were calculated a total of eight volumes, defined by the 3D model and the plans with known Z elevation, from 102.50 m above sea level and continuing with the appropriate equidistance of 0.5 m, up to 106.00 m above sea level, dam crown height (tab. 2).

The volumes can be viewed or not, this setting is made using the Om3D command and using the **Vol** command the flat and sloping surfaces are calculated, corresponding to the objects that make up this volume. The areas for 0.5 m equidistance contour lines (areas in the plan), determined with TopoLT software, are listed in tab. 2.

The comparative analysis of the horizontal surfaces defined by contour lines with 0.5 m equidistance, resultated from measurements on the site plan in the CAD software and those calculated with TopoLT software, revealed differences between the areas up to 1.95% in range, which are within the statistical limit of error.

Table 2

The surfaces resultated from measurements and those calculated with TopoLT

| Elevation<br>[m] | Surfaces          |                   | Differences       |       |
|------------------|-------------------|-------------------|-------------------|-------|
|                  | Measured          | TopoLT            | [m <sup>2</sup> ] | %     |
|                  | [m <sup>2</sup> ] | [m <sup>2</sup> ] |                   |       |
| 102.0            | 0                 | 0                 | -                 | -     |
| 102.5            | 4609              | 4697              | -88.29            | -1.92 |
| 103.0            | 55940             | 54848             | 1092.34           | 1.95  |
| 103.5            | 123730            | 122512            | 1218.21           | 0.98  |
| 104.0            | 158323            | 158253            | 69.81             | 0.04  |
| 104.5            | 174050            | 174213            | -162.81           | -0.09 |
| 105.0            | 192840            | 192061            | 778.90            | 0.40  |
| 105.5            | 211350            | 211405            | -55.33            | -0.03 |
| 106.0            | 226450            | 227719            | -1268.55          | -0.56 |

Table 3 show a comparative analysis of the water volumes calculated with the average areas practical formula and of those calculated by TopoLT software. The volumes differences range up to 4.20%, statistical was fitting in the error's limits, except for the first two volumes that correspond to the 102.50 m and 103.00 m elevation above sea level contour line, for which there were differences of 73.22% and 13.36% respectively.

Since in practice is used a simplified formula of the volume, the result is a volume approximated to reality. The TopoLT software calculates the water volumes as a sum of 3D objects (trunks prism) which are obtained by spatial intersections of the 3D model with the constant elevation plan, thereby achieving values much closer to reality.

This is obvious for the first volume in part because through the simplified formula of the volume, the geometric body is considered a pyramid (the thalweg elevation surface is considered null), which in reality it is impossible, resulting a gross error (73.22%). For the other partial volumes, the

errors due to approximation with a regular geometric body are not removed, but are relatively minor.

Table 3

The water volumes calculated using practical formulas and TopoLT software

| Elevation<br>[m] | Water volumes                |                          | Differences       |        |
|------------------|------------------------------|--------------------------|-------------------|--------|
|                  | Calculated [m <sup>3</sup> ] | TopoLT [m <sup>3</sup> ] | [m <sup>3</sup> ] | %      |
| 102.0            | 0                            | 0                        | -                 | -      |
| 102.5            | 1152.25                      | 309                      | 843.64            | 73.22  |
| 103.0            | 15137.25                     | 17159                    | -2021.75          | -13.36 |
| 103.5            | 60054.75                     | 62579                    | -2524.21          | -4.20  |
| 104.0            | 131699.75                    | 133628                   | -1927.82          | -1.46  |
| 104.5            | 215924.75                    | 216662                   | -737.10           | -0.34  |
| 105.0            | 307647.25                    | 308340                   | -692.33           | -0.23  |
| 105.5            | 408694.75                    | 409608                   | -913.50           | -0.22  |
| 106.0            | 518144.75                    | 519511                   | -1366.37          | -0.26  |

## CONCLUSIONS

1. The TopoLT software creates 3D terrain models that can be easily manipulated, viewed, analyzed and modified according to various requirements and demands.

2. The 3D model can be successfully used to perform calculations needed in the design process; because it offers a very good accuracy and the design process can be performed in a rapid way directly on the 3D model, comparative to the traditional design process in 2D space.

3. This modern computer system implementation will lead to high economic efficiency and obtaining high precise results comparing with the classical methods.

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# RESEARCH ON EXPERIMENT THE HYDROCYCLON SLV02 FOR THE PARTIAL SEPARATION OF THE MUST

## CERCETĂRI PRIVIND EXPERIMENTAREA HIDROCICLONULUI SLV02 PENTRU SEPARAREA PARȚIALĂ A MUSTULUI

BĂETU M.M.<sup>1</sup>, ȚENU I.<sup>1</sup>, CÂRLESCU P.<sup>1</sup>,  
PAHONI C.<sup>1</sup>, BARCAN (BĂETU) Alina – Loredana<sup>1</sup>  
e-mail: marius.baetu@yahoo.com

**Abstract.** *In the wine-making industry are cases in which the must has a high content of suspensions with density exceeded by far the density of the liquid phase. A solution for removing these impurities is to use hydrocyclons which are part of centrifugal separators category. The experiment is to test a new type of hidrociclon with a special construction and has pursued the efficiency the partial separation process from must impurities, depending on the change of speed in suspension equipment. Research has been carried out in "Vasile Adamachi" Teaching Experimental Farm, Iași. Following the obtained results interpretation, has been established the experimental version that is leading to a optimal process improvement.*

**Key words:** must, centrifugal separation, hydrocyclon

**Rezumat.** *În industria vinificației se întâlnesc cazuri în care mustul are un conținut ridicat de suspensii a căror densități depășesc cu mult densitatea fazei lichide. O soluție pentru îndepărtarea acestor impurități este folosirea hidrocicloanelor ce fac parte din categoria separatoarelor centrifugale. Experimentul constă în încercarea unui nou tip de hidrociclon de o construcție specială și s-a urmărit eficiența procesului de separare parțială a impurităților din must, în funcție de variația vitezei de alimentare cu suspensie în utilaj. Cercetările au fost efectuate în Ferma Didactică Experimentală „Vasile Adamachi” Iași. În urma interpretării rezultatelor obținute, s-a stabilit varianta experimentală optimă, ce duce la îmbunătățirea procesului de separare.*

**Cuvinte cheie:** must, separare centrifugală, hidrociclon

### INTRODUCTION

During the technological process of winemaking are cases in which wine has a high content of suspended particles whose density exceeds the density of liquid phase (Luca, 1997).

A solution for removing these impurities is to use centrifugal separators (Svarovsky, 1984, Țârdea et. al., 2010).

The separators are used for the partially clarified of must (Luca, 1997), reducing the risk of completely removing the microorganisms that help to start the alcoholic fermentation.

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<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

## MATERIAL AND METHODS

Research has been carried out in "Vasile Adamachi" Teaching Experimental Farm, Iași. In order to obtain the must, the grapes are processed by their passage through the declustering crushers and pneumatic presses.

In the experiments, to remove the sediments using the hydrocyclone, was used must obtained without pressing the pomace, must from press I (obtained at a pressure of 1.5 barr) and must from press II (2 barr). The more, pomace was subject to greater pressure for a long time, more the must was enriched in suspension.

The musts obtained at various pressures, were separately subjected to separation process by using hydrocyclone SLV02 (fig. 1).



**Fig.1.** Hydrocyclone SLV02:

1 – cylindrical body; 2 – input connection for must; 3 – output connection for the partially cleared must; 4 – connection for sediment purging

Must obtained from draining or pressing, can be separated from impurities in decanted and thin sediment settling in a centrifugal force field created by the tangential entry unit (2), with its high speed (Panturu et. al., 1996).

Mixture performs a rotating helical downward to the bottom of the machine and then moving upward all helical, coaxial with the descent movement. Meanwhile, the particles are deposited on the lower walls and easy phase moves to center, through the tube between the two bodies, following an upward motion, eliminating at the top of the connector 3.

For supplying the must in the hydrocyclone was used a multistage centrifugal pump. By varying pump speed, has achieved different turbidities of samples obtained in the study.

The suspension efficiency separation of must can be determined by measuring the nephelometric turbidity unit (NTU) with Turbo 555 turbidimeter. Nephelometric turbidity unit value is lower as the liquid is less feculent.

## RESULTS AND DISCUSSIONS

For the three experimental variants studied, nephelometric turbidity unit was measured at the beginning and end of the separation process. The variation threshold speed, due to the requirements of the equipment company producing, was between 1200 and 2100 rpm (revolution per minute).

In these conditions, the NTU value, for the must obtained without pressing the pomace at the input connection was 1151,4, for the must from press I was 1274,7, while from press II was 1435.3.

In table 1 are presents turbidity results for the must obtained without pressing the pomace.

*Table 1*

**Turbidity values for the must obtained without pressing the pomace**

| <b>Revolution (rpm)</b> | <b>Must turbidity at the hydrocyclone feeder (NTU)</b> | <b>Cleared must turbidity (NTU)</b> |
|-------------------------|--|-------------------------------------|
| 1200                    | 1151,4   | 982,3                               |
| 1500                    |  | 472,5                               |
| 1800                    |  | 280,1                               |
| 2100                    |  | 753,2                               |

We can observe in table above, that the speed of 1200 rpm and 2100 rpm, turbidity values (NTU 982,3, respectively 753,2 NTU) are superior to those obtained at speeds of 1500 and 1800 rpm (472,5 NTU, respectively 280,1 NTU).

From these data it can be deduced that at low speed (1200 rpm) is not sufficient to create a centrifugal force to separate particles of liquid.

At the speed of 2100 rpm is observed that the final value must (753,2 NTU) is close to the original (1151,4 NTU). This may be due to excessive flow velocity of must in a short period of time through the hydrocyclone, unable to separate particles from must.

The lowest turbidity, respectively 280,1 NTU is obtained at a pump speed of 1800 rpm, being a distinctive difference from the value of must turbidity on feeding the machine.

In table 2 and 3 are the must turbidity values obtained from press I, respectively must turbidity values obtained from press II.

*Table 2*

**Turbidity values for the must obtained at press I**

| <b>Revolution (rpm)</b> | <b>Must turbidity at the hydrocyclone feeder (NTU)</b> | <b>Cleared must turbidity (NTU)</b> |
|-------------------------|--|-------------------------------------|
| 1200                    | 1274,7   | 1041,7                              |
| 1500                    |  | 532,4                               |
| 1800                    |  | 339,5                               |
| 2100                    |  | 812,3                               |

From table 2, we can observe that the lowest turbidity, 339,5 NTU, was obtained by varying the speed to 1800 rpm. Moreover, it can identify also in table 3 that at the same speed of 1800 rpm can be obtain the best turbidity, respectively 425,9 NTU.

Table 3

**Turbidity values for the must obtained at press II**

| <b>Revolution (rpm)</b> | <b>Must turbidity at the hydrocyclone feeder (NTU)</b> | <b>Cleared must turbidity (NTU)</b> |
|-------------------------|--|-------------------------------------|
| 1200                    | 1435,3   | 1135,8                              |
| 1500                    |  | 670,2                               |
| 1800                    |  | 425,9                               |
| 2100                    |  | 845,3                               |

## CONCLUSIONS

1. For partial clarification of musts we recommend to use centrifugal separators.
2. The optimal solution to use hydrocyclone SLV02 for partial clearing of must, is to use the pump at speed of 1800 rpm.
3. The hydrocyclones can be used for clarification must, because of the minimal maintenance costs, the main advantage being the lack of moving parts, resulting a more efficient cleaning equipment.

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# RESEARCH ON OPTIMIZING THE PRESSING PROCESS ON THE VERTICAL HYDROPRESS WITH BELLOWS

## CERCETĂRI PRIVIND OPTIMIZAREA PROCESULUI DE PRESARE A HIDROPRESEI VERTICALE CU BURDUF

**BĂETU M.M.,<sup>1</sup> ȚENU I.<sup>1</sup>, PAHONI C. <sup>1</sup>,  
BARCAN (BĂETU) Alina-Loredana <sup>1</sup>**  
e-mail: marius.baetu@yahoo.com

**Abstract.** *Rapid increase of pressure working on grape pulp is not permitted, because it causes the local compaction and powerful kicks, increasing loss of hydraulic pressure in the layers of pulp and membrane separation. The research has the purpose to highlight the pressing process efficiency by swelling the bellows press with water under pressure in the first variant, and swelling the bellows with compressed air in the second variant. By analyzing the results on the yields obtained by varying the values of pressure and the process duration, it was established the optimal variant of using vertical bellows hydropress.*

**Key words:** hydropress, must yield, optimization

**Rezumat.** *Creșterea rapidă a presiunii de lucru asupra pulpei de struguri nu este admisă, deoarece provoacă compactizări locale și lovituri hidraulice puternice, măbind căderea de presiune în straturile de pulpă și pe membrana de separare. Cercetările au ca scop scoaterea în evidență a eficienței procesului de presare prin umflarea burdufului cu apă sub presiune, în primă variantă, și umflarea burdufului cu aer comprimat în cea de-a doua variantă. Prin analiza rezultatelor cu privire la randamentele obținute prin variația valorilor presiunii și duratei procesului, s-a stabilit varianta optimă de utilizare a hidropresei verticale cu burduf.*

**Cuvinte cheie:** hidropresă, randament în must, optimizare

### INTRODUCTION

In order to obtain quality wines, pressing the pomace is important to make in the staple presses with inflatable bellows or pneumatic presses (Popa and Mureșan, 2007).

The must yield, at pressing the pomace, depends on the pressure, the specific growth rate of the pressure on the pulp and the duration of pressing process (Bălan, 1977).

The rapid growth of the working pressure of grape pulp caused local compaction and strong hydraulic strikes, increasing the pressure drop in the layers of pulp and on the separation membrane. All this leads to enrichment of musts in suspensions and phenolic substances (Luca, 1997).

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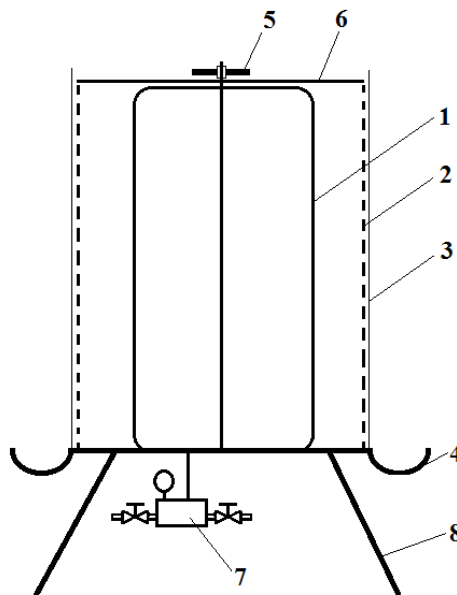
<sup>1</sup>University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

By introducing compressed air into the bellows, the pressure is radial, progressive and flexible. Through the existence of elastic pressure surface, swelling the bellows with spare air subjected pressing material, which leads to a must with less suspensions and poor in substance from the solid parts of grapes (Cotea et al. 2010).

## MATERIAL AND METHODS

The research took place in Teaching Experimental Farm "Vasile Adamachi" Iasi. To study the pressing process of the staple presses, experiments were made on a vertical press with bellows (figure 1).

The press includes a rubber bellows, that can be swell with water or air under pressure, inside a perforated stainless steel cart. The pomace is introduced into the space between the bellows and cart. To prevent the squirt of the must, the cart is doubled on the outside with a plastic pouch. After loading the press, it set the cowl, then will open the water/air feeder and adjust the pressure. To discharge the marc, it's just stand on the perforated metal basket.



**Fig. 1** - Vertical hydro-press with bellows

1 – bellows; 2 – stainless steel cart; 3 – pouch; 4 – channel for must collection; 5 – clamping device; 6 – cowl; 7 – pressure adjustment device; 8 – metal frame.

There have been two experiences where keep track the pomace pressing efficiently. In the first experiment, the bellows was filled with water, and in the second experiment, the bellows was inflated with compressed air.

For filling the bellows we use water from city water supply. Having a direct supply and the pressure is unstable, in the pressing process, there be strong hydraulic strikes. In this case we can not ensure a slow increase of the pressure.



In order to provide a controlled pressure and avoid hydraulic shocks, we used compressed air obtained from an air compressor.

For each experiment, were subjected to pressing different amounts of grapes (V1), pomace obtained by crushing the grapes (V2) and pomace obtained by crushing and declustering the grapes (V3).

Swelling of the bellows with air was achieved by gradually increasing the operating pressure from 1 to 2.5 barr (from 0.5 to 0.5 barr).

Depending on must yields obtained in the experiments, it was established the best solution to use the vertical bellows hydropress.

## RESULTS AND DISCUSSIONS

In table 1 are presented experimental results obtained by pressing the grapes and pomace using the hydropress filled with water.

It can be observed that, the must yields are higher at the pressed pomace obtained only by crushing the grapes (74,3 %), compared to yields obtained from pressing whole grapes (61,92 %) and pomace obtained by crushing and declustering the grapes (71,02%).

Higher yields can be caused by repeated and powerful hydraulic strikes, resulting from the water pressure surge in the bellows.

Table 1

**Yield obtained by pressing whole grapes and pomace using water for filling the hydropress bellows**

| Experimental variant | Grape/pomace weight (kg) | Must weight (kg) | Solid fraction weight (kg) | Must yield (%) |
|----------------------|--------------------------|------------------|----------------------------|----------------|
| V1                   | 10                       | 6,38             | 3,62                       | 63,8           |
|                      | 11                       | 6,87             | 5                          | 62,5           |
|                      | 12                       | 7,35             | 4,65                       | 61,3           |
|                      | 13                       | 7,81             | 5,19                       | 60,1           |
| <i>Average</i>       | <i>11,5</i>              | <i>7,10</i>      | <i>4,61</i>                | <i>61,92</i>   |
| V2                   | 23                       | 17,5             | 5,5                        | 76,1           |
|                      | 24                       | 17,92            | 6,07                       | 74,7           |
|                      | 25                       | 18,45            | 6,55                       | 73,8           |
|                      | 26                       | 18,87            | 7,13                       | 72,6           |
| <i>Average</i>       | <i>24,5</i>              | <i>18,18</i>     | <i>6,31</i>                | <i>74,3</i>    |
| V3                   | 23                       | 16,65            | 6,1                        | 72,4           |
|                      | 24                       | 17,23            | 6,56                       | 71,8           |
|                      | 25                       | 17,65            | 7,35                       | 70,6           |
|                      | 26                       | 18,01            | 7,98                       | 69,3           |
| <i>Average</i>       | <i>24,5</i>              | <i>17,38</i>     | <i>6,99</i>                | <i>71,02</i>   |

Analysing the results in table 2, are presented the experimental results on the three experimental variants (V1, V2 and V3), only as the bellows was inflated with air, we identify the same effect as we used water.

Yields must are higher in experimental variant V2 (72,2%), followed by V3 (68,92%) and V1 (59,82%).

Based on the results achieved in the two tables, it shows that using water to increase pressure on the pomace, there are obtaining higher yields compared with the case where has been used compressed air.

Also it may reveal that by increasing the amount of grape/pomace pressing subjected, yields must reduce results that can lead to decreased productivity, for obtaining a wine with high quality.

Table 2

**Yield obtained by pressing whole grapes and pomace using compressed air for filling the hydropress bellows**

| Experimental variant | Grape/pomace weight (kg) | Must weight (kg) | Solid fraction weight (kg) | Must yield (%) |
|----------------------|--------------------------|------------------|----------------------------|----------------|
| V1                   | 10                       | 6,17             | 3,83                       | 61,7           |
|                      | 11                       | 6,94             | 4,35                       | 60,4           |
|                      | 12                       | 7,1              | 4,9                        | 59,2           |
|                      | 13                       | 7,54             | 5,46                       | 58             |
| <i>Average</i>       | <i>11,5</i>              | <i>6,86</i>      | <i>4,63</i>                | <i>59,82</i>   |
| V2                   | 23                       | 17,02            | 5,98                       | 74             |
|                      | 24                       | 17,42            | 6,58                       | 72,6           |
|                      | 25                       | 17,92            | 7,08                       | 71,7           |
|                      | 26                       | 18,33            | 7,67                       | 70,5           |
| <i>Average</i>       | <i>24,5</i>              | <i>17,67</i>     | <i>6,82</i>                | <i>72,2</i>    |
| V3                   | 23                       | 16,17            | 6,83                       | 70,3           |
|                      | 24                       | 16,72            | 7,27                       | 69,7           |
|                      | 25                       | 17,12            | 7,87                       | 68,5           |
|                      | 26                       | 17,47            | 8,52                       | 67,2           |
| <i>Average</i>       | <i>24,5</i>              | <i>16,87</i>     | <i>7,62</i>                | <i>68,92</i>   |

## CONCLUSIONS

1. Using water to fill the bellows press is obtained higher must yields (70%) compared to yields obtained using air (67%).

2. For obtaining quality wines, it is recommended to use the musts obtained by pressing whole grapes with lower yields.

3. The optimal variant to use the vertical hydropress with bellows, obtaining the best performance in the must yield, is to fill the bellows with water and pressing the pomace resulted from crushing grapes.

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# RESEARCH REGARDING THE DETERMINATION OF THE WORKING INDEXES FOR THE GRAIN COMBINES HARVESTERS IN WHEAT AND BARLEY HARVESTING

## CERCETĂRI PRIVIND DETERMINAREA INDICILOR DE LUCRU AI COMBINELOR DE RECOLTAT CEREALE PĂIOASE LA RECOLTAREA CULTURILOR DE GRÂU ȘI ORZ

PAHONI C.<sup>1</sup>, ȚENU I.<sup>1</sup>, BĂETU M. M.<sup>1</sup>  
e-mail: pahoni\_corneliu@yahoo.com

**Abstract.** *This work presents the results of laboratory experiments and field studies in harvesting wheat and barley by two combine harvesters: Claas Lexion 560 and Claas Lexion 750, both made in Germany. There are differences between this two combines at both constructive level and the degree of automation. This work has tracked the impact of the existing automation elements in the construction of the combines on the growth of their performance, by determining the work quality indexes and the energetic and operating indexes of the combines studied. The major difference has been done by both the values of the working capacities and the values of the operating coefficients, which were higher for the Claas Lexion 750 combine, to the values obtained by the Claas Lexion 560 combine. Overall, the Claas Lexion 750 combine has proven better performance, due to the higher level of automation.*

**Keywords:** grain combine harvesters, work quality indexes, the energetic and operating indexes.

**Rezumat.** *În această lucrare sunt prezentate rezultatele experiențelor de laborator și în câmp, efectuate la recoltarea culturilor de grâu și orz, cu două combine de recoltat cereale: Claas Lexion 560 și Claas Lexion 750, ambele fabricate în Germania. Între cele două combine există diferențe atât la nivel constructiv cât și la gradul lor de automatizare. Lucrarea de față urmărește influența elementelor de automatizare existente în construcția combinelor asupra creșterii performanțelor lor prin determinarea indicilor calitativi de lucru și a indicilor energetici și de exploatare ale combinelor. În ansamblu, combina Claas Lexion 750, a înregistrat performanțe mai bune, diferența majoră făcând-o valorile capacităților de lucru și valorile coeficienților de exploatare, care au fost superiori celor obținuți de combina Claas Lexion 560, nivelul de automatizare mai ridicat al combinei Claas Lexion 750 făcând diferența de performanță.*

**Cuvinte cheie:** combine de recoltat cereale, indici calitativi de lucru, indici energetici și de exploatare.

### INTRODUCTION

Since the straw cereal harvest period is relatively short, it is important that all harvesting operations run on time to avoid significant grain losses. In order to use straw cereals combine harvesters effectively, the combines need to satisfy certain parameters such as: providing the plants cut at a 70 - 260

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

mm height, cutting the plants without shaking off their spikes, ensuring at least 98% purity percentage of the grains collected (Neculăiasa, 2002). Moreover, it is necessary that the grain losses from the remaining unthreshed spikes does not exceed 1.5%, the shaken grains on the top of the the ground to be less than 1 g/m<sup>2</sup> and the percentage of broken grains to be below 2% (Toma and Sin, 1987).

## MATERIAL ŞI METHODS

The experimental research were conducted in 2011 at " SA Zimbrul SA", in the farm no. 3 of the "Ialomita Pond" (Făcăeni - Ialomita) in winter wheat crops, respectively winter barley crops with the biological characteristics presented in table 1, both crops being located on the level ground.

In order to determine the combines' technical and functional performance in field laboratory and in the operating conditions, we studied the behavior in exploitation of two combines harvesters: CLAAS LEXION 560 and CLAAS LEXION 750, both manufactured by CLAAS in Germany. The main technical characteristics of the combines tested are listed in table 2.

Table 1

The crops' biological characteristics

| Specification                        | The measurement unit | The average value of the biological characteristics |           |
|--------------------------------------|----------------------|---|-----------|
|                                      |                      | Wheat   | Barley    |
| Variety                              | -                    | Alex  | Cristalia |
| Grain moisture                       | %                    | 14.6  | 14.8      |
| Plants' density                      | buc/m <sup>2</sup>   | 374   | 365       |
| Plants' average height               | cm                   | 74.5  | 77.6      |
| Total mass of the plants:            | g/m <sup>2</sup>     | 989.45  | 896.09    |
| - grain mass                         | g/m <sup>2</sup>     | 437.62  | 405.86    |
| - straw mass                         | g/m <sup>2</sup>     | 469.58  | 450.45    |
| - weed mass                          | g/m <sup>2</sup>     | 82.25   | 39.78     |
| Recumbent plants percentage          | %                    | 0.67  | 0.52      |
| Grain production per hectare         | kg/ha                | 4376.2  | 4058.6    |
| Average straw production per hectare | kg/ha                | 4695.8  | 4504.55   |
| Grain-straw ratio                    | -                    | 0.93  | 0.90      |
| The 1000 seeds mass                  | g                    | 46.65   | 42.4      |
| Mass per storage volume              | kg/hl                | 78.4  | 63.4      |

The measurement and the calculation of the work quality indexes, the energetic and operating indexes of the Claas Lexion 560 and the Claas Lexion 750 combines have been done according to the specifications. The measurements were taken in three variants, represented by the three feeding flows of each combine. For each variant a total of three repetitions were performed. The working capacity indexes have been measured and calculated on a 8 hours shift, at a normal working combines' speed, which provided an optimal feeding flow, specific to each combine.

Table 2

## The main technical and functional characteristics of the combines tested

| The characteristics' name       | M. U.          | Combine             |                  |
|---------------------------------|----------------|---------------------|------------------|
|                                 |                | CLAAS LEXION 750    | CLAAS LEXION 560 |
| Header – working width          | m              | 7.5                 | 7.5              |
| Beater                          | -              |                     |                  |
| - width                         | mm             | 1680                | 1680             |
| - diameter                      | mm             | 600                 | 600              |
| - number of rails               | -              | 8                   | 8                |
| - rotation speed                | rot/min        | 395-1150            | 395-1150         |
| concave                         | -              |                     |                  |
| - number of rails               | -              | 10                  | 10               |
| - wrapping angle                | degrees        | 90                  | 90               |
| Rotary separator                |                | yes                 | no               |
| First cleaning                  |                |                     |                  |
| - upper sieve                   | -              | yes                 | yes              |
| - bottom sieves kit             | -              | yes                 | yes              |
| - cleaning area                 | m <sup>2</sup> | 5.80                | 5.80             |
| Second cleaning                 | -              | yes                 | yes              |
| - number of shackers            |                | 2 rotary separators | 6                |
| - separation area               | m <sup>2</sup> | 3.00                | 9.85             |
| Chopping equipment              | -              | yes                 | yes              |
| Bunker - capacity               | l              | 10500               | 10500            |
| Engine - model                  | -              | Caterpillar C-13    | Caterpillar C-10 |
| - cylinders' capacity           | l              | 12.5                | 10.3             |
| - Fuel tank capacity            | l              | 800                 | 800              |
| Transmission - number of speeds | -              | 3x2                 | 3x2              |
| -rear tires' size               |                | 600/55 - 26.5       | 16.5/85 - 24 IMP |
| -front tires' size              | -              | 800/65 R 32         | 650/75 R32 R1    |
| -length with header             | m              | 11.42               | 11.42            |
| - length without header         | m              | 9.2                 | 9.2              |
| -width                          | m              | 3.5                 | 3.5              |
| - maximum height                | m              | 4.85                | 4.85             |
| Combine's mass                  | kg             | 17320               | 15655            |

Operating measurements were conducted under normal production conditions. The experiments took place in plots with shapes and sizes that could ensure the mechanized harvesting.

Grain moisture was within acceptable limits of 14-16% during the harvest (Toma and Bianu, 1987).

The combines' working process stages were photographed daily and the daily worksheets were prepared. In these records were recorded: the experiments' location, the crop, the time when the work started, the operations and the period of each operation, the time when the work has ended, the plot's drawing and harvested area, the quantity of the grains harvested, the cutting height, the fuel consumption and the grain moisture.

In the experimental research were also used: metric frame, electronic scale, moisture meter, sheet to collect grains, straws and chaff, plastic bags to collect the grain samples, stopwatch, daily monitoring sheets.

## RESULTS AND DISCUSSIONS

The working quality indexes were calculated based on the feeding flows of the combines. The working quality indexes obtained by the combines in wheat and barley harvesting are grouped in table 3.

For the feeding flows on which the combines were tested, the losses value did not exceed the maximum allowable limit of 2.5%. There were, however, significant losses on both combines for the biggest feeding flows in the experiments. Yet, although both combine reported losses to bigger feeding flows, losses percentage were higher in Claas Lexion 560 case, for both crops.

*Table 3*

**The work quality indexes obtained by the Claas Lexion 560 and the Claas Lexion 750 combines in wheat and barley harvesting**

| Specification                            | M.U. | Average values |       |       |            |       |       |            |       |       |            |      |      |
|--|------|----------------|-------|-------|------------|-------|-------|------------|-------|-------|------------|------|------|
|  |      | Wheat          |       |       |            |       |       | Barley     |       |       |            |      |      |
| Crop                                     | -    | Lexion 560     |       |       | Lexion 750 |       |       | Lexion 560 |       |       | Lexion 750 |      |      |
| Combine                                  | -    | Lexion 560     |       |       | Lexion 750 |       |       | Lexion 560 |       |       | Lexion 750 |      |      |
| Average speed                            | Km/h | 3.22           | 4.33  | 5.52  | 3.98       | 6.00  | 7.64  | 3.00       | 4.04  | 5.16  | 3.70       | 5.57 | 7.09 |
| Moisture                                 | %    | 14.6           | 14.6  | 14.6  | 14.6       | 14.6  | 14.6  | 14.8       | 14.8  | 14.8  | 14.8       | 14.8 | 14.8 |
| Feeding flow                             | kg/s | 3.32           | 5.00  | 6.37  | 4.23       | 5.70  | 7.27  | 3.08       | 4.64  | 5.91  | 3.95       | 5.32 | 6.79 |
| Header losses                            | %    | 0.31           | 0.75  | 1.00  | 0.28       | 0.45  | 0.77  | 0.44       | 1.04  | 1.33  | 0.42       | 0.60 | 1.19 |
| - on the soil-free grains                | %    | 0.14           | 0.24  | 0.24  | 0.13       | 0.11  | 0.31  | 0.12       | 0.35  | 0.32  | 0.26       | 0.39 | 0.59 |
| - grains in the cut-off fallen spikes    | %    | 0.17           | 0.51  | 0.63  | 0.15       | 0.19  | 0.33  | 0.22       | 0.69  | 0.79  | 0.16       | 0.20 | 0.30 |
| - grains in the uncut spikes on the soil | %    | -              | -     | 0.13  | -          | 0.15  | 0.13  | 0.10       | -     | 0.22  | -          | 0.01 | 0.30 |
| Thresher loss                            | %    | 0.43           | 0.59  | 1.13  | 0.16       | 0.32  | 0.72  | 0.60       | 0.75  | 1.14  | 0.31       | 0.58 | 1.00 |
| - free grains in chaff and straws        | %    | 0.43           | 0.48  | 0.98  | 0.16       | 0.32  | 0.56  | 0.60       | 0.75  | 0.90  | 0.31       | 0.58 | 0.79 |
| - grains in unthreshed spikes            | %    | -              | 0.11  | 0.15  | -          | -     | 0.15  | -          | -     | 0.24  | -          | -    | 0.21 |
| Total losses                             | %    | 0.74           | 1.34  | 2.13  | 0.44       | 0.77  | 1.49  | 1.04       | 1.79  | 2.47  | 0.73       | 1.18 | 2.19 |
| Purity                                   | %    | 99.01          | 98.83 | 98.09 | 99.14      | 98.91 | 97.94 | 99.1       | 98.97 | 98.00 | 99.1       | 98.9 | 97.4 |
| Broken grains                            | %    | 3.39           | 2.99  | 2.49  | 3.48       | 2.69  | 2.27  | 3.72       | 2.90  | 2.98  | 3.45       | 2.83 | 2.57 |

To be mentioned the fact that for a feeding flow of 7.27 kg/s achieved by the Claas Lexion 750 combine in winter wheat harvesting and for a feeding flow of 6.79 kg/s performed by the same combine in winter barley harvesting, the grain purity value fell below the minimum allowable limit of 98%.

The operating indexes of the combines tested were calculated based on the timing sheets prepared during the operating experiments.

The average values of the operating coefficients of the combines studied

are shown in table 4.

Table 4

**The operating coefficients of the Claas Lexion 560 and the Claas Lexion 750 combines in wheat and barley harvesting**

| Specification                      | Symbol | Combine    |        |            |        |
|------------------------------------|--------|------------|--------|------------|--------|
|                                    |        | Lexion 560 |        | Lexion 750 |        |
|                                    |        | Wheat      | Barley | Wheat      | Barley |
| Operational time usage coefficient | K02    | 0.87       | 0.86   | 0.87       | 0.87   |
| Production time usage coefficient  | K04    | 0.80       | 0.78   | 0.82       | 0.80   |
| Shift time usage coefficient       | K07    | 0.71       | 0.68   | 0.73       | 0.71   |
| Turns coefficient                  | K21    | 0.94       | 0.93   | 0.95       | 0.94   |
| Technological service coefficient  | K23    | 0.91       | 0.89   | 0.92       | 0.90   |
| Technical care coefficient         | K31    | 0.92       | 0.95   | 0.95       | 0.95   |
| Technological safety coefficient   | K41    | 0.98       | 0.96   | 0.98       | 0.97   |
| Technical safety coefficient       | K42    | 0.98       | 0.94   | 0.98       | 0.93   |
| Operational safety coefficient     | K4     | 0.97       | 0.90   | 0.96       | 0.93   |

As seen on table 4, the Claas Lexion 750 combine recorded higher values at almost all of the coefficients compared to the Claas Lexion 560 combine. The Claas Lexion 750 combine registered lower values for the operational safety coefficient in winter wheat harvesting and for the technical safety coefficient in winter barley harvesting, but the differences were insignificant.

The work capacities and the fuel consumption average values of the both combines can be found in table 5.

Table 5

**The work capacities and the fuel consumption of the Claas Lexion 560 and the Claas Lexion 750 combines in wheat and barley harvesting**

| Specification                           | Symbol | M.U.  | Specification |        |         |        |
|---|--------|-------|---------------|--------|---------|--------|
|   |        |       | Combine       |        | Combine |        |
|   |        |       | Wheat         | Barley | Wheat   | Barley |
| Hourly work capacity on effective time  | Wef    | t/h   | 10.15         | 9.42   | 16.72   | 15.61  |
| Hourly work capacity on operative time  | W02    | t/h   | 8.92          | 8.28   | 14.73   | 13.75  |
| Hourly work capacity on production time | W04    | t/h   | 8.01          | 7.44   | 13.31   | 12.42  |
| Hourly work capacity on shift time      | W07    | t/h   | 7.07          | 6.56   | 11.62   | 10.85  |
| Work capacity on a 8 hours shift        | Wsch   | t/sch | 56.56         | 52.48  | 92.96   | 86.80  |
| Fuel consumption per reference unit     | Gc     | l/ha  | 8.85          | 8.22   | 9.82    | 9.17   |

The Claas Lexion 750 combine had registered better values on the work capacity indexes, compared to the values recorded by the Claas Lexion 560 combine in both winter wheat and winter barley harvesting. (tab. 5).

The fuel consumption per reference unit was higher on the Claas Lexion 750 combine, then the one registered on Claas Lexion 560 combine, mainly due to the bigger quantity of material threshed and to the bigger cylinders' capacity of the Claas Lexion 750 combine's engine.

## CONCLUSIONS

1. From experimental research results can be observed a directly proportional relationship between the feeding flow and the total grains losses of the combines, and a reverse relationship between the feeding flow and the percentage of broken grains.

2. The grains losses did not exceed the maximum allowable limit of 2.5%, this fact proving a quality and a reliability of both combines' construction, especially on threshing and cleaning systems' performance.

3. The Claas Lexion 750 combine, had performed better than the Claas Lexion 560 combine, the major difference being made by the working capacity indexes and the operating coefficients values. Higher values of those indexes registered in operation by the Claas Lexion 750 combine were due to the existence in its construction of the separating rotors that have replaced the classic walkers and the existence of the automation elements, namely: the combine's automatic routing by the chain's edge, the automatic control system of the working speed according to the chain's characteristics and the automatic control of grains losses.

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# RESEARCH REGARDING THE DETERMINATION OF THE WORKING INDEXES FOR THE NEW HOLLAND TC 5050 COMBINE IN MAIZE GRAIN HARVESTING

## CERCETĂRI PRIVIND DETERMINAREA INDICILOR DE LUCRU AI COMBINEI NEW HOLLAND TC 5050 LA RECOLTAREA PORUMBULUI PENTRU BOABE

**PAHONI C.<sup>1</sup>, ȚENU I.<sup>1</sup>, BĂETU M. M.<sup>1</sup>**  
e-mail: pahoni\_corneliu@yahoo.com

**Abstract.** *The work presents the results of laboratory experiments and field studies in maize harvesting with the New Holland TC 5050 combine harvester. The combine's harvesting performance was monitored on the level and slope field by determining the work quality indexes and the energetic and operating indexes of the combine studied. When driving on sloping land on the valley -hill direction, grain losses have exceeded the maximum allowable limit, reaching values over 9.5%. When movement was along level curves, the grain loss had registered values close to maximum allowed limit (2.5%), being therefore the recommended method. Fuel consumption has increased considerably when driving on sloping land compared with driving on the level field. In order to achieve a quality harvesting work, combine harvesters should be adjusted and operated in accordance with land condition and with the agrobiological characteristics of the crops.*

**Keywords:** combine harvester, maize grain, grain losses, fuel consumption.

**Rezumat.** *În lucrare sunt prezentate rezultatele experiențelor de laborator și în câmp, efectuate la recoltarea culturii de porumb de către combina New Holland TC 5050. A fost urmărită comportarea combinei la recoltarea pe teren plan și pe teren în pantă, prin determinarea indicilor calitativi de lucru și a indicilor energetici și de exploatare ai combinei. La deplasarea pe terenurile în pantă pe direcția vale-deal, pierderile de boabe au depășit limita maximă admisibilă, ajungând la valori de peste 9,5%. Când deplasarea s-a făcut de-a lungul curbelor de nivel, pierderile de boabe au înregistrat valori apropiate de limita maximă admisă (2,5%), fiind în consecință, metoda recomandată. Consumul de combustibil a crescut considerabil la deplasarea pe terenurile în pantă față de deplasarea pe teren plan. Pentru realizarea unei lucrări de recoltare de calitate, combinele trebuie să fie adaptate și exploatate în conformitate cu starea terenului și cu caracteristicile agrobiologice ale culturilor.*

**Cuvinte cheie:** combină, porumb pentru boabe, pierderi de boabe, consum de combustibil.

### INTRODUCTION

The straw grains combine harvesters are complex agricultural machinery capable to perform all the technological stages that are needed to obtain the main

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

product (grains). It should be noted that even if those machines were initially designed only for mechanized harvesting of the straw grains, over the years they have been gradually equipped with working boddies in order to harvest other crops: maize, sunflower, soybean, rapeseed, beans, peas, etc (Neculăiasa, 2002).

This paper studies the performance of the TC 5050 New Holland combine in maize grain harvesting on the level and slope land. A special attention should be paid to the level of grain losses, which tend to be very high when harvesting is done on sloping fields.

This paper aims to identify the optimum operating procedures for harvesting maize grain according to the land condition and to highlight the links and the relationship between the work quality, energetic and operating indexes.

## MATERIAL AND METHODS

The experimental research took place in 2011 at the Ezăreni Farm, which belongs to the University of Agricultural Sciences and Veterinary Medicine "Ion Ionescu de la Brad" Iași, on plots with maize grain hybrid DK 440. The biological characteristics of the maize crop on which the experimental research were carried out are presented in Table 1.

Table 1

The crop's biological characteristics

| Specification                        | The measurement unit | The average value of the biological characteristics |
|--------------------------------------|----------------------|---|
| Grain moisture                       | %                    | 17.2  |
| Plants' density                      | mii de plante/ha     | 56.8  |
| Plants' average height               | cm                   | 175.94  |
| Recumbent plants percentage          | %                    | 4.6   |
| Average grain production per hectare | kg/ha                | 4138.7  |
| The 1000 seeds mass                  | g                    | 246   |

Operating measurements were conducted under normal production conditions. The plots on which the experiments took place had shapes and sizes that would ensure the mechanized harvesting. The grain moisture at harvest was below the regulatory limit (25%).

The combine used in experimental research was the TC 5050 New Holland combine, equipped with header for harvesting maize grain with four work sections.

The work quality indexes, the energetic and operating indexes of the TC 5050 New Holland combine in maize grain harvesting were measured.

The main technical and functional features of the TC 5050 New Holland combine can be found in table 2.

Daily stages of the combine working process were photographed and daily worksheets have been prepared. In those particular sheets were recorded the following: experimentants' location, crop, time when the work has started, the operations and the duration of each operation, the time of completion of the work, the plot draw and the plot's area which have been harvested, the quantity of the grains harvested, cutting height, the fuel consumption and the grain moisture.

Table 2

**The main technical and functional characteristics of the combine tested**

| The characteristic name     | M.U.           | New Holland TC 5050    |
|-----------------------------|----------------|------------------------|
| Header – number of rows     | -              | 4                      |
| Beater                      | -              |                        |
| - width                     | mm             | 1040                   |
| - diameter                  | mm             | 607                    |
| - number of rails           | -              | 8                      |
| - rotation speed            | rot/min        | 430-1037               |
| concave                     | -              |                        |
| - number of rails           |                | 14                     |
| - wrapping angle            | degrees        | 111                    |
| Rotary separator            |                | nu                     |
| Cleaning                    |                |                        |
| - cleaning surface          | m <sup>2</sup> | 5.80                   |
| - total area under air flow | m <sup>2</sup> | 3.27                   |
| -cleaning area              | m <sup>2</sup> | 4.00                   |
| - number of shackers        | -              | 4                      |
| Chopping equipment          | -              | yes                    |
| Bunker - capacity           | l              | 4000                   |
| Engine                      | -              | New Holland            |
| - cylinders' capacity       | l              | 6.80                   |
| - fuel tank capacity        | l              | 300                    |
| Transmission                | -              | hidrostatic – 3 speeds |
| -length with header         | m              | 10.72                  |
| - length without header     | m              | 8.13                   |
| -width                      | m              | 3.00                   |
| - maximum height            | m              | 4.00                   |
| Combine's mass              | kg             | 7600                   |

The combine was tested in operation conditions on the level and slope land: on the hill – valley direction, the valley – hill direction and along the contour, in first gear and the second gear. For each gear were used rotation speeds of 1500 rpm and of 1800 rpm for each rotation speed a number of three repetitions were done (Corduneanu I., 1977).

In the experimental research were also used: metric frame, electronic scale, moisture meter, tilt to collect grains and vegetal residues, plastic bags to collect the grain samples, stopwatch, daily monitoring sheets.

The working capacities have been measured and calculated on a 8 hours shift, at a normal working combines' speed, which provided an optimal feeding flow, specific to each combine (Neagu and Sandru, 2004).

## RESULTS AND DISCUSSIONS

The work quality indexes were calculated according to the land's slope, to the combine's movement direction and to the combine's speed. The average values of the work quality indexes obtained by the TC 5050 New Holland combine in maize grain harvesting are grouped in table 3.

When driving on sloping land on the hill-valley direction, grain losses have exceeded the maximum allowable limit, reaching values over 9.5%.

When movement was along the contour, grain losses have been close to the maximum allowable limit (2.5%).

Table 3

**The work quality indexes obtained by the TC 5050 New Holland combine in maize grain harvesting**

| Working speed |      |      | Total losses (%) |               |               |               | Purity (%) |               |               |               | Broken grains (%) |               |               |               |
|---------------|------|------|------------------|---------------|---------------|---------------|------------|---------------|---------------|---------------|-------------------|---------------|---------------|---------------|
| Gear          | rpm  | Km/h | Level land       | Level cur-ves | Hill - valley | Valley - hill | Level land | Level cur-ves | Hill - valley | Valley - hill | Level land        | Level cur-ves | Hill - valley | Valley - hill |
| I             | 1500 | 4.2  | 0.89             | 2.64          | 8.70          | 9.28          | 98.73      | 96.55         | 94.33         | 95.78         | 3.30              | 3.39          | 3.66          | 3.59          |
|               | 1800 | 5.1  | 0.72             | 3.22          | 8.89          | 9.58          | 98.08      | 96.28         | 94.05         | 95.05         | 3.22              | 3.13          | 3.44          | 3.22          |
| II            | 1500 | 6.5  | 0.64             | 2.59          | 8.59          | 9.07          | 99.10      | 97.14         | 94.29         | 96.01         | 2.88              | 2.93          | 3.13          | 3.09          |
|               | 1800 | 9.0  | 1.09             | 3.34          | 9.11          | 9.63          | 98.32      | 96.40         | 93.74         | 95.65         | 2.44              | 2.54          | 2.77          | 2.61          |

The purity of the grains was very high when the combine's movement was on the level land, but fell below the minimum allowable limit of 98% when combine's movement was on the slope field, reaching values below 94%. The percentage of broken grains showed higher values when the combine have been droven at lower speeds, due to the weak flow of grains which have been feeding the threshing apparatus.

The operating indexes of the combines tested were calculated based on the timing sheets prepared during the operating experiments.

The average values of the operating coefficients of the combines studied are shown in table 4.

Table 4

**The operating coefficients of the TC 5050 New Holland combine in maize grain harvesting**

| Specification                      | Symbol | Value |
|------------------------------------|--------|-------|
| Operational time usage coefficient | K02    | 0.86  |
| Production time usage coefficient  | K04    | 0.78  |
| Shift time usage coefficient       | K07    | 0.51  |
| Turns coefficient                  | K21    | 0.95  |
| Technological service coefficient  | K23    | 0.89  |
| Technical care coefficient         | K31    | 0.95  |
| Technological safety coefficient   | K41    | 0.96  |
| Technical safety coefficient       | K42    | 0.87  |
| Operational safety coefficient     | K4     | 0.83  |

The values of the main operating coefficients of the TC 5050 New Holland combine were: the operational safety coefficient 0.83; the technological safety coefficient 0.96; the technical safety coefficient 0.87; the turn's coefficient 0.95.

The hourly work capacity on shift time of the TC 5050 New Holland combine has recorded a decrease on average by 10-25% at harvesting on

sloping land compared to on the level land harvesting, due to the slope, which reduced working speed by 21-33% which led to a decrease of the shift time usage coefficient to 0.51.

The fuel consumption of the TC 5050 New Holland combine obtained in maize grain harvesting on the sloping land has increased on average by 19-31% comparing to the fuel consumption obtained on the level land movement.

The work capacities and the fuel consumption average values of the TC 5050 New Holland combine can be found in table 5.

Table 5

**The work capacities and the fuel consumption of the TC 5050 New Holland combine in maize grain harvesting**

| Specification                           | Symbol | M.U.  | Value |
|---|--------|-------|-------|
| Hourly work capacity on effective time  | Wef    | t/h   | 7.92  |
| Hourly work capacity on operative time  | W02    | t/h   | 6.64  |
| Hourly work capacity on production time | W04    | t/h   | 6.12  |
| Hourly work capacity on shift time      | W07    | t/h   | 5.79  |
| Work capacity on a 8 hours shift        | Wsch   | t/sch | 46.32 |
| Fuel consumption per reference unit     | Gc     | l/t   | 6.37  |

During the experimental research, we have seen that the combine's safety is not ensured on the hill-valley movement balance facing valley, especially on slopes above 22% and when the bunker has a load over 1500 kg of grains.

## CONCLUSIONS

1. The TC 5050 New Holland combine had the total grain losses below the allowable limit of 2.5% when driving on the level and just above this limit when the combine's movement was on the slope land along the contour. When driving the combine on the hill-valley, and on the valley-hill directions, the combine's total grain losses have exceeded the allowed limit, reaching values of 9.63%.

2. The percentage of broken grains was reverse proportional to the speed of the combine and has not been explicitly influenced by the slope.

3. In the experimental research, the 5050 TC New Holland combine has reduced its work capacity by 10-25% and has increased fuel consumption by 19-31% when driving on the slope land comparing to the level land movement.

4. The time for technical fixes has immobilized the combine 29-37 min/shift on average, which drew reduction of the shift time usage coefficient to 0.51, and the operational safety coefficient to 0.83.

5. In order to achieve a quality harvest work, the combines should be adjusted and operated in accordance with land condition and with the agrobiological characteristics of the crops.

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# INFLUENCE OF DETHATCHING ON TURFGRASS DENSITY

## INFLUENȚA SCARIFICĂRII ASUPRA DENSITĂȚII GAZONULUI

**POPOVICI C. I.<sup>1</sup>, VÎNTU V.<sup>1</sup>,  
SAMUIL C.<sup>1</sup>, STAVARACHE M.<sup>1</sup>, CIOBANU C.<sup>1</sup>**  
e-mail: iulian\_vici@yahoo.com

**Abstract.** Lawns are subjected to thatch development due to dry shoots, stolons, rhizomes or clippings. A thin layer of thatch is desirable, protecting rootzone from extreme heat, but a layer thicker than 1.5 cm has a negative influence on turf, as water and fertilizer does not infiltrate as easily in soil. The aim of this study was to evaluate the effect of different types of dethatching methods on turfgrass density. The experiment was established at Ezareni farm from city of Iasi. The evaluation of turfgrass density was done using the visual rating scale elaborated by the National Turfgrass Evaluation Program from USA. The best dethatching treatment was AA+AS where the biggest differences were observed compared with control. Among varieties, the most significant changes were registered at *Poa pratensis* species where turf density increased in average from 6.5 to 8.1.

**Key words:** lawn aerification, turfgrass quality.

**Rezumat.** La suprafața solului se formează un strat de țelină datorat lăstarilor uscați, stolonilor aeriени, dar și frunzelor tăiate prin tuns. Un strat subțire de țelină este benefic, protejând solul împotriva temperaturilor ridicate din vară, dar un strat mai gros de 1,5 cm devine dăunător, îngreunând pătrunderea apei și a fertilizanților în sol. Scopul acestui studiu a fost de a determina efectul unor tipuri de lucrări care vizează țelina gazonului. Experimentările au fost efectuate în cadrul fermei Ezăreni din Iași. Evaluarea densității gazonului s-a făcut cu ajutorul scării de apreciere vizuală elaborată de National Turfgrass Evaluation Program din USA. Cea mai bună variantă experimentată a fost AA+AS unde s-au înregistrat cele mai mari diferențe ale densității comparativ cu varianta martor. Dintre soiurile de gazon, răspunsul cel mai puternic s-a observat la specia *Poa pratensis*, la care densitatea a crescut în medie de la 6,5 la 8,1.

**Cuvinte cheie:** aerarea gazonului, calitatea gazonului.

### INTRODUCTION

Lawns are subjected to thatch development which is caused by dried shoots and leaves, runners, rhizomes and clippings. A thin layer of thatch is desirable, protecting rootzone from extreme heat, but a layer thicker than 1.5 cm has a negative influence on turf, as water and fertilizer does not infiltrate as easily in soil (Christians, 2004; Dunn and Diesburg, 2004). Excess thatch is removed by superficial scarification thus allowing deep rooting with positive effects on turf growth and hardiness, also reducing the demands for irrigation (Carrow, 2003).

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

Aeration and scarification equipment varies by mode. There are aerators based on water injection, which penetrate the soil to a depth of 20 cm, by means of powerful water jets. (Raturi et al., 2003). Hanna W. (2005) believes that verticutting machines can be used successfully to reduce thatch.

## MATERIAL AND METHODS

The experiment was setup at the experimental fields in Ezareni farm from Iasi. The design used was a split plot design with three replicates. The following factors were studied:

Factor A – dethatching method:

A1 – control plot (C)

A2 – verticutting (AA)

A3 – power raking (AS)

A4 – verticutting + power raking (AA+AS)

Factor B – 19 turfgrass varieties from the following species: *Lolium perenne* (L.p.), *Festuca arundinacea* (F.a.), *Festuca rubra rubra* (F.r.r.), *Festuca rubra tricophylla* (F.r.t.), *Festuca rubra commutata* (F.r.c.), *Poa pratensis* (P.p.), *Festuca ovina duriuscula* (F.o.d.).

Turfgrass density evaluation was done using the visual rating scale elaborated by the National Turfgrass Evaluation Program from USA (Morris, 2006). Statistical interpretation of the data was done by ANOVA test.

## RESULTS AND DISCUSSIONS

Turfgrass varieties have registered rather good values of density in the spring, when C and AA treatments were rated 7, while AS and AA+AS treatments were rated 7.2 and 7.3 (table 1).

Table 1

The influence of dethatching on turfgrass density (1-low density; 9-high density)

| Spring           |     |     |       | Summer           |     |     |       | Fall             |     |     |       | Annual average   |     |     |       |
|------------------|-----|-----|-------|------------------|-----|-----|-------|------------------|-----|-----|-------|------------------|-----|-----|-------|
| C                | AA  | AS  | AA AS | C                | AA  | AS  | AA AS | C                | AA  | AS  | AA AS | C                | AA  | AS  | AA AS |
| 7.0 <sup>M</sup> | 7.0 | 7.2 | 7.3   | 6.2 <sup>M</sup> | 6.6 | 7.0 | 7.2   | 6.6 <sup>M</sup> | 6.6 | 7.5 | 7.5   | 6.6 <sup>M</sup> | 6.7 | 7.2 | 7.4   |
| LSD 5% = 0.3     |     |     |       | LSD 5% = 0.2     |     |     |       | LSD 5% = 0.1     |     |     |       | LSD 5% = 0.5     |     |     |       |
| LSD 1% = 0.4     |     |     |       | LSD 1% = 0.3     |     |     |       | LSD 1% = 0.2     |     |     |       | LSD 1% = 0.7     |     |     |       |
| LSD 0.1% = 0.6   |     |     |       | LSD 0.1% = 0.5   |     |     |       | LSD 0.1% = 0.4   |     |     |       | LSD 0.1% = 1.1   |     |     |       |

In the summer we observed different effects among dethatching treatments, the most efficient was AA+AS where density was rated 7.2, followed by AS which was rated 7, AA rated 6.6 and C rated 6. In the fall AS and AA+AS treatments had the best results rated 7.5 while AA and C registered lower densities being rated with 6.6.

Comparing turfgrass varieties response to dethatching we observed a varied effect of treatments, some of these varieties reacted better to aerification and some reacted poorer (table 2). Among varieties with better results we can mention Capri, Ponderosa and Esquire which belong to *Lolium perenne* species, Rosita, Calliope, Celianna and Maxima 1 belonging to *Festuca rubra* species, Panduro and Conni belonging to *Poa pratensis* species.



Table 2

The interaction between dethatching method and variety on turfgrass density (1-low density; 9-high density)

| Turfgrass varieties         | Spring           |        |        |        | Summer           |        |        |        | Fall             |        |        |        | Annual average   |        |        |        |
|-----------------------------|------------------|--------|--------|--------|------------------|--------|--------|--------|------------------|--------|--------|--------|------------------|--------|--------|--------|
|                             | C                | AA     | AS     | AA AS  | C                | AA     | AS     | AA AS  | C                | AA     | AS     | AA AS  | C                | AA     | AS     | AA AS  |
| Mara                        | 6.0 <sup>C</sup> | 6.7    | 6.0    | 6.0    | 3.7 <sup>C</sup> | 4.0    | 4.3    | 4.3    | 4.7 <sup>C</sup> | 5.0    | 5.3    | 5.7**  | 4.8 <sup>C</sup> | 5.2    | 5.2    | 5.3    |
| Margarita ( <i>L.p.</i> )   | 7.0*             | 7.3**  | 7.3**  | 7.3**  | 6.3***           | 7.0*** | 6.3*** | 6.3*** | 6.3***           | 6.3*** | 7.0*** | 7.0*** | 6.5**            | 6.9*** | 6.9*** | 6.9*** |
| Capri ( <i>L.p.</i> )       | 6.7              | 6.3    | 6.3    | 6.3    | 5.3**            | 5.3**  | 6.3*** | 6.0*** | 5.0              | 5.0    | 6.0*** | 6.0*** | 5.7              | 5.5    | 6.2*   | 6.1*   |
| Ponderosa ( <i>L.p.</i> )   | 6.7              | 7.0*   | 7.3**  | 7.3*   | 5.3**            | 5.3**  | 6.0*** | 6.0*** | 5.3              | 5.3    | 6.3*** | 6.3*** | 5.8              | 5.9    | 6.5**  | 6.5**  |
| Esquire ( <i>L.p.</i> )     | 7.7***           | 7.0*   | 7.7*** | 7.7*** | 5.7***           | 5.7*** | 6.3*** | 6.3*** | 6.0***           | 6.0*** | 7.0*** | 7.0*** | 6.5**            | 6.2*   | 7.0*** | 7.0*** |
| Keystone ( <i>L.p.</i> )    | 7.3**            | 6.7    | 7.7*** | 7.7*** | 6.0***           | 6.3*** | 6.7*** | 7.0*** | 7.0***           | 7.3*** | 7.7*** | 7.7*** | 6.8**            | 6.8**  | 7.4*** | 7.5*** |
| Rosita ( <i>F.r.t.</i> )    | 6.0              | 6.0    | 6.7    | 6.7    | 6.7***           | 6.7*** | 7.3*** | 7.3*** | 6.3***           | 6.3*** | 7.7*** | 7.0*** | 6.3*             | 6.3*   | 7.2*** | 7.0*** |
| Smirna ( <i>F.r.t.</i> )    | 6.7              | 6.7    | 6.7    | 7.0*   | 6.0***           | 6.0*** | 6.3*** | 6.3*** | 6.7***           | 7.0*** | 7.0*** | 7.0*** | 6.5***           | 6.6**  | 6.7**  | 6.8**  |
| Calliope ( <i>F.r.c.</i> )  | 8.0***           | 8.0*** | 8.0*** | 8.0*** | 5.3**            | 6.0*** | 6.3*** | 6.7*** | 7.0***           | 7.0*** | 8.0*** | 8.0*** | 6.8**            | 7.0*** | 7.4*** | 7.6*** |
| Celianna ( <i>F.r.r.</i> )  | 6.7              | 7.0*   | 6.0    | 6.0    | 6.3***           | 6.3*** | 7.7*** | 8.0*** | 6.0***           | 6.0*** | 7.0*** | 7.0*** | 6.3*             | 6.4**  | 6.9*** | 7.0*** |
| Legende ( <i>F.r.c.</i> )   | 7.0*             | 7.0*   | 7.0*   | 7.3**  | 6.3***           | 6.7*** | 6.3*** | 7.0*** | 8.0***           | 8.0*** | 9.0*** | 9.0*** | 7.1***           | 7.2*** | 7.4*** | 7.8*** |
| Capriccio ( <i>F.r.c.</i> ) | 8.0***           | 8.0*** | 8.0*** | 8.0*** | 8.0***           | 8.0*** | 8.3*** | 9.0*** | 7.0***           | 7.0*** | 8.0*** | 8.0*** | 7.7***           | 7.7*** | 8.1*** | 8.3*** |
| Maxima 1 ( <i>F.r.r.</i> )  | 5.7              | 5.7    | 6.0    | 6.3    | 6.7***           | 6.7*** | 7.7*** | 7.7*** | 6.0***           | 6.0*** | 7.0*** | 7.0*** | 6.1*             | 6.1*   | 6.9*** | 7.0*** |
| Dumas 1 ( <i>F.o.d.</i> )   | 7.0*             | 7.0*   | 8.0*** | 8.3*** | 6.0***           | 6.7*** | 7.0*** | 7.7*** | 7.7***           | 7.7*** | 8.7*** | 8.7*** | 6.9***           | 7.1*** | 7.9*** | 8.2*** |
| Montserrat ( <i>F.a.</i> )  | 7.0*             | 7.0*   | 6.7    | 7.3**  | 6.7***           | 7.0*** | 7.7*** | 8.0*** | 8.0***           | 8.0*** | 9.0*** | 9.0*** | 7.2***           | 7.3*** | 7.8*** | 8.1*** |
| Starlett ( <i>F.a.</i> )    | 7.0*             | 7.0*   | 7.0*   | 7.0*   | 8.0***           | 8.7*** | 8.3*** | 8.7*** | 8.0***           | 8.0*** | 9.0*** | 9.0*** | 7.7***           | 7.9*** | 8.1*** | 8.2*** |
| Panduro ( <i>P.p.</i> )     | 6.7              | 6.7    | 7.0*   | 8.0*** | 7.0***           | 8.0*** | 8.3*** | 8.7*** | 5.7**            | 5.7**  | 6.7*** | 6.7*** | 6.5**            | 6.8**  | 7.3*** | 7.8*** |
| Conni ( <i>P.p.</i> )       | 7.7***           | 7.7*** | 8.0*** | 8.7*** | 5.7***           | 6.3*** | 7.7*** | 7.7*** | 6.0***           | 6.0*** | 8.0*** | 8.0*** | 6.5**            | 6.7**  | 7.9*** | 8.1*** |
| Penn G6 ( <i>A.s.</i> )     | 8.7***           | 8.7*** | 8.7*** | 8.7*** | 7.3***           | 8.3*** | 8.7*** | 9.0*** | 8.0***           | 8.0*** | 9.0*** | 9.0*** | 8.0***           | 8.3*** | 8.8*** | 8.9*** |
| LSD 5%                      |                  |        | 0.9    |        |                  |        | 1.0    |        |                  |        | 0.6    |        |                  |        | 1.2    |        |
| LSD 1%                      |                  |        | 1.2    |        |                  |        | 1.3    |        |                  |        | 0.8    |        |                  |        | 1.6    |        |
| LSD 0.1%                    |                  |        | 1.6    |        |                  |        | 1.7    |        |                  |        | 1.0    |        |                  |        | 2.0    |        |

A poorer reaction to dethatching has been observed at Mara and Margarita varieties (*Lolium perenne*), Smirna variety (*Festuca rubra*), and Starlett variety (*Festuca arundinacea*). Some turfgrass varieties did not benefit from aerification immediately after the treatments were performed. But the effect was visible after two or three months. For example the Capri variety had a better density in the spring at control plot (6.7). The dethatching treatments resulting in a slightly loss of density for all three treatments (AA, AS and AA+AS) rated 6.3. In the summer however, AS treatment registered the best density (6.3) while the control plot was rated only 5.3. A different case was the one of bluegrass varieties Panduro and Conni, which benefited right after the aerification treatments were applied in the spring. Panduro variety had a spring density at control variant rated 6.7 and 8.0 at AA+AS. The species *Agrostis stolonifera* benefited the most in the summer when it registered the biggest difference between control (7.3) and AA+AS treatment (9.0).

## CONCLUSIONS

1. The best dethatching treatment was AA+AS that produced the best turfgrass density compared with control plot.
2. The highest difference between aerification treatments and control plot was observed in the summer at the treatment AA+AS rated 7.2 while the control plot was rated only 6.2.
3. Among turfgrass varieties the strongest reaction to aerification was observed at *Poa pratensis* varieties, where density increased from 6.5 to 8.1.

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**PRELIMINARY RESULTS REGARDING VIRULENCE TEST  
OF SOME *VERTICILLIUM LONGISPORUM* STAINS ON  
*BRASSICA NAPUS***

**REZULTATE PRELIMINARE PRIVIND TESTAREA VIRULENTEI  
UNOR TULPINI DE *VERTICILLIUM LONGISPORUM* LA *BRASSICA  
NAPUS***

**BURLACU (ARSENE) Mădălina Cristina<sup>1</sup>, LEONTE C.<sup>1</sup>,  
LIPSA F.<sup>1</sup>, LAZARESCU E.<sup>1</sup>**  
e-mail: cleonte@uaiasi.ro

**Abstract.** *Verticillium longisporum* is soil-borne vascular fungal pathogens with host specificity to cruciferous plants such as oilseed rape (Eynck et al. 2008). The fungus is able to survive in soil for several years through the production of microsclerotia and because there are no approved fungicides for these diseases, it is important to cultivate some resistant cultivars. The aim of this study was to test the virulence of 3 *Verticillium longisporum* isolates, in order to identify which is the most aggressive isolate for *Brassica napus*. For this purpose, we used two oilseed rape cultivars one which is less susceptible to the disease (Express) and one, which is highly susceptible to the disease (Falcon). The plants were artificially infected with each *V. longisporum* isolate by root deep inoculation under controlled conditions. For four weeks, the plants were scored for the symptoms using an assessment key with nine classes. Based on the obtained results the most aggressive strain proved to be VL43.

**Key words:** virulence, test, *Verticillium longisporum*

**Rezumat.** *Verticillium longisporum* este un agent patogen vascular care se găsește în sol și are plante gazda plante din familia Crucifere cum ar fi rapița (Eynck et al. 2008). Prin intermediul microscleroțiilor, ciuperca poate supraviețui în sol mai mulți ani, iar datorită faptului că încă nu există un control chimic al bolii, este importantă cultivarea de soiuri cu rezistență la această boală. Scopul acestui studiu a fost testarea a trei tulpini de *Verticillium longisporum* pentru a identifica care este cea mai virulentă la specia *Brassica napus*. Pentru realizarea acestui lucru, s-au utilizat cultivarele de rapiță Express cu rezistență moderată la această boală și Falcon care este foarte sensibil la această boală. Plantele au fost inoculate artificial cu cele trei tulpini de *Verticillium longisporum* prin metoda tăierii rădăcinilor în condiții controlate. Timp de patru săptămâni, plantele au fost bonitate folosind o clasă de bonitare cu nouă note. În urma observațiilor făcute cea mai agresivă tulpina s-a dovedit a fi VL43.

**Cuvinte cheie:** virulentă; testare; *Verticillium longisporum*

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

## INTRODUCTION

Verticillium wilt caused by the fungal pathogen *Verticillium longisporum* is one of the most important pathogens of oilseed rape (*Brassica napus*) from northern Europe (Rygulla et al., 2007). The fungus enters in the vascular system of the plant and moves into the xylem vessels causing wilting symptoms, and in the later stages premature ripening of the seed. In this paper, we tested three isolates of *Verticillium longisporum* in order to identify the most aggressive strain for oilseed rape cultivars.

## MATERIAL AND METHODS

The artificial infection was performed with three isolates of *Verticillium longisporum*, two of them (VL 9802 and VL 4329) provided by BCCM/IHEM Scientific Institute of Public Health Section Mycology and Aerobiology, Brussels and one (VL43) from The Division of Plant Pathology and Plant Protection, Georg August University, Göttingen, Germany.

The inoculum for each isolate was prepared using 250 ml PDB (potato dextrose broth) medium, which was inoculated with 500 µl spore stock solution. The cultures were incubated 7 days at 23°C on a rotary shaker. After 7 days, the culture was filtered and using a haemocytometer, we determined the spore concentration. For the inoculation, we diluted the solution at  $1 \times 10^6$  spore mL<sup>-1</sup>.

As plant material we used two *Brassica napus* cultivars Express which is less susceptible to *V. longisporum* and Falcon which is highly susceptible. The seeds from each cultivar were surface sterilized by immersion in ethanol 70% for 2 minutes and then rinsed two times in distilled water.

The seeds were sown in silica sand and grown in the climatic chamber at 23°C. 10 days old plantlets were carefully washed from sand. The inoculation was made by cutting 2 cm from the root apex and holding them for 30 min in *Verticillium longisporum* spore suspension. We used for each isolate 10 inoculated plants and 10 control plants. At the control plants, the root apex was also cut and the plants were held 30 min in distilled water.

After inoculation the plants were transferred into pots containing a mixture of compost, peat and sand (2:1:1) and grown in a climatic chamber at 23°C with a light/dark cycle of 14/10. Every week we take the disease scores using an assessment key with nine classes as described by Eynck et al 2007 (table 1).

Table 1

Assessment key for scoring disease severity

| Score | Symptom development   |
|-------|---|
| 1     | The plant has no symptoms                                       |
| 2     | The oldest leaves have slight symptoms (yellowing, black veins) |
| 3     | The next younger leaves have slight symptoms                    |
| 4     | About 50% of the leaves have symptoms                           |
| 5     | More than 50% of the leaves have symptoms                       |
| 6     | Up to 50% of the leaves are dead                                |
| 7     | More than 50% of the leaves are dead                            |
| 8     | Only apical meristem is still alive                             |
| 9     | The plant is dead   |

## RESULTS AND DISCUSSIONS

Based on the disease scores that were taken every week we calculated the area under disease curve (AUDPC) in order to determine which is the most aggressive isolate of *Verticillium longisporum* for the species *Brassica napus*.

In case of the isolate VL9802 no typical symptoms were observed during the four weeks of the scoring. In the last week, the disease score of the infected plants were „1” – no symptoms and we observed no difference between the infected plants and the control plants.

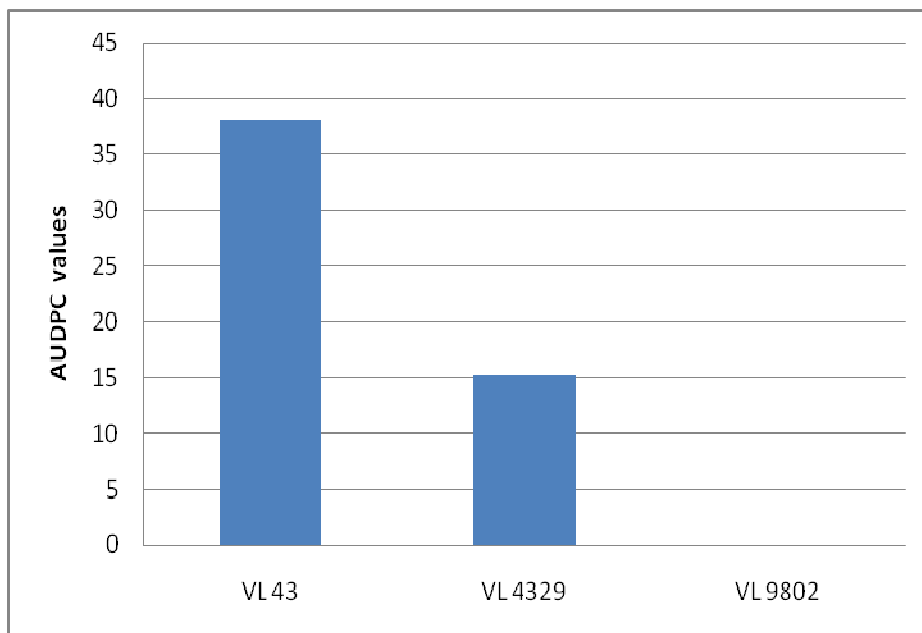
The calculated AUDPC values from the disease scores were 0 in all the infected variants (fig.1).

In the case of the isolate VL4329, typical symptoms like asymmetric yellowing of leaves and early stunting started to be observed at 21 days after infection. The values the calculated AUDPC were higher than at the isolate VL9802 (fig.1). For this isolate the susceptible cultivar Falcon had disease scores between 3-6 after 28 dpi (days after infection) and Express had values between 1 and 3 at 28 dpi.

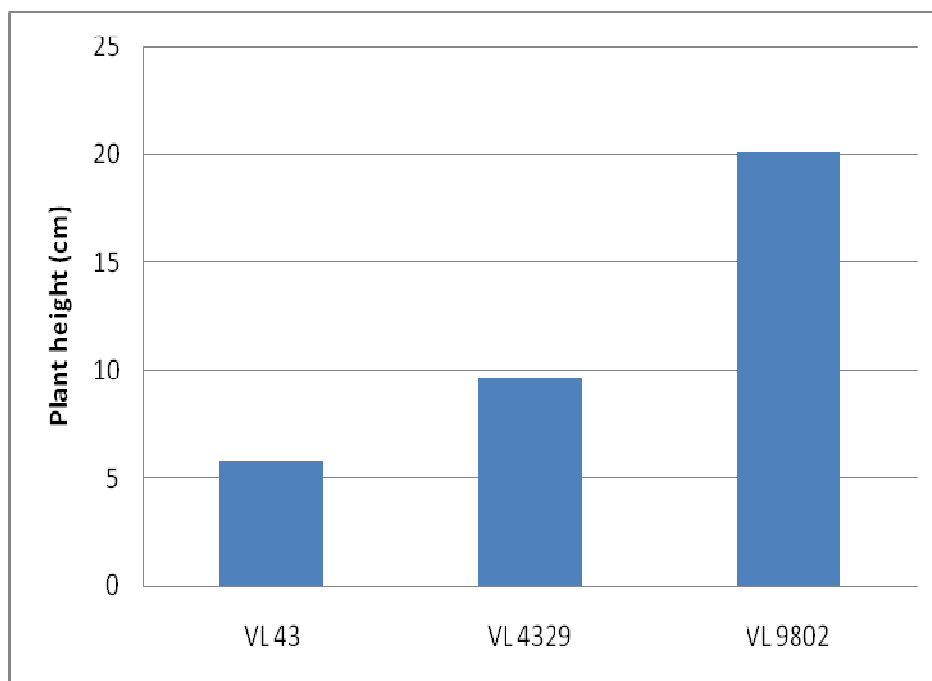
The isolate VL 43 had the higher values of the calculated AUDPC. In this case, typical symptoms started to appear at 14 days after infection. The disease scores and the calculated AUDPC values were the most higher for the both oilseed rape cultivars.

Regarding the evolution of the plant height for all the isolates, the results are presented in fig. 2.

The biggest values for the plant height were in the case of the isolate VL9802 and the smaller values were in the case of the isolate VL43. As it is known, one of the typical symptoms of the infection with *Verticillium longisporum* is the early stunting and as we can observe the evolution of the plant height in the case of the isolate VL43, we can affirm that this isolate was the most aggressive.



**Fig. 1** – The evolution of the virulence of the three isolates of *Verticillium longisporum*



**Fig. 2** – Evolution of the plant height of the inoculated plants with the three isolates of *Verticillium longisporum*

## CONCLUSIONS

In this study we tested three isolates of *Verticillium longisporum* on two *Brassica napus* cultivars in order to identify the most aggressive isolate for this species. Our virulence test performed in the climatic chamber demonstrated that the isolate VL43 originating from Gottingen is the most aggressive producing the most major losses. At this isolate, it could be also observed that the most typical symptoms of the disease like yellow leaves, chlorosis, black veins on the back of the leaves and early stunting started to appear at 14 dpi. Eynck (2007) also reported this and Zhou (2006) in their studies carried out with isolates of both *V. longisporum* and *V. dahlia* on plants. Symptoms of chlorosis and vein blackening were evident after 14 days and stunting was apparent 21 days after inoculation with *V. longisporum*.

In case of the isolate VL9802 that produced no symptoms we can affirm that this isolate is not aggressive for oilseed rape but is may be aggressive for other plants from *Brassicaceae* family. In conclusion, the isolate that we will use for our further studies is VL43 from Gottingen.

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**RESEARCHES REGARDING THE ATTACK OF  
*SCLEROTINIA SCLEROTIUM* ON COTYLEDONS OF  
SOME *BRASSICA NAPUS* L. CULTIVARS**

**CERCETĂRI PRIVIND ATACUL AGENTULUI PATOGEN  
*SCLEROTINIA SCLEROTIUM* ASUPRA COTILEDONELOR LA  
CÂTEVA CULTIVARE DE RAPIȚĂ (*BRASSICA NAPUS* L.)**

**CALISTRU Anca – Elena<sup>1</sup>, LEONTE C.<sup>1</sup>, LAZARESCU E.<sup>1</sup>,  
LIPȘA F.<sup>1</sup>, FITT B. D. L.<sup>2</sup>, HALL Avic<sup>2</sup>**  
e-mail: ancaelenacalistru@gmail.com

**Abstract.** *Sclerotinia sclerotiorum* is a major pathogen for the rapessed crop (*Brassica napus* L.) and for that reason, worldwide, there is a high interest to identify *Brassica* genotypes with resistance to the pathogen (Sarahan et al., 2008). Field testing to identify resistance in the rapeseed germplasm can be difficult and expensive regarding the necessary time and costs. We aimed to examine the reaction of 40 rapeseed cultivars to the attack of *Sclerotinia sclerotiorum*, on cotyledons, in controlled enviromental conditions. For this, we have artificially inoculated the cotyledons, with a solution of macerated mycelium ( $10^4$  mycelial fragments / ml), in liquid PDB media (Garg et al., 2008), from an isolate of the pathogen. The rapeseed cultivars responded with a distinct hypersensitive reaction. The results were statistically interpreted. The cotyledon assay proved to be a rapid and useful method to identify the reaction of the *Brassica napus* cultivars to the attack of *Sclerotinia sclerotiorum*.

**Key words:** *Sclerotinia sclerotiorum*, resistance, artificial infection, cotyledons

**Rezumat.** *Sclerotinia sclerotiorum* este un agent patogen important pentru cultura de rapiță (*Brassica napus* L.) și de aceea, la nivel mondial, se dorește identificarea unor cultivare rezistente la boala produsă de acesta (Sarahan et al., 2008). Testarea rezistenței cultivarelor de rapiță în condiții de câmp se poate dovedi dificilă și costisitoare din punct de vedere al timpului necesar și al cheltuielilor. S-a examinat comportarea a 40 de cultivare de rapiță la atacul de *Sclerotinia sclerotiorum*, pe cotiledoane, în condiții de laborator. Astfel, s-a realizat infecția artificială utilizându-se soluție de miceliu macerat ( $10^4$  fragmente de miceliu / ml), în mediu lichid PDB (Garg et al., 2008), de la un izolat al agentului patogen. Cultivarele luate în studiu au prezentat sensibilitate diferită la atac. Rezultatele obținute au fost interpretate statistic. Metoda de infecție artificială pe cotiledoane s-a dovedit a fi o modalitate rapidă și utilă de identificare a reacției cultivarelor de *B. napus* la atacul de *Sclerotinia sclerotiorum*.

**Cuvinte cheie:** *Sclerotinia sclerotiorum*, rezistență, infecție artificială, cotiledoane

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

<sup>2</sup> University of Hertfordshire, United Kingdom

## INTRODUCTION

Stem rot, caused by the fungus *Sclerotinia sclerotiorum*, is one of the most important diseases of rapeseed, and can lead to significant losses of yield worldwide (Zhao et al., 2004). The pathogen can attack more than 400 plant species from 75 different families (Bolland et al. 1994). The methods used for controlling this disease include chemical control, cultural control and varietal resistance (Bardin et al., 2001). Selection of the host resistance is the only economic and sustainable means of controlling the disease (Zhao et al., 2004).

Field evaluation for selection of resistant cultivars provides variable results, because the response of the plants is dependent on environmental conditions (Abawi et al., 1979). The results are variable also because under field conditions, the *Brassica* genotypes may differ in their maturity and architecture. In contrast, screening for resistance in controlled environmental conditions (laboratory, green house) is more likely to be solely to physiological resistance, because the disease escape mechanisms are very little involved.

Various controlled environment screening methods have been used to evaluate resistance in *Brassica napus*: petiole inoculation (Zhao et al., 2004, Bradley et al., 2006), detached leaf inoculation (Bradley et al., 2006), oxalic acid assay (Bradley et al., 2006), but they don't positively correlate with the field results.

Some researchers used cotyledon tests in genotypes of alfalfa (Pratt et al., 1998) and soybean (Hartman et al., 2000; Kim et al., 2000, Kull et al., 2003) and the results were correlated to field tests. Due to its economic importance, it's important to develop a reliable screening technique for *Brassica napus*, which can rapidly predict the reaction of the genotypes against *Sclerotinia sclerotiorum*.

## MATERIAL AND METHOD

*Test conditions.* The *Brassica napus* genotypes used for screening were grown in 38 x 24 x 5 trays, each having 40 cells and containing a compost mixture. Three seeds of each genotype were sown in each cell and thinned to a single seedling per cell after emergence.

A complete randomised block design was used with three replications and two plants per genotype per replication. All experiments were conducted in the growth room, under controlled environmental conditions ( $18 \pm 1^\circ$  C during the day and  $14 \pm 1^\circ$  C during the night, with a light intensity of  $150 \mu\text{E}/\text{m}^2.\text{s}$  (Garg et al., 2008).

Seedlings were grown until cotyledons were fully expanded (growth stage 1.00, on the scale given by Sylvester-Bradley and Makepeace, 1984).

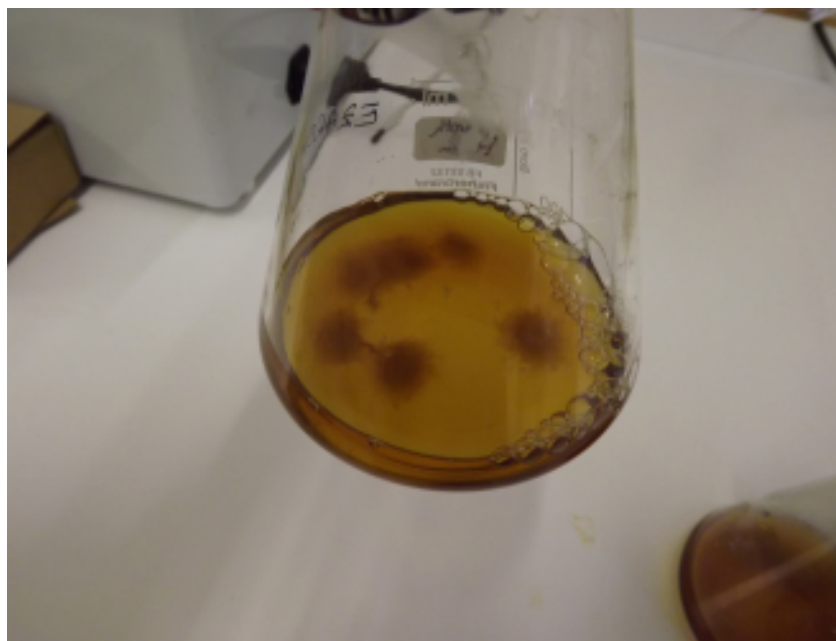
*Genotypes tested.* The 40 genotypes tested were provided by the Centre of Genetic Resources of Netherlands.

**Inoculum production.** A single sclerotium of *Sclerotinia sclerotioru* was surface sterilised in 1% (v/v) sodium hypochlorite and 70 % ethanol for 4 min followed by two washes in sterile distilled water for 1 min (Clarkson et al., 2003). The sclerotium was cut in half and placed on potato dextrose agar (PDA).

*S.sclerotiorum* was subcultured and maintained in an incubator at 20° Con PDA. Seven agar plug discs (each 5 mm in diameter) were cut from the actively growing margin of a 3-day-old colony and transferred to a 25m ml flask containing 75 ml of a sterilised liquid medium of PDB (potato dextrose broth 24 g, peptone 10 g, H<sub>2</sub>O 1 l).

Flasks were rotated on a platform shaker, at 120 rpm / min. After 3 days, colonies were harvested and washed twice with sterilised water (Fig. 1). The fungal mats obtained were transferred to □125 ml of the same liquid medium and the mycelia macerated in a food grinder for 3 min.

The mycelial suspension was then filtered through four layers of cheese cloth and the concentration was adjusted to 10<sup>4</sup> fragments /ml using a haemocytometer with the same liquid medium (Garg et al, 2008).



**Fig. 1** - Flask containing *Sclerotinia sclerotiorum* colonies, in liquid PDB medium (original)

**Inoculations.** The inoculation was made when cotyledons were 10 days old. A total of four droplets of mycelial suspension of 10 µl were deposited on every seedling using a micropipette, with a single drop on each cotyledon lobe (Fig. 2). While inoculating, the mycelial suspension must be shaken regularly to maintain the homogeneity of the mixture. A very fine mist of water was sprayed both over cotyledons and on the inside of the lids, with the purpose of maintaining a relative humidity level of □100 %. After the inoculations, the trays covered by lids were placed for 2 days at a low light intensity of □13 µE / m<sup>2</sup> and then returned to the original light intensity (Garg et al, 2008).



**Fig. 2** - Rapeseed seedling, artificially inoculated with *Sclerotinia sclerotiorum* (original)

*Disease assessment.* Typical hypersensitive and or / necrotic lesions were apparent by 1-2 days post-inoculation. At 4 days post-inoculation, the lids were removed and the diameter of the lesions (mm) was measured with a linear ruler (fig. 3).



**Fig. 3** - Lesions measured on the infected cotyledons (original)

## RESULTS AND DISCUSSIONS

Typical necrotic and / or water – soaked lesions appeared on cotyledons of susceptible genotypes infected. The size of the lesions varied between the tested genotypes, from 1,2 for the *Brassica napus* group WOSR cultivar to 4,7 mm for the Lirama cultivar (Fig. 4). From all the tested genotypes, 2 of them (*Brassica napus* group WOSR and Kodakskii) showed distinct significant differences, and 3 of them (Kievskii 216, Diana and Olymp) presented significant differences compared to the control (the average of all the values for the isolate). For those genotypes, the diameter of the lesions was  $\leq 3.06$  mm. Four genotypes (Ligora, Lirama, Madora, Matador)

presented hypersensitive reaction to the isolate used for infection. The other cultivars presented statistical uninsured differences.

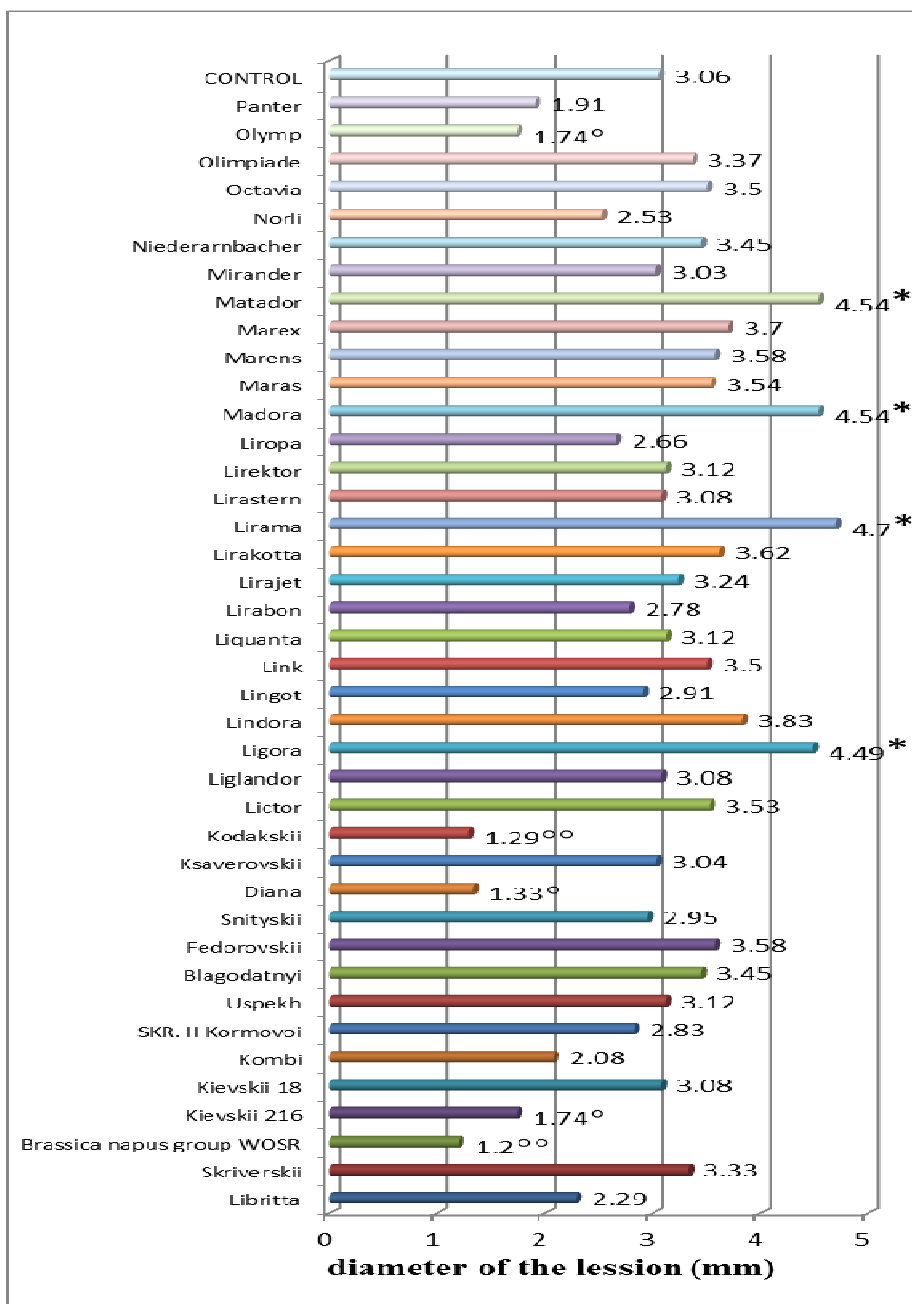


Fig. 4 - Diameter of the lesion measured on the cotyledons

## CONCLUSIONS

1. The cotyledon assay proved to be a reliable method of screening rapeseed genotypes for the resistance to *Sclerotinia sclerotiorum*.
2. From the 40 genotypes tested, only 2 presented a better resistance to the pathogen, compared to the average.
3. Four cultivars showed hypersensitive reaction to the artificial infection.

*Acknowledgments: The work is part of the project No ID 714 POS CCE - Studies of molecular genetics regarding the adaptation of rapeseed to conditions of biotic and abiotic stress, and the optimization of cultivation technology for the extension of cultivating /GENOBRASS, funded by the EU*

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# IN VITRO RELATIONSHIPS BETWEEN FUNGI ISOLATED FROM *RIBES NIGRUM* L. PLANTS

## RELAȚIILE IN VITRO DINTRE UNELE SPECII DE CIUPERCI IZOLATE DE PE PLANTE DE *RIBES NIGRUM* L.

**PETRESCU Eugenia<sup>1</sup>, ȘESAN Tatiana-Eugenia<sup>2</sup>**  
e-mail: petrescu\_eugenia@yahoo.com

**Abstract:** *Fungal isolates from Ribes nigrum L. have been screened in vitro for antagonism against the two pathogens Botrytis cinerea Pers. and Alternaria tenuissima (Kunze)Wiltshire. Among saprophytic isolates tested, strains of Trichoderma sp. were the most competitive against B. cinerea and A. tenuissima pathogenic isolates. The other fungi - Trichothecium roseum Link, Epicoccum nigrum Link and Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams have showed a very slight antagonistic action or lack of antagonism towards the two phytopathogens of blackcurrant.*

**Key words:** *Ribes nigrum*, phytopathogens, antagonistic fungi, biological control

**Rezumat:** *Cercetările au urmărit evaluarea capacității antagonice in vitro a unor ciuperci izolate de pe plante de Ribes nigrum față de ciupercile fitopatogene Botrytis cinerea Pers. și Alternaria tenuissima (Kunze)Wiltshire. Dintre ciupercile testate față de cei doi patogeni ai coacăzului, tulpinile de Trichoderma sp. au manifestat cea mai puternică acțiune antagonistă față de cei doi fitopatogeni-test. Față de Botrytis cinerea, ciupercile Epicoccum nigrum Link și Clonostachys rosea (Link) Schroers, Samuels, Seifert & W. Gams au prezentat o activitate slab antagonistă, iar Trichothecium roseum Link. nu a manifestat antagonism. Față de Alternaria tenuissima, izolatele de T. roseum și C. rosea au dovedit un antagonism de valoare medie, în timp ce E. nigrum a fost antagonistul cel mai puțin eficient.*

**Cuvinte cheie:** *Ribes nigrum*, ciuperci fitopatogene, ciuperci antagoniste, control biologic

### INTRODUCTION

In the last years, due to development in the world and in Romania of gemotherapy, as new field of phytotherapy, is seeking for the glycerol-hydroalcoholic extracts from fresh meristematic plant tissues. For this purpose there are used black currant buds (Raiciu, 2011). Recent studies of Romanian researchers have demonstrated antimicrobial activity of some gemoderivatives containing buds extracts of *R. nigrum* (Mihele, 2007), and the ability of some *R. nigrum* essential oils to reduce inert substrate adhesion of bacteria by inhibiting the expression of microbial adhesins (Oprea, 2008). It is considered that the plant

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<sup>1</sup> Research-Development Institute for Plant Protection Bucharest, Romania

<sup>2</sup> University of Bucharest, Romania

organs with a therapeutic value are not only the buds, but also the leaves, fruits or seeds (Bojor, 2003).

Since the quality of these products used to obtain phytopharmaceutical products may be impaired by various pathogens, especially fungi both in vegetation period of culture, and in the storage period, the establishment of biological control measures of these pathogens it is necessary.

Our researches have been focused on the use of some fungi antagonistic to black currant crop pathogens *Alternaria tenuissima* (Kunze) Wiltshire and *Botrytis cinerea* Pers., for integration in sustainable crop protection.

## MATERIAL AND METHODS

In vitro testing was performed by dual culture method (Jouan, 1964), which is the method used in the work of Romanian researchers to study the relationships of different species of fungi (Hulea, 1973; Drăgoescu, 1996; Raicu, 1970; Şesan, 1985 to present). Each variant had 3-5 repetitions. In petri plates, whereby it was assigned malt-extract-agar medium (MEA), at equal distances from the centre of the vessel, it was inoculated the test pathogenic fungus (marked A) and potential antagonistic fungus (marked B).

To assess the degree of antagonism, it was used the value of the  $x$  ratio between of the internal rays ( $i$ ) and external rays ( $e$ ) of the test pathogenic fungus (A) and the potential antagonistic fungus (B), by the formula  $x=iA/iB \times eB/eA$ , whereby  $iA$ = internal radius of the colony of the phytopathogenic fungus test A;  $iB$  = internal radius of the colony of the antagonistic fungus B;  $eB$  = external ray of the colony of antagonistic fungus B;  $eA$  = external ray of the colony of the phytopathogenic fungus test A.

The ratio  $x>1$  indicates no mutual interaction or antagonism between the two fungi;  $x<1$  indicates a phenomenon of varying degrees of antagonism.

As biological material we used the test phytopathogenic fungi like *Botrytis cinerea*, isolated from the fruits of black currant, and *Alternaria tenuissima*, isolated from the leaves of currant in experimental plots of S.C. Hofigal S.A. Bucharest.

Fungi of which antagonistic activity was evaluated were: *Trichoderma viride* Pers. – Td1 strain, *Trichotecium roseum* Link, *Epicoccum nigrum* Link, *Clonostachys rosea* (Link) Schroers, Samuels, Seifert & W. Gams, isolated from the leaves of black currant plantation S.C. Hofigal S.A. Bucharest, and the strain of *Trichoderma viride* Td2, isolated from black currant plantation SC Hofigal S.A. Bucharest, and the strain of *Trichoderma viride* Td 2, isolated from *Ribes nigrum* leaves from the plantation of Furculeşti, Teleorman County.

A first assessment of the relationship between the two colonies of fungi that grow in the same petri vessel was based on macroscopic analysis from contact line between the two fungal isolates tested.

## RESULTS AND DISCUSSIONS

In all cases whereby the action of *Trichoderma* isolates was tested against the phytopathogens *Botrytis cinerea* and *Alternaria tenuissima*, there was initially a slight distance action on pathogenic fungi colony by the persistence of a very narrow area of mycelium weakly developed, that can be considered a zone of inhibition. Concavity of the contact line was directed



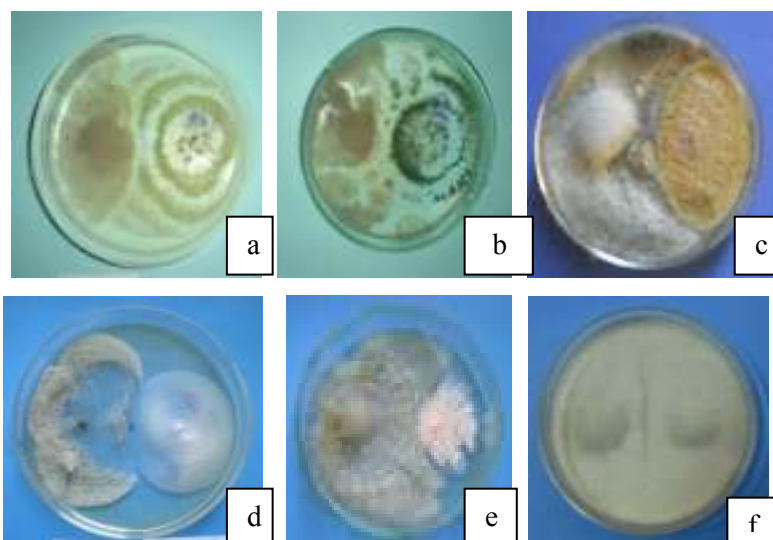
towards *T. roseum*, which had a slower growth. (fig. 1a,b; fig. 2a,b). In the case of test fungus *B. cinerea* and *Clonostachys rosea* and of the potential antagonist *Trichothecium roseum*, the contact curve concavity was directed to *T. roseum*, which had a slower growth (fig. 1e).

In the experimental variant *B. cinerea* and *Clonostachys rosea* it was noticed a slight concavity oriented towards the fungus *C. rosea*, which had a slower growth. Although the phytopathogen increased faster, the portion of colony oriented towards the antagonist presents a more rarefied aspect of growth compared to the portion of colony from the wall of Petri vessel (fig. 1d).

In the variant whereby it was tested the action of fungus isolate *E. nigrum* on the *B. cinerea*, the concavity of marking area was more pronounced and oriented to the antagonist fungus *E. nigrum*, as consequence of the faster growth rate of the colony of phytopathogenic fungus (fig. 1c). When it was tested the influence of antagonist *E. nigrum* on *A. tenuissima*, the marking curve was smoother, the concavity being forwarded to the colony of pathogenic fungus (fig. 2c).

In control variants (phytopathogenic fungi placed face to face with itself), contact line was straight, sign of uniform growth of both colonies placed in the same petri dish (fig. 1f, fig. 2f).

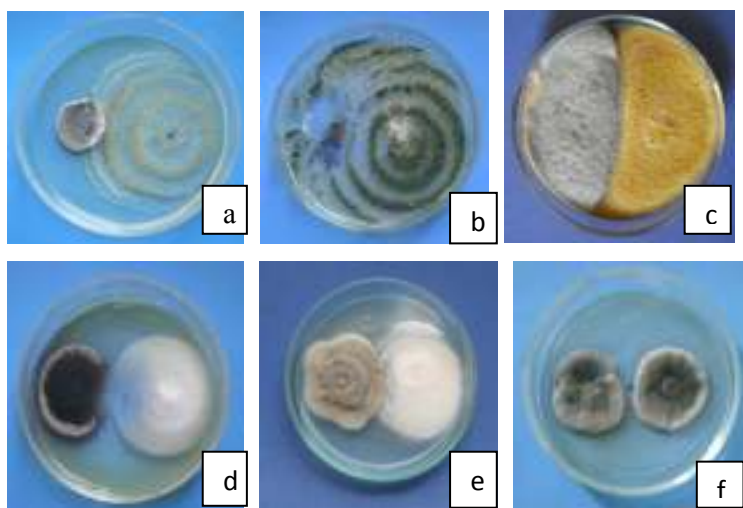
There was a linearly correlation between the linear aspect of the contact area in control and value equal to unity of the x coefficient between external and internal rays of the colonies, both in the version that was tested fungus *B. cinerea* influence by itself and in version that was tested isolate of *A. tenuissima* (tab.1, tab. 2).



**Fig. 1** – Macroscopic *in vitro* aspects of the antagonism of Td1 (a), Td2 (b), *Epicoccum nigrum* (c), *Clonostachys rosea* (d), *Trichothecium roseum* (e) și *Botrytis cinerea* (f) isolates of the phytopathogen *Botrytis cinerea*, on MEA medium

Analysis of x coefficient value shows that strains of *Trichoderma* have expressed antagonism to the pathogen *B. cinerea*. (tab. 1). The stronger antagonistic strain was the fungus isolate from leaves from currant plantation S.C. Hofigal S.A. of Furculești, Teleorman County.

The fungi *E.nigrum* and *C. roseum* showed slight antagonistic activity, whereby they obtained average values of the coefficient x close to the control. In case of the confrontation of fungi *B. cinerea* and *T. roseum* was obtained a higher than one value of coefficient x, which showed that *T. roseum* strain expressed no antagonism against *B. cinerea* isolate (Tab. 1).



**Fig. 2** – Macroscopic *in vitro* aspects of the antagonism of Td1 (a), Td2 (b), *Epicoccum nigrum* (c), *Clonostachys rosea* (d), *Trichothecium roseum* (e) și *Botrytis cinerea* (f) isolates of the phytopathogen *Alternaria tenuissima*, on MEA medium

Table 1

**The *in vitro* relationships between *Botrytis cinerea* and some antagonistic fungi, expressed by the x coefficient, calculated at 7 days, after Jouan and colab. (1964)**

|        | Variant   | x    | Behavior |
|--------|---|------|----------|
| 1.     | <i>B. cinerea</i> / <i>Trichoderma</i> 1        | 0,63 | A        |
| 2.     | <i>B. cinerea</i> / <i>Trichoderma</i> 2        | 0,59 | A        |
| 3.     | <i>B. cinerea</i> / <i>Clonostachys rosea</i>   | 0,88 | SA       |
| 4.     | <i>B. cinerea</i> / <i>Trichothecium roseum</i> | 1,25 | N        |
| 5.     | <i>B. cinerea</i> / <i>Epicoccum nigrum</i>     | 0,97 | SA       |
| martor | <i>B. cinerea</i> / <i>Botrytis cinerea</i>     | 1,00 | I        |

Legend.: x > 1 absent antagonism, nonantagonist izolat e (N);  
x < 1 antagonism (A), strong antagonist (PA), weak antagonist (SA);  
x = 1 absence of mutual influences, indifferently (I).

Against pathogen *Alternaria tenuissima* (table 2), both strains of *Trichoderma* tested showed antagonism. The strain of *Trichoderma* Td 2, Furculești origin, showed the strongest antagonistic, while the strain Td,

provenance Bucharest, was less effective. Isolates of *T. roseum* and *C. rosea* showed antagonism, but of mean value. The fungus *E. nigrum* showed no antagonism against *Alternaria tenuissima*, so the average of coefficient x is close to that of the control (tab. 2).

Table 2

**The *in vitro* relationships between *Alternaria tenuissima* and some antagonistic fungi, expressed by the x coefficient, calculated at 7 days, after Jouan and colab. (1964)**

| Variant |   | x    | Behavior |
|---------|---|------|----------|
| 1.      | <i>A. tenuissima</i> / <i>Trichoderma</i> 1         | 0,72 | A        |
| 2.      | <i>A. tenuissima</i> / <i>Trichoderma</i> 2         | 0,50 | A        |
| 3.      | <i>A. tenuissima</i> / <i>Clonostachys rosea</i>    | 0,81 | A        |
| 4.      | <i>A. tenuissima</i> / <i>Trichothecium roseum</i>  | 0,80 | A        |
| 5.      | <i>A. tenuissima</i> / <i>Epicoccum nigrum</i>      | 1,06 | N        |
| marcor  | <i>A. tenuissima</i> / <i>Alternaria tenuissima</i> | 1,00 | I        |

Legend: x > 1 absent antagonism, nonantagonist isolate (N);  
 x < 1 antagonism (A), strong antagonist (PA), weak antagonist (SA);  
 x = 1 absence of mutual influences, indifferently (I).

## CONCLUSIONS

1. Against pathogen *Botrytis cinerea*: 1.1. both strains of *Trichoderma* tested – Td1 and Td2- showed antagonism on malt-extract-agar medium; the strain with stronger antagonistic action was Td2, the strain isolated from the leaves of currant plantation S.C. Hofigal S.A. from Furculești, Teleorman County; 1.2. the isolates of *E. nigrum* and *C. rosea* were slight antagonistic activity against the fungus *Botrytis cinerea*; 1.3. *T. roseum* showed no antagonism against the pathogen *Botrytis cinerea*.

2. Against pathogen *Alternaria tenuissima*: 2.1. The two strains of *Trichoderma* isolated from *Ribes nigrum* leaves behaved differently: Td 1 Furculești origin, showed the strongest antagonistic action; 2.2. Isolates of *T. roseum* and *C. rosea* showed a mean antagonism to pathogen *Alternaria tenuissima*; 2.3. Fungus *E. nigrum* showed no antagonism against *A. tenuissima*;

3. From the strains with an antagonist potential tested *in vitro* on the two pathogens of the black currant culture, those of *Trichoderma* had the most powerful biological activity. There were registered differences of the two isolated antagonistic depending on their origin. Strain Td 2 Furculești origin was stronger antagonistic to both pathogens tested - *B. cinerea* and *A. tenuissima*.

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# RESEARCHES REGARDING THE LEAVES DISEASES AT WILLOW IN SRC CROPS – MEASURES OF PREVENTION AND CONTROL IN ECOLOGICAL CONDITIONS OF SOUTH WEST OF OLTENIA

## CERCETĂRI PRIVIND BOLILE FOLIARE LA SALCIE ÎN PLANTAȚII DE TIP SRC – MĂSURI DE PREVENIRE ȘI COMBATERE ÎN CONDIȚIILE ECOLOGICE DIN SUD VESTUL OLTENIEI

**PREDA Silvia<sup>1</sup>, GIORGOTA Andreea<sup>1</sup>, ALECU Anca<sup>1</sup>, MITREA I.<sup>2</sup>**  
e-mail: andreea\_giorgota@yahoo.com

***Abstract** Lately in our country, the willow culture intensified as a result of increasing requests for obtaining biomass. The intensification of culture, as of any other plants determined the mass propagation of pathogens, which sometimes contributes to undermining culture. In the South-western Oltenia conditions, were identified pathogens that cause staining black leaf willow (*Marssonina salicicola*), rust (*Melampsora salicicola*) and willow tar spot fungus (*Rhystima salicinum*). Phytosanitary treatments applied to the earliest stage of the attack with fungicides as: Dithane M 45 – 0,2%, Topsin WP - 0,1 %, Alert SC - 0.1%, Merpan 50 WP – 0.2%, have ensured the phytosanitary protection of plants.*

**Key words:** willow, pathogen, prevention, fungicides

***Rezumat:** În România în ultima vreme, cultura salciei s-a intensificat, ca urmare a solicitărilor crescânde pentru producerea de biomasă. Intensificarea culturii, ca a oricărei alte plante a tras după sine înmulțirea în masă a agenților patogeni, care contribuie uneori la compromiterea culturii. În condițiile din sud – vestul Olteniei, au fost identificați agenți patogeni care produc pătarea neagră a frunzelor de salcie (*Marssonina salicicola*), rugina (*Melampsora salicicola*) și pecinginea (*Rhystima salicinum*). Tratamentele fitosanitare aplicate în faza incipientă a atacului cu fungicidele: Dithane M 45 – 0,2%, Topsin WP - 0,1 %, Alert SC - 0.1%, Merpan 50 WP – 0.2% , au asigurat o protecție fitosanitară a plantelor.*

**Cuvinte cheie:** salcie, patogen, combatere, fungicide

### INTRODUCTION

Willow, as forest species with wide spread around the globe, is responsible to a large extent of needs of current and future human society.

Due to rapid growth, the willow crops realise in a short time a major amount of wood, creating conditions for satisfying the increasing needs of wood.

Enhancing culture, like any other plants attract the mass propagation of pathogens contributing to undermining the plant sometimes (Filat, Chira, 2004).

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<sup>1</sup> Research and Development Station for Fruit Tree Growing of Vâlcea, Romania

<sup>2</sup> University of Craiova, Romania

The most common criptogamic infections are produced by species of *Melampsora*, *Marsonia* and *Rhystima*. The whole vegetative apparatus of these fungi is represented by an endoparazit, intercellular mycelium (rarly intracellular), yellow due to the presence of lipocrom, primary (n) haploidal and secondary dicariotic (n + n), which predominates. In addition to active parazitic mycelium, there is a resistance mycelium in different organs of perennials plants (Marcu, 2005).

The aim of present work is presenting the research on the symptomatology of the attack of foliare diseases of willow and the measures for preventing and controlling the pathogens .

## MATERIAL AND METHODS

The biological material under study was made up of 27 varieties and byotypes of *Salix* from fields of the University of Craiova – SCDP Vâlcea.

Observations were made on the symptoms produced by patogens as: *Marssonina salicicola*, *Melampsora salicicola* and *Rhystima salicicola*. Also have studied some aspects of the biology of patogens as well as methods of preventing and controlling them. Depending on the disease symptomatology was established the frequency of attack ( $F\% = [n \times 100] : N$ ).

It was tested the efficacy of biological products, fungicides as: Dithane M 45 conc. 0,2 %, Topsin M70 WP conc. 0,1 %, Alert SC – 0,1%, Merpan 50 WP conc. 0,2 %, Systhane 12 EC conc. 0,05 %.

Fungicides have been associated with the insecticide Sumialpha 5 EC conc. 0,02% for the control of pests.

Phytosanitary treatments have been applied by foliare spraying at an interval of 14 days.

## RESULTS AND DISCUSSIONS

The attack of staining black leaf willow (*Marssonina* sp.) manifests in all phases of vegetation of the plant, in conditions of high humidity (over 70%) and optimum temperature of 22-24°C.

The disease has been reported in our country for the first time by Teodorescu and Mocanu in Brăila in 1968.

Characteristic symptoms appeared on the leaves during the growing season starting from June. On the surface of the leaf blade are observed in the early stages of attack, yellow-greenish spots, small point. These spots are small at first, rare, colored uniform, then shall be increased by 1-3 mm in diameter, increased in number and became brown rust on edges-redheads and yellowish whitish in the central part. The whitish portions develops the fructification of phungus.

At big attacks, spots cover a large area of the foliar blade, appear on the ribs, ribbed and offshots (fig. 1).



**Fig. 1** - Attack of *Marssonina salicicola* at willow (original)

Willow leaf rust (*Melampsora salicicola*), has been reported in the country by Tr. Săvulescu in counties Prahova and Suceava.. Biological material was analyzed in terms of this disease symptomatology, presented on the leaves starting from June – yellowish greenish very small spots (0.5-1 mm) circular or irregular that grow and join them in time. On these spots were noticed some small orange spots which until autumn comes the appearance of crusts, which are rust brown with frutification of phungus. (fig. 2).



**Fig. 2** - Attack of *Melampsora salicicola* at willow (original)

*Rhystima salicinum* which produces willow tar spot fungus on leaves has been reported in Corabia, Turnu Măgurele and Ploiești by C.C. Georgescu and M. Badea, in 1935.

The disease manifests in vegetation starting with June, when on the leaves appear yellowish spots at first, then grey-black. These black point spots of 0.1-0.2 cm, with time become curved with glossy crusts appearance and unregulate surface (fig. 3).



**Fig. 3 - Attack on leaves of *Rhystima salicinum***

The data in table 1, shows that the genotypes of plant have resistance (R) or have different levels of sensitivity to *Marssonina salicicola* fungus attack. Thus, willow varieties as Tordis and Tora and biotypes Cozia 1, Arpașu de sus 1, Arpașu de sus 2, Arpașu de sus 5, Pesceana 1, Pesceana 2, Pesceana 3, Pesceana 4 și Pesceana 7, have shown resistance to this disease.

*Table 1*

**The reaction of willow biotypes against phytopathogenic fungi attack**

| No crt. | Specifica-tion  | <i>Marssonina salicicola</i> |           | <i>Melampsora salicicola</i> |           | <i>Rhystima salicicola</i> |            |
|---------|-----------------|------------------------------|-----------|------------------------------|-----------|----------------------------|------------|
|         |                 | Attack fequency %            | Qualifier | Attack fequency %            | Qualifier | Attack fequency %          | Qualifi er |
| 1       | Tordis          | 0                            | R         | 40                           | MS        | 0                          | R          |
| 2       | Tora            | 0                            | R         | 0                            | R         | 0                          | R          |
| 3       | Inger           | 20                           | PS        | 20                           | PS        | 0                          | R          |
| 4       | Jorr            | 20                           | PS        | 30                           | MS        | 0                          | R          |
| 5       | Lădești         | 20                           | PS        | 50                           | MS        | 10                         | PS         |
| 6       | Cozia 1         | 0                            | R         | 20                           | PS        | 0                          | R          |
| 7       | Cozia 2         | 20                           | PS        | 20                           | PS        | 0                          | R          |
| 8       | Cozia 3         | 20                           | PS        | 40                           | MS        | 0                          | R          |
| 9       | Cozia casantă   | 0                            | R         | 0                            | R         | 0                          | R          |
| 10      | Robești 1       | 20                           | PS        | 0                            | R         | 15                         | PS         |
| 11      | Drăgășani       | 0                            | R         | 20                           | PS        | 15                         | PS         |
| 12      | Gibești         | 20                           | PS        | 30                           | MS        | 0                          | R          |
| 13      | Arpașu de sus 1 | 0                            | R         | 30                           | PS        | 0                          | R          |
| 14      | Arpașu de sus 2 | 0                            | R         | 30                           | PS        | 0                          | R          |
| 15      | Arpașu de sus 3 | 20                           | PS        | 40                           | MS        | 10                         | PS         |



|    |                 |    |    |    |    |    |    |
|----|-----------------|----|----|----|----|----|----|
| 16 | Arpașu de sus 4 | 20 | PS | 40 | MS | 10 | PS |
| 17 | Arpașu de sus 5 | 0  | R  | 0  | R  | 0  | R  |
| 18 | Arpașu de sus 6 | 20 | PS | 30 | PS | 0  | R  |
| 19 | Arpașu de sus 7 | 20 | PS | 30 | PS | 0  | R  |
| 20 | Pesceana 1      | 0  | R  | 40 | MS | 10 | PS |
| 21 | Pesceana 2      | 0  | R  | 40 | MS | 10 | PS |
| 22 | Pesceana 3      | 0  | R  | 30 | MS | 0  | R  |
| 23 | Pesceana 4      | 0  | R  | 40 | MS | 0  | R  |
| 24 | Pesceana 5      | 20 | PS | 50 | MS | 10 | PS |
| 25 | Pesceana 6      | 20 | PS | 50 | MS | 10 | PS |
| 26 | Pesceana 7      | 0  | R  | 0  | R  | 0  | R  |
| 27 | Pesceana 8      | 20 | PS | 0  | R  | 0  | R  |

Legend:

0% attacked plants = R (rezistent),

0-20% attacked plants = PS (less sensitive),

21-50% attacked plants = MS (moderate sensitive),

51-60 % attacked plants = S (sensitive)

Well behaved, proving the resistance (R) to attack of fungus on the leaves that produces willow rust (*Melampsora* spp.) varieties as Tora și Jorr and biotypes as Cozia casantă, Robești 1, Arpașu de sus 5, Pescaana 7 și Pescaana 8.

Compared with willow tar spot fungus *Rhystima salicinum*, the biological material proved resistant (R), at this type of fungus. Have reacted negatively genotypes as : Tordis, Tora, Inger, Jorr, Cozia1, Cozia 2, Cozia 3, Cozia casantă, Gibești, Arpașu de sus 1, Arpașu de sus 2, Arpașu de sus 5, Arpașu de sus 6, Arpașu de sus 7, Pescaana 3, Pescaana 4, Pescaana 7 și Pescaana 8.

Less sensitive (PS) and moderate sensitive (MS), to attack the three pathogens proved to be genotypes: Cozia 1, Cozia 2, Drăgășani 1, Arpașu de sus 3, Arpașu de sus 4, Arpașu de sus 6, Arpașu de sus 7, Pescaana 5 and Pescaana 7.

In the table 2 the data presents the efficacy of fungicides against staining black leaf willow attack (*Marssonina salicicola*) all products tested have shown good capacity (75,0-90,0%), but showed very good, product Systhane 12 EC conc. 0,05% (90,0%).

Table 2

**The effectiveness of fungicides to combat willow diseases produced by *Marssonina salicicola*, *Melampsora salicicola* and *Rhystima salicicola***

| No. Crt. | Product used    | Conc. % | Effectiveness of fungicide of the attack of : |                              |                            |
|----------|-----------------|---------|---|------------------------------|----------------------------|
|          |                 |         | <i>Marssonina salicicola</i>                  | <i>Melampsora salicicola</i> | <i>Rhystima salicicola</i> |
| 1        | DITHANE M 45    | 0,2     | 80  | 90                           | 90                         |
| 2        | TOPSIN M70 WP   | 0,1     | 85  | 80                           | 80                         |
| 3        | ALERT SC        | 0,1     | 75  | 75                           | 90                         |
| 4        | MERPAN 50 WP    | 0,2     | 85  | 80                           | 90                         |
| 5        | SYSTHANE 12 EC  | 0,05    | 90  | 80                           | 80                         |
| 6        | ZEAMĂ BORDELEZĂ | 0,5     | 85  | 85                           | 90                         |

In preventing and controlling that rust (*Melampsora salicicola*) all products used have had a very good effectiveness(80,0-90,0%), but showed very good, product Dithane M45 conc. 0,2 % (90,0%). Frequency of attack of *Rhystima salicicola* is very low, at willow plants, fungicides used to combat the three pathogens have ensured an optimum protection of plants with good efficacy (80-90 %).

## CONCLUSIONS

1. Staining black leaf willow (*Marssonina salicicola*), willow leaf rust (*Melampsora salicicola*) and willow tar spot fungus on willow leaves (*Rhystima salicicola*) are pathogens met in willow cultures, may occur separately, but also at the same time;

2. In order to combat these pathogens in short coppice rotation cultures (SRC) of willow for biomass, it is recommended the use of resistant genotypes with reduced sensitivity to the attack;

3. During the period of vegetation in these types of crops is important to pick up the infected plants (hygiene culture), in order to prevent the transmission of the pathogen from one plant to another;

4. Plant protection treatments in the earliest stage of the attack with one of its fungicides: Dithane M45- 0,2%, Topsin WP – 0,1 %, Alert SC - 0.1%, Merpan 50 WP– 0,2%, Zeamă bordeleză – 0,5%, have ensured that the phytosanitary protection of plants.

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**RESEARCH REGARDING THE BIOLOGY AND ECOLOGY  
OF THE *EURYTOMA SCREINERII* SCHR. AT S.C.D.P.  
VÂLCEA**

**CERCETĂRI PRIVIND BIOLOGIA ȘI ECOLOGIA SPECIEI *EURYTOMA  
SCHREINERI* SCHR. LA S.C.D.P. VALCEA**

**BANCĂ Gh.<sup>1</sup>, MITREA I.<sup>1</sup>**  
e-mail: mitreaion@yahoo.com

**Abstract.** *Eurytoma screineri* Schr. is one of the most dangerous species encountered in the plum orchards in Romania. Pest is present in all areas of culture of plum, damage can reach 90% of the fruit. Within the integrated pest control, knowing the biology and ecology is an essential component without which cannot be made the schemes for control treatment. In the climate of Romania *Eurytoma schreineri* Schr. has one generation per year hibernating as larva in plum stones. In the climatic conditions at S.C.D.P. Valcea, the larvae transform to pupae takes place in late March and early adult emergence occurred in the third decade of April, after the temperature exceeded 15<sup>0</sup> C.

**Key words:** *Eurytoma schreineri* Schr., biology, ecology

**Rezumat.** *Eurytoma screineri* Schr. este una dintre cele mai periculoase specii întâlnită în livezile de prun din România. Dăunătorul este prezent în toate zonele de cultură a prunului, pagubele produse putând ajunge până la 90% din producția de fructe. În cadrul combaterii integrate cunoașterea biologiei și ecologiei dăunătorilor este o verigă esențială, fără de care nu pot fi realizate schemele pentru tratamentele de combatere. În condițiile climatice din România *Eurytoma schreineri* Schr. are o singura generația pe an, iernând în stadiul de larvă în sămburii de prun. În condițiile climatice de la S.D. Vâlcea, transformarea larvelor în pupe are loc la sfârșitul luni Martie, iar apariția primilor adulți a avut loc în a treia decadă a lunii Aprilie, după ce temperatura a depășit 15<sup>0</sup> C.

**Cuvinte cheie:** *Eurytoma schreineri* Schr., biologie, ecologie

### INTRODUCTION

Plum is one of the most important fruit species grown in the county Valcea (59.7%). It is a valuable species because trees have relatively low requirements to ecological conditions, requiring a relatively simple agricultural technique with a constant high productive potential (Mitrea, 2005).

The production potential can be reduced by 20-30% or sometimes totally compromised because of disease and pest attack (Gava, 1999).

*Eurytoma screineri* Schr. is one of the most dangerous species found in the plum orchards in Romania (Copaescu, 1987).

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<sup>1</sup> University of Craiova, Romania

Description: wasp measuring 4-6 mm long, is black with whitish piliferous, rare on head and thorax, abdomen with a strong luster.

The head is large, wide, oval eyes, brown. Antenna is composed of 13 articles.

Pedunculate abdomen is short and smooth, with metallic luster.

Ovipositor is yellow, the legs are brown.

The egg is oval and measures 0.3 mm, with an elongated pedicel at one end, in excess of 2 times the length of the egg.

Larva is apod and encephalon white, with body bulging in the middle and thin at the ends, white brain capsule - yellow and brown mandibles.

White pupa, with yellow legs and wings, attached to the body (Perju et al. 1980).

## MATERIAL AND METHODS

Research and observations on the biology of wasps plum stones were performed in SDCP Valcea, under natural conditions, using growth cages.

To determine the biological reserve we have collected 20 seeds from 5 trees, which were analyzed macroscopically.

Climatic conditions for the period of research, were generally favorable for development of the seeds plum stone sawfly.

Research regarding the morphology of the seeds wasp plum stone were performed on biological material collected from plum orchard at SDCP Valcea and examined under laboratory conditions.

The moment of adults emergence, has been surveilled by placing the growing cages under the crown of the trees. Also ha been established the duration of transforming larvae into pupae, incubation period and duration of larval development.

On the inside of the growth cages were placed attacked stones, harvested in autumn. Biological material from the growth cages was examined daily.

## RESULTS AND DISCUSSIONS

Experiences on the biology and ecology of the species *Eurytoma schreiner* Schr. (wasp plum stone) were performed in 2010, on Stanley variety.

For monitoring pest development stages were collected randomized 100 seeds, from under the crown of the trees. Stones that were examined contained larvae of wasp plum stones, then were monitorized the larvae which came into diapause.

Following our observations, it was found that of the 100 stones collected, 90 showed symptoms of wasps plum stones attack.

The data presented show that, in 2010 from the examined larvae 77% were alive and 23% dead.

According to collected data it came out that from all the examined attacked stones have passed diapause 37.7% of the total number of seeds attacked examined.

The transformation of the larvae into pupa took place in early spring, when average daily temperature began to exceed 10°C. According to our research, this period coincided with plum buds swelling. To determine adult flight, during

spring, there were made observations in growth cages, and results are presented in Table 1. After finishing the pupa stage, 47% of adults have died and 53% of adults flew from seeds attacked.

Table 1

**Evolution of the development stages of *Eurytoma schreiner* Schr. species at S.C.D.P. Vâlcea**

| Examined seeds | Attacked seeds | Larva |       |          | Pupa | Adults |      |
|----------------|----------------|-------|-------|----------|------|--------|------|
|                |                | Dead  | Alive | Diapause |      | Alive  | Dead |
| 100            | 90             | 21    | 69    | 34       | 13   | 7      | 6    |

### The phenology of the wasps plum stones

In order to establish schemes for treatments in an integrated control of wasp plum stones a very important role it's represented by the study of the pest phenology (Georgescu et.al. 1998).

Following our observations made on the biology of wasps plum stones it came out that in 2010, beginning of the transformation of the larvae into pupa occurred later (7. IV. in 2010), because in March and early April prevailed lower temperature than the normal average temperature, which had a negative influence effect on insect development (table 2).

Low temperatures in the first decade of April 5.7°C, determine the appearance of the first adults of wasp plum stones in the second decade of April, on April 25.

The beginning of eggs laying it is largely influenced by the action of several factors, such as climatic conditions, food, etc. (Talmaciu et. al., 2006). Thus, in 2010 the beginning of laying eggs has been recorded on April 25. During the observations there has been discovered eggs layed even on the day of mating individuals. Embryonic development lasted 26 days. Hatching larvae occurred in the second half of May.

Table 2

**Phenology of the *Eurytoma schreineri* Schr. species at S.C.D.P. Vâlcea, 2010**

| Development stages              | Cronology |
|---------------------------------|-----------|
| Begining of the pupa stage      | 7. IV     |
| Mass pupating                   | 15-27. IV |
| Ending of the pupa stage        | 3. V      |
| Transforming pupa in adults     | 21. IV    |
| Appearance of male              | 25. IV    |
| Appearance of female            | 26. IV    |
| Ending of the adults appearance | 20. V     |
| Beginning of laying eggs        | 25. IV    |
| Ending of laying eggs           | 18. V     |
| Hatching larvae                 | 20. V     |
| Last stage larvae               | 13.VI     |

## CONCLUSIONS

In the climatic conditions of S.C.D.P. Valcea *Eurytoma schreiner* Sch. species, has a single generation.

The percentage of viable larvae of the wasp plum stones was 77% and 37% of them entered in diapause, and after finishing the pupa stage 53% of adults were living.

Regarding the phenology of *Eurytoma schreiner* species Sch. we draw the following conclusions:

- the beginning of transforming larvae into pupa began early in the first decade of April;
- adult emergence phased over 26 days (25.IV – 20. V);
- beginning of laying eggs recorded in the third decade of April, and hatching larvae occurred in the third decade of May

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# ÉTUDE DE LA DIVERSITÉ GÉNÉTIQUE D'*APROCEROS LEUCOPODA*

## STUDIUL PRIVIND DIVERSITATEA GENETICĂ A INSECTEI *APROCEROS LEUCOPODA*

CARDAȘ G.<sup>1</sup>, CIORNEI C.<sup>2</sup>, LUPAȘTEAN Daniela<sup>1</sup>,  
BADOUIN A.<sup>3</sup>, PURNELLE B.<sup>3</sup>  
e-mail : gcardas@yahoo.com

**Abstract.** *L'étude present est orienté à l'établissement ou la propagation de l'espèce invasive Aproceros leucopoda Takeuchi, 1939. L'objectif est de déterminer la diversité génétique de quatre populations d'A. leucopoda de Roumanie. De plus nous avons eu l'occasion de comparer la diversité génétique des spécimens Roumain avec un individu venant d'Hokkaido au Japon. Le séquençage des ITS2 ainsi que les RAMS ne montrent pratiquement aucune diversité aussi bien au sein des populations qu'entre les différentes populations. Il semblerait donc que la Roumanie aie subit une introduction ponctuel. Il va donc de soit que si A. leucopoda a été introduit depuis peu, la population n'a pas eu le temps d'évoluer fortement.*

**Key words:** *Aproceros leucopoda*, diversité génétique, propagation des espèces invasives

**Rezumat.** *În lucrarea de față se realizează un studiu privind prezența și răspândirea speciei Aproceros leucopoda Takeuchi, 1939. S-a evaluat diversitatea genetică prezentă în patru populații de A. leucopoda din România. În plus, a fost valorificată posibilitatea de a compara diversitatea genetică a exemplarelor din România cu cea a indivizilor din Hokkaido, Japonia. Secvențierea ITS2 și RAMS arată că nu există aproape nici o diversitate, atât în cadrul populațiilor și nici între populații diferite. Acest lucru sugerează că România a cunoscut o introducere unică, cu câțiva indivizi de la început. Această lipsă de variabilitate genetică ar putea fi explicată prin introducerea recentă a A. leucopoda în România, populația neavând timp să evolueze în mod semnificativ.*

**Cuvinte cheie:** *Aproceros leucopoda*, diversitate genetică, răspândire specii invazive

### INTRODUCTION

Les processus génétiques et évolutifs peuvent être les caractéristiques clés permettant l'établissement ou la propagation d'une espèce invasive. Cependant, la génétique et l'évolution de ces espèces a reçu beaucoup moins d'attention que leur écologie. Les espèces invasives peuvent évoluer, à la fois, durant le processus initial d'établissement et durant leur propagation. Tout particulièrement en

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<sup>1</sup> Université "Ștefan cel Mare" Suceava, Roumanie

<sup>2</sup> Institute de Recherche Forestière Bacău, Roumanie

<sup>3</sup> Université Catholique de Louvain, Belgique

réponse aux pressions de sélections générées par leur nouvel environnement (Sakai et al, 2001).

Les marqueurs moléculaires peuvent s'avérer très utile dans l'étude du déroulement d'une invasion et de la structure la population d'une espèce invasive (Sakai et al, 2001). Les marqueurs génétiques peuvent être utilisés pour mesurer la diversité génétique dans une population invasive et donne une « empreinte » génétique (Sakai et al., 2001). Ils procurent également une indication sur la quantité de diversité perdue au cours de la colonisation ou fournissent la preuve d'une source d'introduction multiple (Sakai et al, 2001).

## MATÉRIEL ET MÉTHODES

Parmi la large de gamme de marqueurs moléculaires disponibles, nous avons décidé d'utiliser les microsatellites, d'après l'étude réalisée par Heidema *et al.* (2004) sur une espèce d'une famille voisine. Les microsatellites sont définis comme étant des répétitions courtes en tandem, contenant entre 2 et 6 nucléotides. Ils représentent une excellente source de polymorphisme car le nombre d'unité répétée peut augmenter ou diminuer à cause de glissement des brins d'ADN durant la réplication par l'ADN polymérase (Neppelenbroek et al., 2006). N'existant pas de microsatellite connu chez *A. leucopoda*, nous avons décidé d'évaluer la diversité génétique chez cette espèce à l'aide d'une technique alternative appelée « Random Amplified Microsatellite » (RAMS). L'utilisation de RAMS se base sur l'utilisation de la PCR. (la réaction de polymérisation en chaîne).

La PCR amplifie de manière sélective un fragment d'ADN flanqué par deux séquences complémentaires au primer. Habituellement les deux primers sont fabriqués sur base des séquences connues du locus d'intérêt. Cependant, dans le cas des RAMS les primers utilisés, sont de courtes séquences « aléatoires », l'amplification de plusieurs loci aléatoires peut donc avoir lieu. On obtient par conséquent, un produits PCR représentant un pattern caractéristique qui peut être utilisé comme signature de l'ADN analysé (Zietkiewicz et al., 1993). Cette technique permet donc d'étudier la variabilité génétique intra-ou inter- spécifique sans nécessité aucune information préalable sur les séquences qui seront amplifiées.

D'autre part, pour avoir une idée encore plus précise de la variabilité génétique présente au sein de la population d'*A. leucopoda* en Roumanie, nous avons utilisé un deuxième marqueur, l'ITS2. Chez les insectes, l'ADN ribosomal (ADNr) est constitué d'unités de transcription répétées en tandem avec chaque unité contenant les gènes pour l'ARN ribosomal (ARNr) 18S, 5.8S et 28S. Les ITS ( internal transcribed spacer) 1 et 2 flanquent le gène 5.8S le séparant ainsi du 18S et 28S et un ETS ( external transcribed spacer) sur l'extrémité 5' du gène 18S complète l'unité de transcription. L'unité de base de l'ADNr, appelée ADNr array, est donc constituée de l'unité de transcription et d'un spacer qui n'est pas transcrit. L'ADNr array varie en nombre, et ces répétitions n'évoluent pas indépendamment, mais évolue selon une vitesse homogène au sein d'une population ou d'une espèce. Alors que la région codante reste relativement conservée, les ITS peuvent évoluer plus librement et divergent même chez des individus apparentés. Par conséquent les ITS s'avèrent très utile pour étudier la variabilité génétique à différent niveau taxonomique (Marinucci et al., 1999).

Les séquences d'ITS2 obtenues furent alignées à l'aide du logiciel ClustalX 2.0.11. Un alignement multiple a donc été réalisé en utilisant la matrice de score IUB.



Une fois les séquences alignées, le logiciel DnaSP 5.10 fut utilisé pour réaliser un test visant à déterminer le nombre de site polymorphique constitué de deux, trois ou quatre nucléotides (DnaSP Version 5 Help Contents).

Pour ce qui est des séquences obtenues avec AM1, l'alignement réalisé par ClustalX 2.0.11 est presque parfait sur les 390 premières paires de base et le score est maximal pour chaque colonne de l'alignement. L'analyse des sites polymorphiques réalisée avec DnaSP 5.10 ne dénombre aucun site polymorphique.

D'autre part, lors de la mise au point de la technique de séquence, nous avons séquencé l'ITS2 des individus B7 et V2 à l'aide du primer AM2. Grâce à cette séquence, nous avons pu évaluer la variabilité génétique dans la partie qui n'était pas spécifique lorsque l'on utilise le primer AM1.

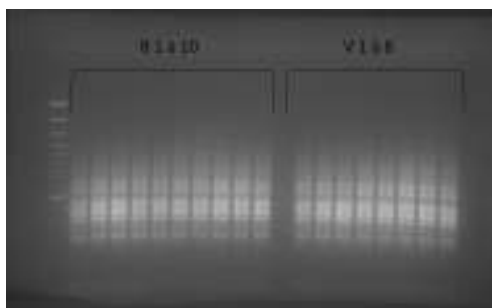
De plus nous avons pu comparer ces séquences avec celle obtenue à l'aide du primer AM1. Pour ce faire nous avons inversé les séquences B7 et V2 obtenues avec le primer AM2 pour ensuite calculer leur séquence complémentaire. Ces séquences complémentaires ont été alignées avec leur séquence respective obtenue avec le primer AM1. Un indice de similarité a alors pu être calculé. Toutes ces manipulations ont été réalisées à l'aide du logiciel BioEdit version 7.0.9.0

## RÉSULTATS ET DISCUSSION

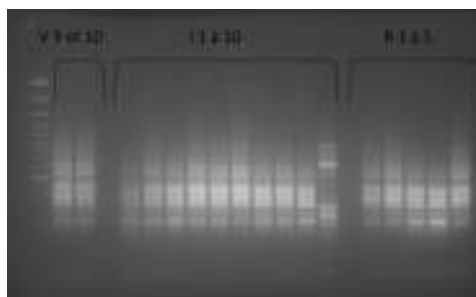
L'ADN obtenu après extraction avait une concentration allant de 3,9 à 335 ng/ $\mu$ l. L'ADN pouvait également être considéré comme « pure » puisque les ratios 260/280 et 260/230 étaient, tout deux, en moyenne égale 2. Grâce au séquençage, nous avons pu obtenir une séquence d'ADN de 629 paires de base. Cependant, le séquençage n'est plus spécifique au delà des 390 paires de base, aussi bien pour les séquences obtenues à partir d'AM1 que d'AM2. De plus les pics observés sur le chromatogramme sont encore moins marqués pour les séquences obtenues avec AM2. Les analyses ne se sont donc portées que sur les 390 nucléotides amplifiés.

En observant les photos prises des gels (fig. 1-4) on peut observer que le pattern est bien similaire d'une expérience à l'autre. De plus on peut remarquer que tous les individus, sauf un, présentent un pattern composé de 6 bandes distinctes (fig. 4). Même l'individu venant du Japon présente le même pattern (fig. 5). La première bande est constituée environ de 600 paires de base, la seconde de 550, la troisième de 400, la quatrième se situe à légèrement au dessus de 300 paires de base, la cinquième est à 300 et la dernière se situe entre 200 et 300 paires de base.

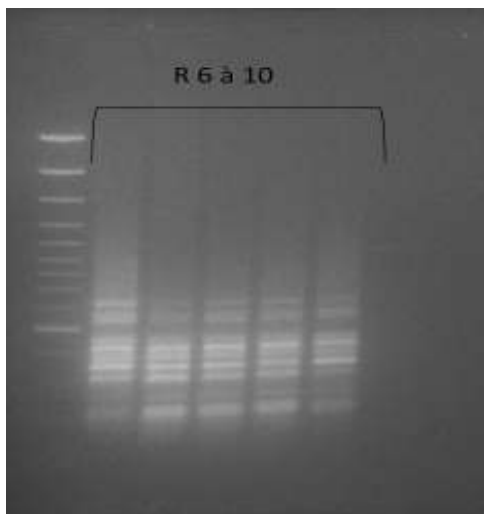
Seul l'individu I10 présente un pattern différent des autres (fig. 4). On peut observer chez ces dernières 9 bandes distinctes. La première fait environ 900 paires de base, la seconde 800, la troisième environ 700, ensuite deux bandes très proches aux environ à 600, et à 550, la septième se situe à 300 paires de base, la huitième est légèrement en dessous de 300 et la dernière se situe entre 200 et 300 paires de base.



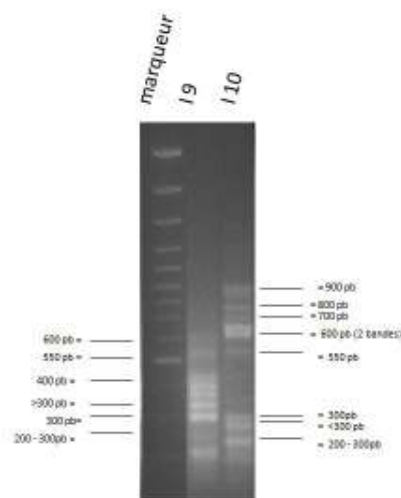
**Fig. 1** - Pattern RAMS observé à 10 individus venant de Brodoc IV,64 et à 8 individus venant de Vaslui IV,40A



**Fig. 2** - Pattern RAMS observé à 2 individus venant de Vaslui IV,40A, à 10 individus venant de Iași III,55/87 et à 5 individus venant de Răducăneni III,6B



**Fig. 3** - Pattern RAMS observé à 5 individus venant de Răducăneni III,6B

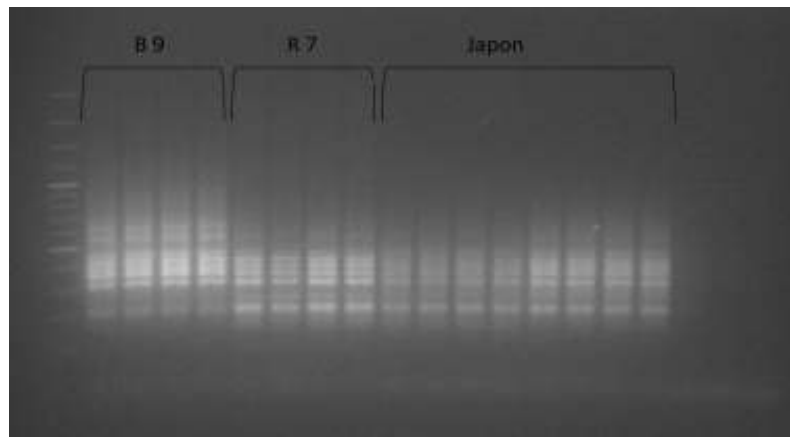


**Fig. 4** - Comparaison de pattern RAMS à individus numéro 9 et 10 venant de Iași III,55/87

On peut donc calculer l'indice de similarité génétique (GS) entre ces deux individus. Les deux individus ont en commun 4 bandes, l'individu I9 possède 2 bandes qui lui sont propres tandis que l'individu I10 possède 5 bandes qui lui sont propres. GS est donc égale à 1,142. GS calculé sur les autres individus sera égale à 1 puisque le pattern des RAMS est identique.

Le séquençage des ITS2 ainsi que les RAMS ne montrent pratiquement aucune diversité aussi bien au sein des populations qu'entre les différentes populations. Seul l'individu I10 présente un pattern RAMS différent des autres individus, pourtant sa séquence ITS2 est identique au autre pour la région spécifique.

Il semblerait donc que la Roumanie aie subit une introduction ponctuel. Selon Blank et al. (2009) des cocons auraient été importés de Chine ou du Japon sur des ormes utilisés dans l'horticulture.



**Fig. 5** - Pattern RAMS observé à l'individu 9 venant de Brodoc IV,64, à l'individu 7 venant de Răducăneni III, 6B et à l'individu venant de Japonia

De nombreuses études (Allendorf et Lundquist, 2003 ; Dlugosh et Parker, 2008 ; Hufbauer, 2008) montrent que les espèces invasives dont l'introduction s'est réalisée en une fois présente une variabilité génétique amoindrie. De plus, les conditions climatiques sur l'île d'Hokkaido au Japon sont similaires à celle rencontrées en Europe centrale. Cependant l'hiver est généralement plus froid et l'été plus chaud, avec des précipitations annuelles plus élevées (Blank et al., 2009). Il semblerait donc normale que la variabilité génétique observée entre les individus roumains et l'individu japonais soit réduite étant donné que *A. leucopoda* n'a dû que légèrement s'adapter aux conditions climatiques roumaines. Il a été montré que de nombreuses espèces invasives sont pré-adaptées à leur nouvel habitat (Sakai et al., 2001) et malgré le fait qu'elles doivent s'adapter à leur nouvel environnement elles sont capables d'être plus compétitives que les espèces indigènes (Allendorf et Lundquist, 2003). De plus nous avons eu l'occasion de comparer la diversité génétique des spécimens roumains avec un seul individu venant d'Hokkaido au Japon.

Une autre explication potentielle qui conforterait la faible diversité génétique observée chez *A. leucopoda* est son mode de reproduction. Au vu du fait que cette espèce possède un mode de reproduction parthénogénétique, on ne s'attend pas à ce que la variabilité génétique soit importante au sein de la population (Castagnone-Sereno, 2006). Cette absence de variabilité génétique pourrait également être expliquée par l'introduction récente d'*A. leucopoda* en Roumanie. Cela est confirmé par les résultats obtenus chez l'individu venant du Japon, aussi bien les RAMS que la séquence de l'ITS2. Il va donc de soit que si *A. leucopoda* a été introduit depuis peu, avant 2003 selon Blank et al. (2009), la population n'a pas eu le temps d'évoluer fortement.

Dernièrement, l'absence de diversité des ITS2 pourrait tout simplement être une caractéristique d'*A. leucopoda*. En effet certaines espèces présentent une très forte variabilité dans la séquence et dans la longueur de leur ITS tandis que

d'autres n'en possède presque pas. De plus, un processus appelé évolution concertée peut maintenir l'intégrité des séquences d'ADNr grâce aux mécanismes d'homogénéisation des séquences telle que la conversion des gènes ou le crossing-over inégal (Beebe et al., 2000). Néanmoins, il n'est pas exclu que la population d'*A. leucopoda* roumaine soit pourvue de variabilité génétique. Il se peut tout simplement que les marqueurs ne l'ont simplement pas mis en évidence.

## CONCLUSIONS

1. Les résultats de l'étude de la diversité génétique d'*A. leucopoda* ne montrent pratiquement aucune diversité au sein des populations.
2. Néanmoins, il n'est pas exclu que la population d'*A. leucopoda* roumaine soit pourvue de variabilité génétique. Il se peut tout simplement que les marqueurs ne l'ai simplement pas mis en évidence.
3. On considère la perspective de déterminer la population d'origine et d'analyser la diversité génétique de cette population.

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# RESEARCH ON THE EFFICIENCY OF THE METHODS OF COMBATING THE HARMFUL ENTOMOFAUNA THAT ARE CURRENTLY APPLIED IN FOREST NURSERIES IN THE NE OF MOLDOVA

## CERCETĂRI PRIVIND EFICIENȚA METODELOR DE COMBATERE A ENTOMOFAUNEI DĂUNĂTOARE APLICATE ÎN PREZENT ÎN PEPINIERELE SILVICE DIN N – E MOLDOVEI

GRĂDINARIU F.<sup>1</sup>, TĂLMACIU M.<sup>1</sup>, BODESCU C.<sup>1</sup>

e-mail: mtalmaciu@uaiasi.ro

**Abstract.** *When you want to realize a sustainable management of the forests it's absolutely necessary to resolve some important problems like assuring high quality wood saplings. For this reason, it's mandatory that beside the technical works that must be done and that are foreseen by the existing instructions, to apply modern methods of preventing and controlling the pests existing in the forest nurseries and forest cultures. The research has as a purpose to determine the efficiency of current pest control methods applied in nurseries of the Forest Directorate Botosani and Iasi. ROMSILVA National Administration of Forests records in present a weak point in terms of production of seedlings and pest control, control methods applied are outdated, and substances used are highly toxic and ineffective.*

**Key words:** pest, forest nurseries, root insects, saplings

**Rezumat.** *Pentru a realiza un management durabil al pădurilor este absolut necesar rezolvarea unei probleme de mare importanță și anume asigurarea de puieți forestieri de calitate superioară. Din acest motiv, se impune ca pe lângă lucrările tehnice ce trebuie efectuate în baza instrucțiunilor și normelor silvice în vigoare, să fie aplicate metode moderne de prevenire și combatere a dăunătorilor prezenți în pepinierele și culturile silvice care provoacă cel mai adesea pagube însemnate. Cercetările au avut în vedere stabilirea eficienței metodelor actuale de combatere a dăunătorilor aplicate în pepinierele din cadrul Direcțiilor Silvice Botoșani și Iași. Regia Națională a Pădurilor înregistrează în prezent un punct slab la capitolul producerii de puieți și combaterii dăunătorilor acestora, metodele de combatere aplicate fiind învechite, iar substanțele folosite au un grad ridicat de toxicitate.*

**Cuvinte cheie:** dăunători, pepiniere forestiere, insecte de rădăcină, puieți

### INTRODUCTION

The research has as a purpose to determine the effectiveness of current pest control methods applied in nurseries of the Forest Directorate Botosani and Iasi. In essence, the research analyzed the dynamics of the species of pests found

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

in the nurseries and also the applied methods of preventing and controlling (Brudea V., 2007).

## MATERIAL AND METHODS

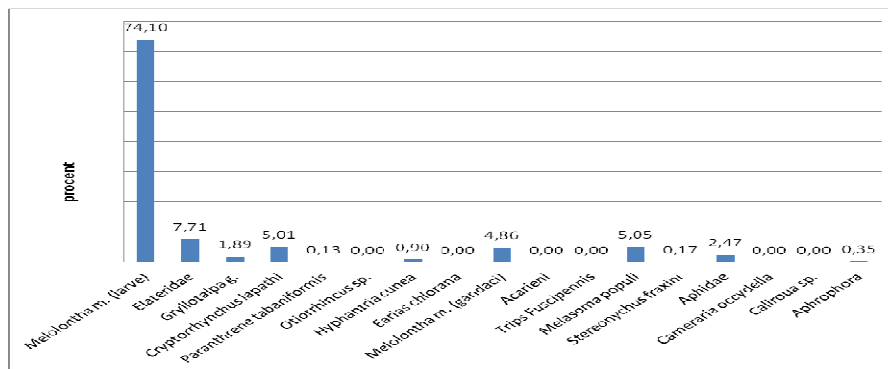
Were collected and processed statistically data from results of surveys conducted in autumn 2011 in a number of 48 nurseries forests from Botosani and Iasi counties; programs and projects for protection of forests for the years 2007, 2008, 2009, 2010 and 2011 made by specialized staff, on the basis of statistics of forest pest and the dynamics of the active outbreaks in nurseries and solariums and pre-measurement with pre-calculation of the protection works (2007-2011). Data was analyzed according to the area affected by pests and intensity of their attack against the control measures applied by RNP staff.

## RESULTS AND DISCUSSIONS

Between the years 2007 – 2011 the harmful insects detected in the nurseries inside Iasi and Botosani forest administrations were classified after the nature of their attack:

1. insects that attack the root: *Melolontha melolontha* – cockchafer larvae; *Agriotes spp.* Elateridae family – wireworms; *Gryllotalpa gryllotalpa* – mole cricket;
2. insects that attack the stem: *Cryptorrhynchus lapathi* – poplar and willow's moth; *Paranthrene tabaniformis*; *Otiorhynchus sp.*;
3. defoliating insects: *Hyphantria cunea*, *Earias chlorana*, *Melolontha melolontha* – beetles, *Melasoma populi*, mites, *Trips fuscipennis*, *Stereonychus fraxini* – ash leaves weevil; (Brudea, 2007).
4. sucking insects: *Aphrophora*, *Caliroa spp.*, *Cameraria ohridella*.

The highest rate among the harmful insects as infested area are the root insects which represent 87.20% from the total of the harmful insects and among these, the most popular pests are *Melolontha melolontha* larva with 74.10% followed by the species from *Elateridae* family – wireworms with 7.71%, as shown in fig.1.



**Fig. 1** - Percentage of insect species found in forest nurseries in Botosani and Iasi between the years 2007-2011 (%)

Measures to prevent and combat were made mostly, mechanical and chemical, but biological missing entirely.

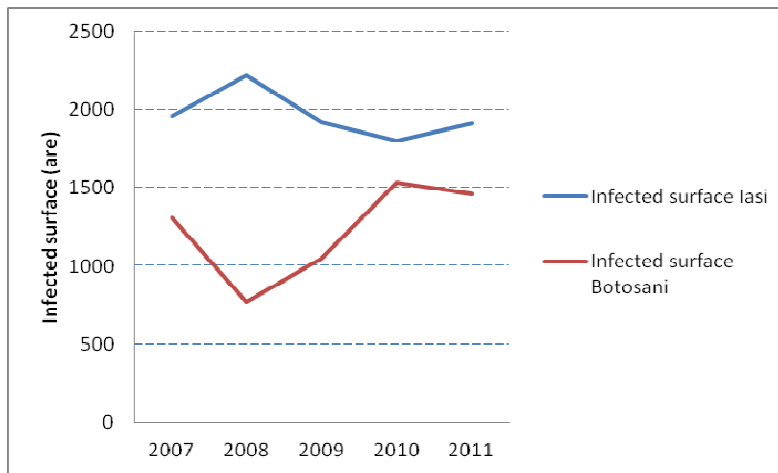
Administration of chemical substances granulated and diffused with the Kyoritz device with Vydate (active material: Oxamyl) in 2009, 2010 and 2011 in general in two halves: first work with norm of 50 kg/ha (10 g/50kg/ha before the onset of spring crops), and the second with a norm of 30 kg/ha (treating the soil between rows of trees at the beginning of the vegetation season). During 2007-2008 for combating larvae of beetles and wire worms, were used substances like Sinolintox (Dimetoat – 40%, Gama Hch -35 %), Sinoratox (Dimetil-S, Dimetoat), Reldan (Clorpirifos metil 400 g/l), Fastac 10 C (Alfacipermetrin 100 g/l), Karate Zeon (Lambda cihalotrin 50g/l) (Tălmăciu, 2005).

Works performed for pest for *Melolontha melolontha* root and *Elateride* species were:

- significantly dusting ground all over with insecticides;
- incorporation into the soil of insecticide between the rows of seedlings;
- executing surveys to determine the degree of infestation;
- collecting defoliation beetles from trees (margins of nurseries with the flight of *Melolontha Melolontha*);
- collecting larvae of the bugs where these were observed.

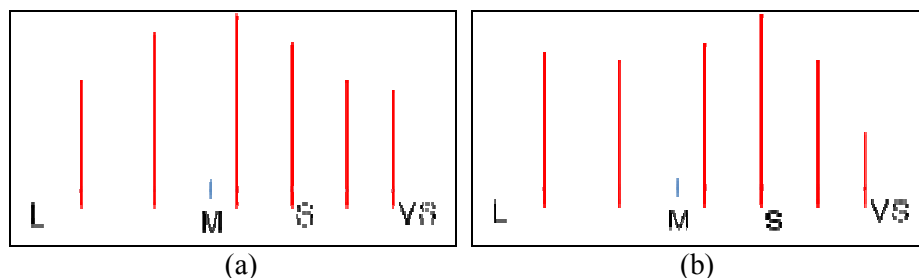
Combating the mole cricket (*Gryllotalpa gryllotalpa*) was achieved by significantly dusting ground all over with insecticides and also by placing toxic baits.

As it can be noticed from fig. 2. the affected surface by root insects in the Botosani Forest Directorate is less in 2011 than the previous year, but compared to 2008 has increased up to 47 %. In Iasi Forest Directorate we can also observe an increase of the infected surface by the *Melolontha melolontha* larva, but this increase is insignificant.



**Fig. 2** - Dynamics of the *Melolontha Melolontha* (larvae) in forest nurseries in Botosani and Iasi during 2007 - 2011

The intensity of the attack of the root insects in the period 2007-2011 illustrated in fig. 3. was from weak to very strong for the *Melolontha melolontha* larva and generally weak for the species from *Elateridae* family, and the intensity of the other categories of harmful insects was registered as being weak and average, very rarely on the small areas has been registered as being powerful or very powerful.



**Fig. 3** - Frequency level of the attack of the *Melolontha melolontha* (larvae) in forest nurseries in Botosani (a) and Iasi (b) during 2007 - 2011

## CONCLUSIONS

1. ROMSILVA National Administration of Forests records now a weak point in terms of production of seedlings and pest control, control methods applied are outdated, and substances used are highly toxic and ineffective.

2. Worrying is the fact that the degree of attack of larval beetles in forest nurseries records higher values over the medium to very strong than over the low to medium.

3. In most cases, deviation from the technical rules of the consumption of insecticide per hectare or ages of treatment application leads to a very low pest control measure efficiency.

4. Substances which are currently used to combat pests affect directly also the useful entomofauna from the nurseries. Is recommended to use some selective biological products to control larvae of beetles based on entomopathogenic fungi (*Beauveria brongniartii*) that are commercially available - Melocont and biological testing of other new products.

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# CONTRIBUTIONS TO THE STUDY OF BEETLES ENTOMOFAUNA IN ANALYZED ORCHARDS USING THE BEATING METHOD

## CONTRIBUȚII LA STUDIUL ENTOMOFAUNEI DE COLEOPTERE DIN CADRUL PLANTAȚIILOR POMICOLE ANALIZATE PRIN APLICAREA METODEI FRAPAJULUI

**PĂDURARU L.B.<sup>1</sup>, TĂLMACIU M.<sup>1</sup>, TĂLMACIU Nela<sup>1</sup>**  
e-mail: mtalmaciu@uaiasi.ro

**Abstract.** The research was conducted in 2008-2010 using beating method and observations were made in four stationary belonging to Iasi and Vaslui counties. In three of these stationarys chemical treatments were performed as follows: stationary V. Adamachi Iasi – treated, Miroszlava Iasi and stationary SC Service Delești SRL Vaslui, untreated apple orchard were performed in Iasi stationary V. Adamachi - untreated. Beating method samples were collected were the sudden shaking of the branches of trees trunks with a rubber stick, which was previously under the trees placed a tarpaulin. To collect useful and harmful entomofauna tree crown, beating method consisted of shaking suddenly two branches of 10 trees in each stationary. For each sampling time of 5 trees was kept constant and marked with distinctive signs early in the season and five randomly chosen trees. After harvesting the biological material it was made in the laboratory where he was recording and then the samples were cleaned and collected insects were determined.

**Key words:** beating method, apple orchards, stationary

**Rezumat.** Cercetările s-au desfășurat în perioada 2008-2010 utilizându-se metoda frapajului, iar observațiile au fost efectuate în patru staționare aparținând județelor Iași și Vaslui. La trei dintre acestea s-au efectuat tratamente chimice și anume: staționarul Vasile Adamachi Iași - tratat, staționarul Miroszlava Iași și staționarului de la ferma SC Loturi Service SRL Delești Vaslui, plantațiile de măr netratate au fost în cadrul staționarului Vasile Adamachi Iași -netratat. Prin metoda frapajului s-au recoltat probe care au constat în scuturarea bruscă a trunchiurilor a ramurilor pomilor cu ajutorul unui baston din cauciuc, unde în prealabil sub pomi a fost așezată o prelată. Pentru colectarea entomofaunei utile și dăunătoare din coroana pomilor, metoda frapajului a constat în scuturarea bruscă a câte două ramuri din 10 pomi din fiecare staționar. De fiecare dată eșantionarea a fost făcută din 5 pomi menținuți constant și marcați cu semne distinctive la începutul sezonului și 5 pomi aleși la întâmplare.

**Cuvinte cheie:** frapaj, livezi de măr, staționare

### INTRODUCTION

As a result of quantitative and qualitative damage caused by pests, on the one hand to increase the number of chemical treatments to combat, something that

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

results in explosive evolution of pests and on the other fauna decreased substantially useful to help maintain the balance pest populations (Tudose and Dobrin, 2002).

## MATERIAL AND METHODS

The research was conducted in 2008-2010 using the beating method and observations were conducted in four counties of Iasi and Vaslui belonging stationary. Three of these chemical treatments were performed as follows: stationary V. Adamachi Iasi – treated, Miroslava Iasi stationary and farm plots Service SC Delești SRL Vaslui, untreated apple orchard were in Iasi V. Adamachi untreated.

Beating method samples were collected were the sudden shaking of the branches of trees trunks with a rubber stick, which was previously under the trees placed a tarpaulin. To collect useful and harmful entomofauna tree crown, beating method consisted of shaking suddenly two branches of 10 trees in each hospital.

For each sampling time of 5 trees was kept constant and marked with distinctive signs early in the season and five randomly chosen trees. After harvesting the biological material it was made in the laboratory where he was recording and then the samples were cleaned and collected insects were determined.

## RESULTS AND DISCUSSIONS

Stationary V. Adamachi Iasi - untreated (table 1) in the apple orchard where no chemical treatments were applied in 2009 have been a number of eight harvests. After harvesting these 212 specimens were collected belonging to eight orders and 25 families. In 2010 the beating method the crown of the trees during the growing and harvesting were carried out five. They collected a total of 55 items that belong to 6 orders and 13 families, of which 24 specimens belonging to species of fauna useful insects and 31 species belonging to the fauna harmful.

*Table 1*  
**The situation regarding the collection of entomofauna from Vasile Adamachi Iași – untreated stationary**

| Year  | Beating method     |                     |           |
|-------|--------------------|---------------------|-----------|
|       | Nr. useful species | No. harmful species | Total no. |
| 2009  | 105                | 107                 | 212       |
| 2010  | 24                 | 31                  | 55        |
| Total | 129                | 138                 | 267       |
| %     | 48,3 %             | 51,7 %              | -         |

Stationary Vasile Adamachi Iasi - treated (table 2) by collecting insects using the beating method in 2009 apple orchard, after eight harvests were collected a total of 90 copies, they belong to eight orders and 25 families. Of the 90 samples, 74 were determined to species of useful entomofauna, and 16 specimens belonging harmful wildlife.

During the observations of 2010, the chemically treated orchard, fauna collected 81 total copies of which 13 specimens belonging to species of fauna useful insects and 68 species belonging to the fauna harmful.

Table 2

**The situation regarding the collection of entomofauna from Vasile Adamachi Iași stationary**

| Year         | Beating method     |                    |            |
|--------------|--------------------|--------------------|------------|
|              | Nr. useful species | Nr. useful species | Total no.  |
| 2009         | 74                 | 16                 | 90         |
| 2010         | 13                 | 68                 | 81         |
| <b>Total</b> | <b>87</b>          | <b>84</b>          | <b>171</b> |
| %            | 50,9 %             | 49,1 %             | -          |

In the Miroslava stationary 2009 (table 3), after eight harvests were collected a total of 176 copies, of which 70 were determined as entomofauna species of useful and harmful fauna belonging to 106 copies.

In 2010 the beating method the crown of the trees during the growing season we have collected a total of 72 samples of which 57 samples belong to the species of fauna useful insects and 15 species belonging to the fauna harmful. In terms of systematically collected fauna belonging to eight orders of insects (Coleoptera, Hymenoptera, Heteroptera, Araneae, Neuroptera, Homoptera, Diptera and Orthoptera) and 13 families.

Table 3

**The situation regarding the collection of entomofauna from Miroslava Iași stationary**

| Year         | Beating method     |                    |            |
|--------------|--------------------|--------------------|------------|
|              | Nr. useful species | Nr. useful species | Total no.  |
| 2009         | 70                 | 106                | 176        |
| 2010         | 57                 | 15                 | 72         |
| <b>Total</b> | <b>129</b>         | <b>121</b>         | <b>248</b> |
| %            | 52 %               | 48,8 %             | -          |

Stationary Delești Vaslui (table 4) in 2009 have collected a number of 97 copies, 55 copies have been determined as of useful entomofauna species and 42 specimens belonging harmful wildlife.

In 2010 the number of specimens collected in the crown of trees during the growing season was 56 copies of which 11 samples belong to the insect fauna species useful and harmful fauna belonging to 45 species.

In terms of systematically collected fauna belongs to a number of 7 orders of insects (Coleoptera, Hymenoptera, Homoptera, Heteroptera, Neuroptera,

Diptera and Neuroptera) and 13 families: *Anthomyidae*, *Miridae*, *Chrysomelidae*, *Curculionidae*, *Formicidae*, *Pteromalidae*, *Aphidiidae*, *Araneidae*, *Chloropidae*, *Cecidomyidae*, *Cixiidae*, *Coccinellidae*, *Cantharidae*.

Table 4

The situation regarding the collection of entomofauna from Delești Vaslui stationary

| Year  | Beating method     |                    |           |
|-------|--------------------|--------------------|-----------|
|       | Nr. useful species | Nr. useful species | Total no. |
| 2009  | 55                 | 42                 | 97        |
| 2010  | 11                 | 45                 | 56        |
| Total | 66                 | 87                 | 153       |
| %     | 43,1 %             | 56,9 %             | -         |

## CONCLUSIONS

1. If untreated variant, the stationary V. Adamachi Iasi - untreated, the number of beetle species collected was higher, 267 copies in both years studied. Useful species represented 48.3% and 51.7% of total specimens harmful.

2. The largest number of copies the package was registered in the stationary V. Adamachi of 267 copies of the relevant species represented 51.7% of the total collected.

3. Most abundant species belonged to the following families: *Aphididae*, *Staphylinidae*, *Miridae*, *Formicidae*, and *Anthomyiidae* *Pyrrhocoridae*

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# CONTRIBUTIONS TO THE STUDY OF BEETLES ENTOMOFAUNA IN ANALYZED ORCHARDS USING THE METHOD TYPE BARBER SOIL TRAPS

## CONTRIBUȚII LA STUDIUL ENTOMOFAUNEI DE COLEOPTERE DIN CADRUL PLANTAȚIILOR POMICOLE ANALIZATE PRIN APLICAREA METODEI CAPCANELOR DE SOL DE TIP BARBER

**PĂDURARU L.B.<sup>1</sup>, TĂLMACIU M., TĂLMACIU Nela, HEREA Monica**  
e-mail: mtalmaciu@uaiasi.ro

**Abstract.** *The research was conducted in 2008-2010 using the method type Barber soil traps and observations were made in four stationary belonging to Iasi and Vaslui counties. In three of these stationaries chemical treatments were performed as follows: stationary V. Adamachi Iasi – treated, Miroslava Iasi and farm plots stationary SC Service Delești SRL Vaslui, untreated apple orchard were performed in Iasi stationary V. Adamachi - untreated. For Barber method we used plastic boxes, with a volume of 500 ml, with a diameter of 10 cm and height 8 cm, and the fixing liquid formaldehyde solution was used in concentrations of 4% in 2009 and in 2010 was used as a fixing liquid salt solution at a concentration of 25%. In studied stationaries, six traps were placed each on a row of trees at the edge inward in a straight line at a distance of 20 m from the edge and from 6 to 8 m between traps at a time.*

**Key words:** soil traps, apple orchards, stationary

**Rezumat.** *Cercetările s-au desfășurat în perioada 2008-2010 utilizându-se metoda capcanelor de sol tip Barber, iar observațiile au fost efectuate în patru staționare aparținând județelor Iași și Vaslui. La trei dintre acestea s-au efectuat tratamente chimice și anume: staționarul Vasile Adamachi Iași - tratat, staționarul Miroslava Iași și staționarului de la ferma SC Loturi Service SRL Delești Vaslui, plantațiile de măr netratate au fost în cadrul staționarului Vasile Adamachi Iași -netratat. Pentru metoda Barber s-au utilizat cutii din material plastic, cu un volum de 500 ml, cu un diametru de 10 cm și înălțimea de 8 cm, iar ca lichid de fixare s-a utilizat o soluție de formol în concentrație de 4% în anul 2009, iar în anul 2010 am utilizat ca și lichid de fixare o soluție de sare în concentrație de 25%. Recoltarea probelor s-a făcut periodic în funcție de metoda de cercetare aplicată, în cursul perioadei de vegetație, pe durata a 2 ani cât s-au desfășurat cercetările.*

**Cuvinte cheie:** capcane de sol, livezi de măr, staționare

### INTRODUCTION

The literature shows that apple trees most important species in the world, but was attacked by a large number of diseases and pests. Percher (Way, 1988 cit. Talmaciu, 2007) has compiled a list that includes no more than 80 diseases that attack this species is caused by viruses, mycoplasmosis, bacteria, 64 species of insects and mites, 8 species of nematodes and at least 3 species of rodents.

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

## MATERIAL AND METHODS

The research was conducted in 2008-2010 using the method type Barber soil traps and observations were conducted in four counties of Iasi and Vaslui belonging stationary. Three of these chemical treatments were performed as follows: stationary Vasile Adamachi Iasi – tratat, Miroslava stationary Iasi and farm plots SC Service Deleşti Vaslui SRL, untreated apple orchard were in Iasi Vasile Adamachi untreated stationary. Method Barber traps soil type was used in research in apple orchard in Iasi and Vaslui, from May to September, two years of observations from 2008 to 2009 and from 2009 to 2010. For Barber method we used plastic boxes, with a volume of 500 ml, with a diameter of 10 cm and height 8 cm, and the fixing liquid formaldehyde solution was used in concentrations of 4% in 2009 and in 2010 was used as a fixing liquid salt solution at a concentration of 25%. In studied stationaries, and six traps were placed each on a row of trees at the edge inward in a straight line at a distance of 20 m from the edge and from 6 to 8 m between traps at a time. Harvesting were made at intervals between 10 and 20 days, a total of 6 harvests in 2009, and four harvests in 2010 (Talmaciu, 2006, Barbara, 1981).

## RESULTS AND DISCUSSIONS

Stationary Vasile Adamachi Iasi - untreated (table 1) from the application method type traps Barber in 2009, we collected a total of 146 specimens of insects, of which 39 species belong copies and 107 copies useful entomofauna belong entomofaunei harmful. In this stationary did not conducted chemical treatments and species with the highest number of collected specimens were *Opatrum sabulosum* 32 copies, 17 copies *Omiias rotundatus*, *Coccinella 7punctata* 10 copies 9 copies *Brachysomus hirtus*, *Meligetes maurus* Strm and *Otiorrhynchus pinastri* with 5 and *Polydrosus flavipes* Deg. with 4 copies. In 2010 the number of specimens collected by Barber soil traps method was 25 copies of which 13 species belong copies of useful entomofauna, and 12 copies belong pest. Species with the highest number of collected specimens were *Harpalus distinguendus* 8 copies *Brachysomus echinatus*, *Harpalus tardus* and *Longitarsus tabidus* 4 copies, 3 copies *Phylotreta vittula* and with one single species collected were *Drilus concolor* and *Melighetes maurus*.

Table 1

The situation regarding collection of entomofauna from Vasile Adamachi Iasi – untreated stationary

| Year  | Barber method      |                     |           |
|-------|--------------------|---------------------|-----------|
|       | No. useful species | No. harmful species | Total no. |
| 2009  | 39                 | 107                 | 146       |
| 2010  | 13                 | 12                  | 25        |
| Total | 52                 | 119                 | 171       |
| %     | 30,4 %             | 69,6 %              | -         |

Stationary Vasile Adamachi Iasi - treated (table 2) after chemical treatment and application type using Barber traps in 2009, were collected a total of 33 species, of which 27 species belong of useful entomofauna and 6 copies belong harmful entomofauna. Species with the highest number of collected

specimens were 6 copies *Carabus coriaceus*, *Harpalus tardus* with 5, *Dermestes lanarius*, *Harpalus calceatus* Duft. and *Opatrum sabulosum* with 3 copies remaining 11 species had between 1 and 2 copies. In 2010 the number of specimens collected by Barber soil traps method was 16 copies of which 6 belong to the species of useful entomofauna copies and 10 copies belong pest. Species with the highest number of collected specimens were *Opatrum sabulosum* 4 copies, 3 copies *Harpalus distinguendus*.

Table 2

**The situation regarding the collection of entomofauna from Vasile Adamachi Iași -treated stationary**

| Year         | Barber method     |                   |           |
|--------------|-------------------|-------------------|-----------|
|              | No.useful species | No.useful species | Total no. |
| <b>2009</b>  | 27                | 6                 | 33        |
| <b>2010</b>  | 6                 | 10                | 16        |
| <b>Total</b> | 33                | 16                | 49        |
| <b>%</b>     | <b>67,4 %</b>     | <b>32,7 %</b>     | -         |

Stationary Miroslava Iasi (table 3) from the application method soil traps Barber type in 2009, due to treatments performed were collected fewer species of 45 species, of which 33 species belong copies of useful entomofauna, 12 copies belong harmful. Species with the highest number of collected specimens were *Coccinella septempunctata* with 6 copies, *Dermestes lanarius* and *Harpalus calceatus* with 5, *Carabus violaceus* and *Otiorrhynchus raucus* 4 copies.

Table 3

**The situation regarding the collection of entomofauna from Miroslava Iași stationary**

| Year         | Barber method     |                   |           |
|--------------|-------------------|-------------------|-----------|
|              | No.useful species | No.useful species | Total no. |
| <b>2009</b>  | 33                | 12                | 45        |
| <b>2010</b>  | 9                 | 4                 | 13        |
| <b>Total</b> | 42                | 16                | 58        |
| <b>%</b>     | <b>72,4 %</b>     | <b>27,6 %</b>     | -         |

In 2010 the number of specimens collected by type Barber soil traps method was much smaller, 13 copies of the nine samples belonging to the species of useful entomofauna and harmful species belonging to four copies. Species with the largest number of specimens collected are: *Calathus fuscipes* and *Harpalus distinguendus* 3 copies.

Stationary Delești Vaslui (table 4) from the application Barber method soil traps in 2009 we collected a total of 347, 310 copies of which belong to the useful entomofauna and and 37 specimens belonging to harmful. Species with the highest number of collected specimens were *Harpalus tardus* cu 88 copies,

*Harpalus calceatus* with 61 copies, *Dermestes lanarius* with 52 copies, *Harpalus distinguendus* with 46 copies and *Harpalus rufipes* *Pseudophonus aeneus* and 12 copies. In 2010 the number of samples collected was 170 of which 161 specimens belonging to useful entomofauna and 9 harmful. Species with the largest number of specimens collected were genus *Harpalus*: *Harpalus distinguendus* 34 copies, *Harpalus tardus*, *Harpalus aeneus* 31 copies and 22 copies. The species *Calathus fuscipes*, and *Harpalus griseus* were harvested 14 and 10 copies. *Harpalus calceatus* and *Amara similaris* species presented at harvest 9 and respectively 8.

Table 4

The situation regarding the collection of entomofauna from Delești Vaslui stationary

| Year  | Barber method     |                   |           |
|-------|-------------------|-------------------|-----------|
|       | No.useful species | No.useful species | Total no. |
| 2009  | 310               | 37                | 347       |
| 2010  | 161               | 9                 | 170       |
| Total | 471               | 46                | 517       |
| %     | 91,1 %            | 8,9 %             | -         |

## CONCLUSIONS

1. If untreated variant, the stationary V. Adamachi Iasi - untreated, the number of beetle species collected was higher, 171 copies in both years studied. Useful species represented 30.4% and 69.6% harmful of total.

2. Highest number of species package was registered in the stationary Delești-Vaslui, 517 copies of the relevant species represented 91.1% of the total.

3. Most abundant species were *Harpalus calceatus*, *Harpalus tardus*, *Dermestes lanarius*, *Pseudophonus rufipes*, *Coccinella septempunctata*, *Carabus violaceus* and *Otiorrhynchus raucus*.

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# HEAVY METALS IN SOILS FROM THE HORTICULTURAL AREAS OF SÂRCA AND HUȘI (ROMANIA)

## METALELE GRELE DIN SOLURILE UNOR AREALE HORTICOLE: SÂRCA ȘI HUȘI (ROMÂNIA)

HUZUM Ramona<sup>1</sup>, PRUNDEANU I. M.<sup>1</sup>, IANCU O.G.<sup>1</sup>

e-mail: ramona.huzum@gmail.com

**Abstract:** *The present study aims at determining the geochemical contents of seven heavy metals (Cd, Co, Cr, Cu, Ni, Pb, and Zn) in soils (haplic and calcareo-calcic chernozems) from the Sârca apple orchard and the Huși vineyard area. The heavy metal contents were established using the AAS and EDXRF methods, the average values being between 0.12-0.88 mg/kg Cd, 11-12 mg/kg Co, 14-128 mg/kg Cr, 26-61 mg/kg Cu, 37-41 mg/kg Ni, 21 mg/kg Pb, and 67-71 mg/kg Zn. Measurements of the pH and the carbonate contents were also recorded, the dominant soil reaction being neutral to weakly alkaline, with an average content of 1.16% CaCO<sub>3</sub> for the soil sampled from the Sârca area, and 5.27% CaCO<sub>3</sub> for the Huși vineyard area.*

**Keywords:** heavy metals, AAS, ICP-MS, vineyard, apple orchard, Sârca, Huși.

**Rezumat** În cadrul acestui studiu au fost analizate concentrațiile a șapte elemente, Cd, Co, Cr, Cu, Ni, Pb, Zn din solurile utilizate în horticultură (cernoziomuri cambice și calcarice) din cadrul bazinului pomicol Sârca și arealului viticol Huși. Conținuturile de metale grele au fost determinate prin metodele AAS și EDXRF obținându-se valori medii între 0.12 -0.88 mg/kg Cd, 11-12 mg/kg Co, 14-128 mg/kg Cr, 26-61 mg/kg Cu, 37-41 mg/kg Ni, 21 mg/kg Pb și 67-71 mg/kg Zn. De asemenea, a fost determinat pH-ul și % de carbonați din sol, reacția predominantă a solului fiind neutră spre slab-alkalină cu un conținut mediu de 1.16% CaCO<sub>3</sub> pentru probele de la Sârca și ceva mai ridicat (5.27% CaCO<sub>3</sub>) pentru solurile viticole de la Huși.

**Cuvinte cheie:** metale grele, AAS, ICP-MS, plantație viticolă, livadă de meri, Sârca, Huși.

## INTRODUCTION

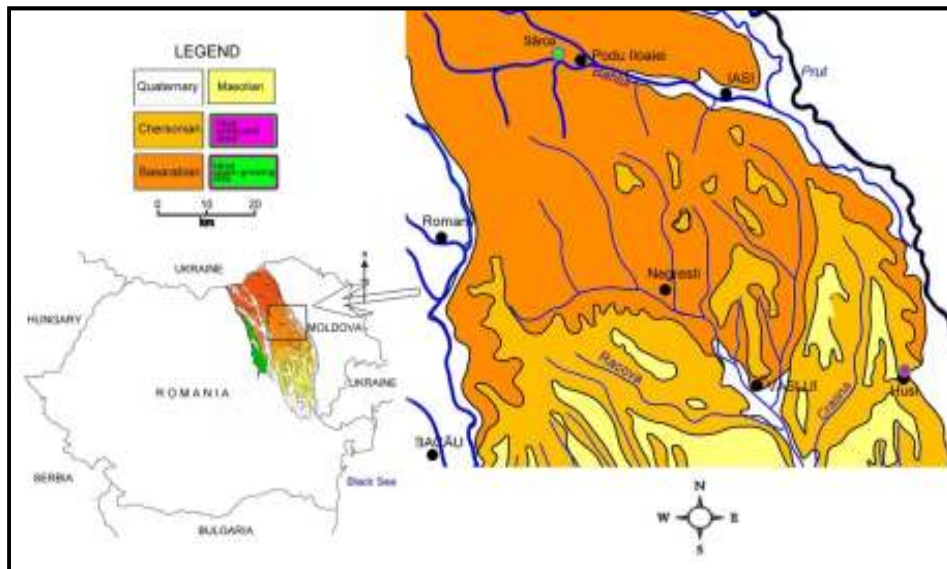
Heavy metals are chemical elements with a specific weight higher than 5 g/cm<sup>3</sup> and an easily changing oxidation state, and which form hard-to-dissolve hydroxides and other chemical compounds with affinity for sulfides. Soil fertility and its pollution are contributing to an increase in the number of studies dealing with heavy metals in relation to their mobility as influenced by pedogenetic factors. The heavy metals in soils have sources that are either natural, deriving from the parental materials from which the soils formed and evolved, or anthropogenic, caused by industry and agricultural practices (Lăcătușu, 2000). In agriculture, the crop yields are influenced mainly by the fertility of the soil.

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<sup>1</sup>“Alexandru Ioan Cuza” University of Iași, Romania

The Sârca apple-growing area is located in the south-west of the Jijia hilly plain, at the boundary with the Iași Coast, being delimited by the Oilor Valley to the north, the Bahluiet Valley to the south, the Hârtop and Fandolica valleys to the west, and by the confluence of the Valea Oilor brook with the Bahluiet River to the east (Boronia, 2010).

The Huși area is located in the eastern part of Vaslui County, in the south-eastern part of the Huși Depression. Geologically, the studied perimeters belong to the Moldavian Platform, which is part of the East-European Platform. The Sârca apple-growing area is located in the central-eastern part, while the Huși vineyard area is located in the east of the Moldavian Platform. The lithology of the Sârca apple-growing area is represented by pelite deposits corresponding to the neritic lithofacies (clays with *Cryptomacra*) (Ionesi et al., 1996). Quaternary deposits consist of recent alluviums and terraces as results of a hydrographical network (Boronia, 2009).



**Fig. 1** – Locations of the studied areas on the geological map of the Moldavian Platform (modified after Ionesi et al., 2005)

The soil samples from the Sârca apple-growing area were collected from the surface of Farm no. 6, on which haplic and calcaro-calcic chernozems are found. In the Huși vineyard area, the sedimentary cover is represented by sand with sandstones layers, sometimes with oolitic texture, and argillaceous sands of Chersonian age. The soil type from the vineyard sampling area of Farm no 16, the Galbena Hill, is calcic chernozem (OJSPA Vaslui, 2006).

## MATERIAL AND METHODS

The soil sampling was carried out using telescoping augers. Samples with weights between 1.5-2 kg were stored in zip-locked bags, clearly marked with the

corresponding identification tag. A soil sample was composed of 5 sub-samples, resembling a square, collected from 4 points one meter away from the center.

From the Sârca apple-growing area, 12 topsoil (0-20 cm) samples were collected from points located in the middle of the apple tree rows, on a sampling grid of 300 m. In the Huși area, the soil was sampled using a 50 m grid, 14 samples being collected from the grape-vine rows and inter-rows, at depths of 0-20 cm.

After drying and the removal of vegetal parts, the samples were ground and sieved through 1 mm mesh. A 1:5 ratio mixture of soil and resin was used to obtain the pressed powder pellets.

The soil and resin mixture was homogenized for 10-12 minutes at 180 rpm, using an agate ball mill. After mixing, 9 g weighted in aluminum capsules were kept for 30 seconds under a 20 t/cm<sup>2</sup> pressure. The pressed pellets were analyzed using an Epsilon-5-type energy-dispersive XRF spectrometer (EDXRF), within the Geology Department of the "Al. I. Cuza" University of Iași.

The total contents of heavy metals from the Sârca apple-growing area were obtained using flame atomic absorption spectrometry (AAS) at the National Institute of Research and Development for Pedology, Agrochemistry and Environment Protection, Bucharest, after sample digestion, in an open system, with a mixture of HNO<sub>3</sub> and HClO<sub>4</sub>.

For all 26 samples, the pH measurements were performed using the potentiometer method, in aqueous solution, with a 2.5:1 water:soil ratio. The carbonate percentages were determined using a Bernard calcimeter.

## RESULTS AND DISCUSSION

In order to obtain the statistical parameters (minimum, maximum, mean, standard deviation, median, and geometric mean), the Statistica 8 software was used (tab. 1). Central statistical parameters (mean, median and geometric mean) are representative for the sets of data which have similar values centered between minimum and maximum limits. This suggests a normal distribution of the two data sets (Reimann et al., 2008).

Cd concentrations range between 0.09-1.88 mg/kg for the samples collected from Sârca (S), while for the samples from the Huși vineyard (H) the range is narrower: 0.06-0.20 mg/kg.

For Co, the contents display similar values for the studied areas, with small variations between 10-13 mg/kg (S), and 11-12 mg/kg (H), respectively.

Greater differences between the two areas regard Cr values: 11-17 mg/kg for (S) and 112-158 mg/kg for (H). This gap is not mainly due to a natural and/or anthropogenic influence. Instead, it is more likely an effect of the two different methods used for concentration measurements and the possible interferences during the EDXRF analyses.

The situation is similar for the Cu contents, where the 19-33 mg/kg range for (S) is clearly below that for (H): 34-104 mg/kg. The differences can be explained by the intensive Cu-based treatments applied to vineyard soils (Ca(OH)<sub>2</sub>+CuSO<sub>4</sub> – Bordeaux mixture).

Table 1

Statistical parameters of heavy metals contents, pH and CaCO<sub>3</sub>

| Statistical parameter | Location                        | Cd    | Co   | Cr   | Cu   | Ni   | Pb   | Zn   | pH   | CaCO <sub>3</sub> |
|-----------------------|---------------------------------|-------|------|------|------|------|------|------|------|-------------------|
|                       |                                 | mg/kg |      |      |      |      |      |      |      |                   |
| min                   | Sârca apple-growing area (n=12) | 0.09  | 10   | 11   | 19   | 31   | 10   | 60   | 6.51 | 0.56              |
| max                   |                                 | 1.88  | 13   | 17   | 33   | 51   | 35   | 88   | 8.04 | 2.52              |
| mean                  |                                 | 0.88  | 11   | 14   | 26   | 37   | 21   | 71   | 7.25 | 1.16              |
| $\sigma$              |                                 | 0.60  | 0.90 | 1.93 | 4.22 | 5.59 | 6.52 | 7.78 | 0.44 | 0.52              |
| G                     |                                 | 0.64  | 11   | 14   | 26   | 37   | 20   | 71   | 7.24 | 1.07              |
| Me                    |                                 | 0.91  | 11   | 14   | 26   | 36   | 21   | 71   | 7.20 | 1.09              |
| min                   | Huși vineyard area (n=14)       | 0.06  | 11   | 112  | 34   | 37   | 19   | 63   | 7.73 | 1.02              |
| max                   |                                 | 0.20  | 12   | 158  | 104  | 43   | 22   | 77   | 8.35 | 10.1              |
| mean                  |                                 | 0.12  | 12   | 128  | 61   | 41   | 21   | 67   | 8.09 | 5.27              |
| $\sigma$              |                                 | 0.05  | 0.47 | 11.8 | 20.7 | 1.72 | 1.14 | 3.66 | 0.14 | 2.81              |
| G                     |                                 | 0.11  | 12   | 128  | 57   | 41   | 21   | 67   | 8.09 | 4.44              |
| Me                    |                                 | 0.13  | 12   | 127  | 59   | 41   | 20   | 67   | 8.12 | 5.05              |
| NV*                   |                                 | 1.00  | 15   | 30   | 20   | 20   | 20   | 100  |      |                   |
| AT*                   |                                 | 3.00  | 30   | 100  | 100  | 75   | 50   | 300  |      |                   |

$x_{\min}$ - minimum,  $x_{\max}$  – maximum,  $\sigma$  – standard deviation, G – geometric mean, Me – mean, NV – normal value in soil, AT – alert threshold for soil with sensitive use, \* - Order no 756/1997 emitted by the Romanian Ministry of Waters, Forests and Environmental Protection.

No significant differences arise in the case of the Ni concentrations, which range between 31-51 mg/kg for (S), and 37-43 mg/kg for (H).

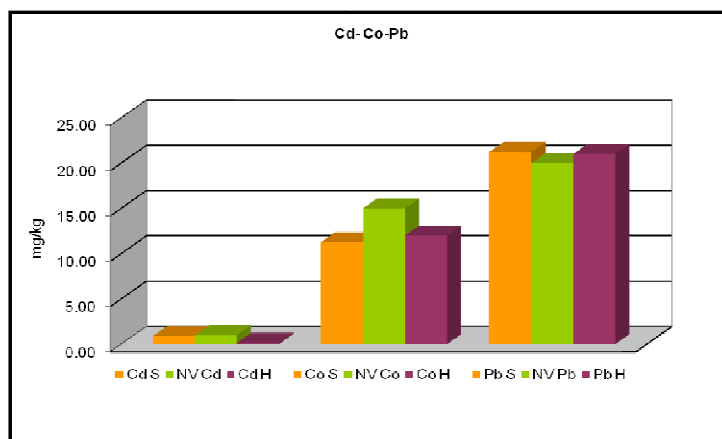
The comparison of the two sets of data yields only slight variations in the case of Pb. However, the range is wider for (S): 10-35 mg/kg than for (H): 19-22 mg/kg.

The same is valid for Zn, which displays a wider range of 60-88 mg/kg for (S), and a narrower one, of 63-77 mg/kg, for (H).

The sequence order for the mean contents for the two studied areas is the following: Cd<Co<Cr<Cu<Pb<Ni<Zn for (S), and Cd<Co<Pb<Ni<Zn<Cu<Cr for (H). The pH values describe a neutral soil reaction, with a mean content of 1.16 % CaCO<sub>3</sub>, for (S), and a weakly alkaline one, with an average of 5.27 % CaCO<sub>3</sub>, for (H).

The mean values of Cd are 0.88 mg/kg for (S), and 0.12 mg/kg for (H), both under the normal reference value in soil, of 1 mg/kg (Fig.3). For Co, the normal reference value in soils is 15 mg/kg (Order no 756/1997), while the mean Co concentrations obtained are under this threshold (11 mg/kg for (S), 12 mg/kg for (H)).

The Pb mean of 21 mg/kg is equal for both sets of data, slightly higher than the normal reference value in Romanian legislative acts. By comparing it with an average continental crust of 11 mg/kg (Rudnick and Gao, 2003), one notices an enrichment in the case of the soils studied (fig. 2).

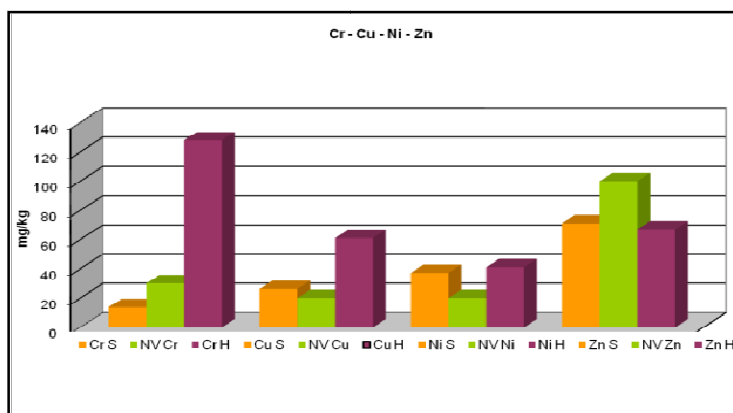


**Fig. 2** – The distribution of Cd, Co and Pb in the Sârca and Huși areas, compared to the normal reference values in soils (Order no 756/1997). S – Sârca fruit-growing area, H – Huși vineyard area, NV – normal values in soils.

The mean contents obtained for Cu are 26 mg/kg for (S) and 61 mg/kg (H), both over the normal reference value (20 mg/kg). Given the differences in the application of Cu-based fungicides, an increase in the Cu concentrations occurs in the case of vineyard soils, where the maximum value exceeds the alert threshold for soil with sensitive use (100 mg/kg).

Due to the two different analysis methods, the Cr contents for the Huși area should be treated carefully when considering a comparison with the Romanian legislation. Regarding the (S) area, the mean value of 14 mg/kg is lower than the normal limit in soil.

The mean values of Zn and Ni are similar for both sets of data: 71 mg/kg (S) - 67 mg/kg (H) Zn and 37 mg/kg (S) - 41 mg/kg (H) Ni, the normal limit in soil being exceeded only by Ni (fig. 3).



**Fig. 3** – The distribution of Cr, Cu, Ni and Zn in the Sârca and Huși areas, compared to the normal reference values in soils (Order no 756/1997). S – Sârca fruit-growing area, H – Huși vineyard area, NV – normal values in soils.

The present study presents the contents of seven heavy metals (Cd, Co, Cr, Cu, Ni, Pb and Zn) in the topsoils from the Sârca apple-growing (Farm no. 6) area and the Huși vineyard area (Farm no. 16).

Although two different analysis methods were used, namely AAS and EDXRF, similar results were obtained, the only difference being registered in the case of Cr.

## CONCLUSIONS

The average Cu concentrations are over the normal reference limit provided by national regulations. An increased accumulation can be observed for the soils sampled from the Huși vineyard area, caused by intensive foliar-spray treatments. The chernozems from the two studied areas display similar concentrations for Cd, Co, Ni, Pb and Zn. Only Ni and Pb exceed the normal limit in soils. The soil reaction ranges from neutral to weakly alkaline, with a mean CaCO<sub>3</sub> content of 1.16 – 5.27%.

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# COMPOSTING OF URBAN SLUDGE BY MIXING WITH PLANT RESIDUES

## COMPOSTAREA NĂMOLURILOR URBANE PRIN AMESTECAREA CU RESTURI VEGETALE

**PĂTRUȘCĂ (CEPOI) Daniela Alina<sup>1</sup>, POPA D.<sup>1</sup>, PĂTROESCU V.<sup>2</sup>**  
e-mail: cepoialinadaniela@yahoo.com

**Abstract.** Sludge arising from municipal treatment plants is a valuable source of trace elements and N, P, K for plants, while improving physical and chemical properties of soil. The purpose of this study was to evaluate the possibility of composting biodegradable waste through anaerobic digestion mixed with plant residues. The results showed the suitability of applying the composting process from a chemical point of view. The study highlights the environmental impact of compost derived from sewage mixed with vegetable scraps that leads to improved resource utilization, conservation and reduction of pollutants.

**Keywords:** organic fertilizer, anaerobic digestion, aerobic composting

**Rezumat:** Nămolurile de la stațiile de epurare municipale constituie o sursă valoroasă de oligoelemente și de N, P, K pentru plante, totodată contribuind și la îmbunătățirea proprietăților fizice și chimice ale solului. Scopul acestui studiu a fost de a evalua posibilitatea de compostare a deșeurilor biodegradabile, prin digestie anaerobă amestecat cu reziduuri de plante. Rezultatele au aratat sustenabilitatea aplicării procesului de compostare din punct de vedere chimic. Studiul subliniază impactul asupra mediului prin utilizarea compostului rezultat din canalizare amestecat cu resturi vegetale, care duce la îmbunătățirea resurselor de utilizare, conservare și de reducere a poluanților.

**Cuvinte cheie:** fertilizare organică, digestie anaerobă, compostare aerobă

### INTRODUCTION

In accordance with European Council Directive on the landfill of waste (European Council Directive, 1999), Member States are required to reduce gradually the amount of biodegradable municipal waste.

Currently, the major methods of waste management are: a) recycling (recovery) of product materials having been used by consumers, b) a composting process aerobic biological degradation of biodegradable organic matter, c) wastewater-a process of treating raw sewage that produces a non-toxic liquid effluent is discharged to rivers or sea, and a semi-product (sludge), which is used to change soil properties, d) burning a combustion process designed to recover energy and reduce the amount of waste that must be eliminated (Domingo et al., 2009).

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<sup>1</sup> University of Craiova, Romania

<sup>2</sup> National Research and Development Institute for Industrial Ecology Bucharest, Romania

Biodegradable waste is not suitable for incineration because of their high water content and creates problems when put in landfills (emanation of gas and leachates (Befa, T. 2002). A promising solution to use biological waste is municipal sewage sludge fermentation. Distribution of treated sludge improves soil agrochemical characteristics.

Should be noted that sludge can contain organic pollutants, inorganic and biological, commercial and industrial establishments and add or compounds formed during the various water treatments (Dominica Del Mundo Dacera et al., 2009).

The purpose was to estimate, harness and label waste compost made from municipal waste mixed with vegetable waste, by studying chemical parameters.

## **MATERIAL AND METHODS**

The fermented sludge samples (obtained by mesophilic anaerobic fermentation at 320 C) and composting (biodegradable vegetable waste resulting from the mixture, represented by leaves, grass, straw 30% residual sludge - sludge mixed with primary sludge fermented biological anaerobic and dehydrated in 70%) were taken and analyzed in the Laboratory Institute of Technology INCD ECOIND Bucharest. The analytical methods used were the standard in Romania. During the experimental periods (28.06.2010-06.07.2010 - fermentation and 08.07.2010-03.09.2010 - for composting) were analyzed fermented and composted sludge properties in accordance with rules laid down in Government Decision 708/2005-the use of the sludge of wastewater treatment plants in agriculture as fertilizer.

Compost from the effluent treatment plant was used as fertilizer, as previously sterilized and stabilized by anaerobic fermentation process.

Quality indicators used to assess anaerobic digestion process and to determine the efficiency of composting determination were determined separately for the samples of sludge and compost.

For samples of sludge were used to determine the pH, moisture, volatile matter, ammonium (NH<sub>4</sub><sup>+</sup>), total Kjeldahl nitrogen (NTK), total phosphorus (P total), sodium (Na), potassium (K), calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>), total iron (Fe total), manganese (Mn), lead (Pb), cadmium (Cd), total chromium (total Cr), copper (Cu), nickel (Ni), zinc (Zn), mercury (Hg), and, compost samples were monitored by following indicators: pH, total organic carbon (TOC), total nitrogen (N total), total phosphorus (P total), the ratio carbon / nitrogen ( C / N), sodium (Na), calcium (Ca<sup>2+</sup>), magnesium (Mg<sup>2+</sup>), lead (Pb), cadmium (Cd), total chromium (Cr total), copper (Cu), nickel (Ni), zinc (Zn), cobalt (Co).

For analysis of fermented sludge from 28.06.2010 - 07.06.2010 have been taken equal instantaneous samples every 8 hours.

- pH was determined according to ISO 10390-99 with a pH meter.  
- Humidity (amount of water determined by the difference between the mass of the sludge before and after drying at 105 o C) was determined in the oven according to EN 12880/2002.

- Dry matter (mass of sludge remaining after evaporation and drying at 105o C) was determined the oven, under ISO 11465-98.



- Volatile substance (amount of dry mass of sludge that is released as volatile as its calcination at 550oC) was determined in the furnace, according to EN 12879/2002.

- Heavy metals (Pb, Cd, Cu, Ni, Zn) were determined according to ISO 8288-01 with atomic absorption spectrophotometer.

- Total nitrogen determined by Kjeldahl method according to ISO 11261-00 take it 0.5 g dry sludge at 105 0 C which is transferred into a digestion tube and aims to determine nitrogen in the form of organic compounds found in sludge fermented by mineralization of the sample with concentrated sulfuric acid in the presence of catalyst and then subjected to alkaline distillation, ammonia released is absorbed in a boric acid solution and titrated with sulfuric acid.

- Total phosphorus was determined according to STAS 7184/14-79. Method for determination of phosphorus in sludge is fermented dry sludge sample in an oven at 1050C until constant mass which is then mineralized by dry or wet to convert various forms of phosphorus in orthophosphate. Orthophosphate ion is converted fosfomolibdenic complex is reduced with ascorbic acid to a blue complex. Absorbance of the blue complex formed is proportional to the concentration of phosphorus and measured photometrically by the spectrometer.

Of dry compost sample in drying oven at 750 C and ground through a sieve with pore size of 2 mm, measurements were made on pH, total N and heavy metals:

- pH was determined using a pH meter of compost aqueous extract prepared by mixing compost with distilled water at a rate of 1/10. The suspension was separated by centrifugation and the supernatant filtered through filter paper was analyzed. pH close to neutral and the final report C / N gives full mature compost, strengthening agronomic quality compost produced from municipal waste by anaerobic fermentation process.

- Total N was determined using a distillation apparatus by the Kjeldahl method.

- Heavy metals were determined by inductively coupled plasma spectrometer with optical emission (ICP-OES).

## RESULTS AND DISCUSSION

The results obtained on the physico-chemical properties of fermented sludge obtained from the Waste water municipal treatment plant Pitesti and compost made from urban waste organic sludge mixed with vegetable waste (leaves, grass, straw) are presented in tables 1 and 2.

*Table 1*

**Physical-chemical properties of fermented sludge**

| <b>Crt. no.</b> | <b>Indicator</b>                        | <b>Value</b> |
|-----------------|---|--------------|
| 1.              | pH                                      | 7,23         |
| 2.              | Humidity %                              | 93,46        |
| 3.              | Volatile substance % from d.m.          | 51,7         |
| 4.              | NH <sub>4</sub> <sup>+</sup> mg/kg d.m. | 924          |
| 5.              | NTK mg/kg d.m.                          | 22316        |
| 6.              | P total mg/kg d.m.                      | 1027         |
| 7.              | Na mg/kg d.m.                           | 2896         |

|     |                     |        |
|-----|---------------------|--------|
| 8.  | K mg/kg d.m.        | 3814   |
| 9.  | Ca mg/kg d.m.       | 12263  |
| 10. | Fe total mg/kg d.m. | 12392  |
| 11. | Mn mg/kg d.m.       | 2213   |
| 12. | Pb mg/kg d.m.       | 98,1   |
| 13. | Cd mg/kg d.m.       | 6,2    |
| 14. | Cr total mg/kg d.m. | 67,5   |
| 15. | Cu mg/kg d.m.       | 146,7  |
| 16. | Ni mg/kg d.m.       | 72,8   |
| 17. | Zn mg/kg d.m.       | 1408,6 |

Content of heavy metal ions from the fermented sludge falls into the maximum concentration limits established by Order 708/2004 on environmental protection and especially when we use sewage sludge in agriculture: Cd = 6.2 mg / kg DM concentration maximum accepted from of 10 mg / kg DM, with = 146.7 mg / kg DM concentration maximum accepted from of 500 mg / kg DM, Ni = 72.8 mg / kg DM concentration maximum accepted from of 100 mg / kg DM, Pb = 98.1 mg / kg DM concentration maximum accepted from of 300 mg / kg DM, Zn = 1408.6 mg / kg DM concentration maximum accepted from of de2000 mg / kg DM, Cr = 67.5 mg / kg DM concentration maximum accepted from of 500 mg / kg DM

Table 2

**Chemical composition of the final compost produced from municipal sewage sludge**

| Crt. no. | Parameter      | Unit of measurement | Value |
|----------|----------------|---------------------|-------|
| 1.       | pH             |                     | 7,33  |
| 2.       | TOC            | % from d.m.         | 21,14 |
| 3.       | N <sub>T</sub> | % from d.m.         | 1,73  |
| 4.       | C/N            | % from d.m.         | 12,21 |
| 5.       | P <sub>T</sub> | % from d.m.         | 0,25  |
| 6.       | Ca             | % from d.m.         | 0,07  |
| 7.       | Mg             | % from d.m.         | 0,65  |
| 8.       | Na             | % from d.m.         | 0,22  |
| 9.       | Cu             | mg/kg d.m.          | 84,1  |
| 10.      | Zn             | mg/kg d.m.          | 358,2 |
| 11.      | Co             | mg/kg d.m.          | 12,6  |
| 12.      | Pb             | mg/kg d.m.          | 128,1 |
| 13.      | Ni             | mg/kg d.m.          | 53,4  |
| 14.      | Cr             | mg/kg d.m.          | 19,4  |
| 15.      | Cr total       | mg/kg d.m.          | 324   |
| 16.      | Cd             | mg/kg d.w.          | 2,0   |

By anaerobic digestion there is a reduction in the proportion of organic matter content of 45-55%. We can consider that the pH value, due to its almost neutral (7.33) reflects its maturation, unlike the immature compost that

has an acidic pH and makes the material product - compost made from municipal sewage sludge - have a potential character inhibitor of the movement of heavy metals in soil and their uptake by plants.

In composted sludge heavy metal concentration is reduced by following three percentages:

- Cd to 6.2 mg / kg DM in fermented sludge reached in compost to 2.0 mg/kg DM, so is reduced by 66.66%;

- Ni at 72.8 mg / kg DM in fermented sludge reached in compost to 53.4 mg/kg DM, reduced by 26.64%;

- With the 146 mg / kg DM in fermented sludge reached in compost 84.1 mg/kg DM, reduced by 42.39%;

- Pb from 98.1 mg / kg DM in fermented sludge reached in compost 128.1 mg/kg DM, so 30.58% increase in compost

- From 1408.6 mg Zn / kg DM in fermented sludge reached in compost 358.2 mg/kg DM, reduced by 74.57%;

- From 67.5 mg Cr / kg DM in fermented sludge reached in compost to 19.4 mg/kg DM, reduced by 71.25%.

## CONCLUSIONS

1. Anaerobic digestion of sludge leads to a removal of the organic matter of 50%. Sludge treated by anaerobic digestion and composted with vegetable residues enables subsequent use as agricultural fertilizers.

2. C / N ratio of 12.21% is a possible recommendation of compost as organic biofertilizers.

3. Since heavy metals in sludge treatment plants are among the decisive factors for use in making compost, compost as fertilizer recommendation is possible in crops without soil pollution risk crops.

4. The content of heavy metals (Cr, Zn, Cd, Cu, Pb) of compost examined falls within the legal limits allowed and are therefore appropriate in terms of environmental protection in order to give an eco-label.

5. The finished compost provides an accurate picture of anaerobic digestion efficiency and quality of final product (compost), increasing the agronomic quality of compost made from municipal waste by anaerobic fermentation process.

6. The data presented are part of a comprehensive study aimed at applying sludge under anaerobic fermentation of compost as flower species.

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# GREEN ROOF OR ROOF GARDEN?

## ACOPERIȘ VERDE SAU ACOPERIȘ GRĂDINĂ?

ANDRIESCU Ioana<sup>1</sup>, CANTOR Maria<sup>1</sup>, DAN V.<sup>1</sup>, HORT Denisa<sup>1</sup>

e-mail: ioanaandriescu@yahoo.com

**Abstract.** Far from being news to civilized countries, green roofs are slowly starting to spread in Romania as well. Vegetation up high becomes a necessity because of the spread of built areas, which diminishes green areas. This can be a solution in the attempt to compensate for the loss caused by the inevitable urban development. There are two concepts available, "the green roof" and the "roof garden". There are several differences between the two, such as place and placement mode, the type of system chosen and adequate vegetation for each. This paper will analyze these two concepts, ending with a set of conclusions and recommendations concerning the purpose and the benefits of each.

**Key words:** ecoroof, landscape over structure, extensive system, intensive system, substrate, rehabilitation

**Rezumat.** Departe de a fi o noutate pentru țările civilizate, înverzirea acoperișurilor își găsește încet locul și în România. Extinderea zonelor construite în detrimentul spațiilor verzi face ca prezența vegetației pe clădiri să devină o necesitate. Se poate astfel compensa ceea ce se pierde prin inevitabila dezvoltare urbană. În acest sens există două concepte, cel de acoperiș verde și cel de acoperiș grădină. Acestea se diferențiază prin mai multe aspecte, printre care locul și modul de realizare, tipul de sistem ales și vegetația care se pretează pentru fiecare în parte. În acest articol se va realiza o analiză a celor două concepte, prezentând în final concluzii și recomandări referitoare la rolul fiecăruia și la beneficiile aduse de acestea mediului înconjurător.

**Cuvinte cheie:** acoperiș înverzit, sistem extensiv, sistem intensiv, substrat, reabilitare

## INTRODUCTION

The idea to grow vegetation on a roof is not contemporary. Information on such a vegetation cover dates back to Ancient times. The first examples come from Mesopotamia, the well-known "hanging gardens" of Babylon, roofs with luxuriant vegetation and vegetative terraces (Velazquez, 2005). In extreme climate countries, either very cold or excessively hot, traditional housing with vegetative rooftops was chosen hundreds of years ago in order to regulate or keep constant interior temperature. In Iceland, for instance, traditional houses of the sodhouse-type were made out of peat. Their roof was covered with grass and during wintertime no other heating was required besides human-generated heat. The roof of these houses was made out of two

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<sup>1</sup> University of Agricultural Science and Veterinary Medicine of Cluj-Napoca, Romania

or three layers of peat laid on tree branches and twigs and covered with a thick layer of grass patches (Minke G., 2010).

An interesting example that clearly influenced the development and spreading of modern vegetative roofs can be found in 19<sup>th</sup> century Berlin. It was then that the so-called *holzzementdach* were placed upon four-store buildings with flat roofs towards an interior backyard. Wooden tar was used in between the card box layers in order to ensure sealing. This layer was covered with gravel and clay for fire resistance. Spontaneous vegetation usually developed onto these roofs. (Minke, 2010).

Complex vegetative systems were implemented in many regions over the time, with various aims. In Germany, the country that laid the foundations of the modern vegetative system and related legislation, the main aims were environmental concerns and the fight against the loss of green areas caused by the expansion of the built-up areas.

In North America, the aims were related to economic efficiency. In Norway, these initiatives tried to re-establish a link between nature and traditional buildings and to ensure the latter's integration in the landscape.

The United Kingdom also started to use the potential of the new technology (Dunnett and Kingsbury, 2008).

This concept is relatively new for Romania and it comes at a favourable time, as architects focus more and more on saving energy and protecting the environment. It can be seen as an attempt to regain the green areas which were lost in the wake of the chaotic urbanisation process during the last few years.

Using this type of vegetative roof for new buildings or existing ones contributes to a rational land use. It also compensates for the loss of green areas due to the expansion of urban built-up areas. Vegetative roofs are not only pleasant to look at, they also create valuable biotopes, improve air quality and have important physical and building-related advantages. 10 to 20% of the total roof area should be covered with vegetation in order to ensure a healthy climate in urban areas. Supposing that one third of the total urban area is covered by buildings and another third by streets and squares, only another third is covered by green areas. Therefore, if every fifth or tenth roof would be covered by vegetation, then urban green areas would double (Minke, 2010).

## MATERIAL AND METHOD

What is a vegetative roof? The concept refers to an entire system which uses vegetation and its growing media in order to improve the performances of a supporting structure (Snodgrass and McIntyre, 2010). There are two types of concepts, defined by the degree of vegetative insertion, namely the **green roof**, an extensive system, and the **roof garden**, an intensive one.

The present paper is a comparative analysis of the two vegetative systems, mainly based on references to existing studies. According to their structure and their role, they both contribute to the development and rehabilitation of the built-up areas and therefore to improving life quality.

## RESULTS AND DISCUSSION

Vegetative roofs can be planted on any type of roof, both on new buildings and existing ones, irrespective of the height and type of supporting structures, flat or sloping. In order to choose the optimal system for each particular case, we have to consider the structural, climatic, and usage restrictions, as well as the aims of the greening process.

The concept of **eco-roof**, **green roof** or **living green roof** implies an **extensive greening system** and it is a modern adaptation of the roof garden. Its benefits are mainly functional, namely to protect the waterproofing layer, by sealing and endurance, and also to retain some of the rainfall water. The reduced width of the substrate imposes a limited variety of plants, which entails a simple design, a lack of flexibility and a bland landscape. This aspect might be compensated by the fact that different species and types of plants can be used in order to bring in variety of texture and colour. The weight of the whole system is relatively low and does not involve a massive supporting structure, or additional reinforcement in the case of existing buildings. The maintenance of the extensive system is not expensive and permanent access during exploitation is not required. The implementation costs are low and the system is suited for any type of roof, be it flat (fig. 1) or sloping (fig. 2).



**Fig. 1** - Extensive green roof – flat supporting structure  
([www.greenroofsolutions.com](http://www.greenroofsolutions.com))



**Fig. 2** - Extensive green roof – sloping supporting structure  
([www.greenroofromania.blogspot.com](http://www.greenroofromania.blogspot.com))

Germany is the country with the most advanced implementation of green roofs. Around 14% out of the total flat roofs (13.5 million square meters) were covered in vegetation in 2001 (Snodgrass and Snodgrass, 2010). The extensive system was used in 80% of cases (Carter and Butler, 2008), proving that the cost-benefit ratio favours simple, extensive green roofs.

**Landscape over structure** or **roof garden** imposes an **intensive vegetative system** which is suitable only for flat supporting structures. The benefits of this system are comparable to those of a proper green area at pavement level, plus the benefits of an extensive green roof. The thickness of the substrate allows for a large variety of plants and better thermal and phonic insulation, plus better results in retaining runoff. The landscape offered is more attractive, as the green areas can be organized in a more flexible and

diverse way (fig. 3). The advantages for the environment are also more significant in comparison to the extensive system. Nonetheless, the intensive system is implemented on a lower scale because it is expensive, needing constant maintenance and irrigation. Moreover, its implementation is more difficult because it weighs more and it adds an extra load to the building resistance structure. This type of system is used for roofs which are accessible to people, which means ensuring easy access and additional protection measures, such as parapets and protection guardrail (fig. 4).



**Fig. 3** - Intensive roof garden  
([www.naturalpatriot.org](http://www.naturalpatriot.org))



**Fig. 4** - Intensive roof garden  
([www.optigreen.com](http://www.optigreen.com))

The structure of a vegetative roof includes the same elements, irrespective of the system chosen: supporting structure, waterproofing membrane, root barrier, drainage panel, filter fabric, substrate (growing media) and vegetation.

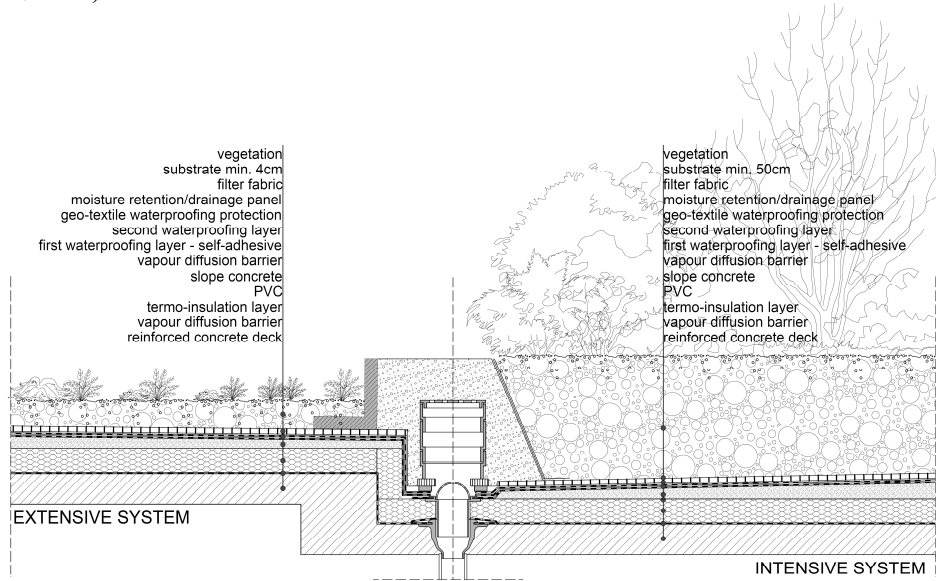
When designing any type of vegetative roof we need to consider the following factors: the climate of the region, local microclimate, annual rainfall pattern, drought periods, frosting periods, direction and speed of dominant wind, whirls and air currents, exposure and sunshine rates, the ability of nearby surfaces to reflect light and the type and intensity of polluting emissions (Dabija et. al., 2011).

The significant difference between the two systems is given by the width and the structure of the substrate and implicitly by the vegetation it can support (fig. 5).

The substrate has to have a high water retention capacity. It has to ensure air permeability and to have a stable structure, exchange capacity and high buffer power (Dabija et. al., 2011). Growing media used for vegetative roofs can be natural, artificial and of mixed origin, according to their composition. Natural ones are rich in organic material and microbial flora and have an instable physical and mechanical structure. Artificial media are obtained by industrial manufacturing of rocks and chemical substances synthesis, organic by-products from oil distillation. They are more uniform, poor in nutrients and organic material. They maintain their structure for a longer period of time and cannot be used without added nutrients. The mixed type includes components from both groups mentioned above, in various quantities. They can be combined in various ways, according to the



specificities of each material and the specific needs the plants have (Dabija et. al., 2011).



**Fig. 5 - Vegetative roof. Structure.**

In the case of extensive green roofs, the minimum thickness of the substrate is 4cm, its specific weight is low and its components include a mixture of peat, perlite, vermiculite, earth from dead leaves, expanded plastic material. Intensive roof gardens have a substrate that depends on the needs the plants have, with a minimum thickness of 50cm (Dabija et. al., 2011).

Several factors must be considered when selecting plants to be grown on a vegetative roof: the thickness of the growing media and its ability to retain water, the slope, wind exposure, orientation in relation to cardinal points, rainfall. The criteria to be complied with when choosing the vegetation are: resistance to frost, resistance in time, growth height, plants which develop vertically and not so much on the horizontal (Minke, 2010).

Extensive green roofs are formed of succulent plants and moss. Usually, local plants are used, since they are adapted to survive in extreme climate conditions and ensure a natural growth of vegetation, without needing special care. The intensive roof garden consists mainly of perennial herbaceous, bulb-rhizome plants, shrubs, bushes and even woody vegetation. The range of design is wide and the same principles as for ground vegetation can apply. (Dabija et. al., 2011).

## CONCLUSIONS

1. A successful vegetative roof depends on the proper choice of the best system that suits the needs of that roof. The extensive *green roof* is better suited

for the rehabilitation of existing buildings, with a flat or sloping roof, since their structure cannot support additional loads. On the other hand, the intensive type, the *roof garden* can be successfully implemented mainly in the case of new, public or private buildings, where the supporting structure can be designed in order to support the extra weight.

2. The benefits of having vegetation and related layers on the roof of a building are obvious: they improve the aesthetic image, purify the air by absorbing carbon dioxide, release oxygen and filter dust and polluting emissions; they increase thermal and phonic comfort due to the additional insulation and therefore reduce costs for maintaining an optimal interior temperature; they reduce humidity variations, retain excessive water runoff, prolong the life of supporting structures and reduce the greenhouse effect.

3. Vegetative roofs are a solution for the rehabilitation of urban areas, a topic which concerns us more and more. They improve life quality and help develop biodiversity and the urban microclimate. In conclusion, green roofs bring multiple benefits for sustainable development in our cities and this investment is one worth making.

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# MODERNIZATION AND REHABILITATION OF THE “VASILE ALECSANDRI” NATIONAL THEATER PUBLIC PARK IASI

## MODERNIZAREA ȘI REABILITAREA PARCULUI PUBLIC AL TEATRULUI NAȚIONAL “VASILE ALECSANDRI” IAȘI

CIOBĂNAȘU C.<sup>1</sup>

e-mail: sc\_arcdesign@yahoo.com

**Abstract.** *This park is in the central area of Iasi Municipality, located inside the protection perimeter of urban architectural and historical values. This area located in the vicinity of National Theater of Iasi is currently unorganized as the result of some major interventions that had other priorities than the preservation and rehabilitation of green areas, the monuments were taken out of context and out of their natural dialogue, due to provisional urban arrangements or arrangements for traffic regulation and finding new parking lots. The proposed rehabilitation solution takes into account the physical features of the location, its urban evolution, the presence of historical monuments (of national value), as well as solving the current and future specific functional needs in the conditions of clearing the stylistic constraints, suggesting a unitary system with an important esthetical function of creating a harmonious urban landscape.*

**Key words:** park, National Theater, green areas, rehabilitation

**Rezumat.** *În zona centrală a municipiului Iași se află acest parc, situat în interiorul perimetrului de protecție a valorilor istorice și arhitectural urbanistice. Această zonă constituită în vecinătatea Teatrului Național Iași, este în prezent dezorganizată, rezultatul unor intervenții majore care au avut ca totul alte priorități decât conservare și reabilitarea spațiilor verzi, monumentele din ansamblu lor fiind scoase din context și din dialogul lor firesc, de amenajări urbane provizorii sau de amenajări pentru reglementarea circulației și găsirea unor spații de parcare. Soluția de reabilitare propusă are în vedere caracteristicile fizice ale amplasamentului, evoluția sa urbană, prezența monumentelor istorice, de valoare națională, precum și rezolvarea necesităților funcționale specifice, prezente și de perspectivă, în condițiile eliberării de constrângerile de ordin stilistic, propunând un sistem unitar, cu o importantă funcție estetică, de realizare a unui peisaj urban armonios.*

**Cuvinte cheie:** parc, Teatrul Național, spații verzi, reabilitare

### INTRODUCTION

The park is located in the central area of Iasi Municipality, delimited by Cuza Vodă, I.C. Brătianu, str. Dancu, b-dul Ștefan cel Mare streets. In its vicinity, a series of buildings included in the List of Historical and Architectural Monuments are located.

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<sup>1</sup> “Gheorghe Asachi” Technical University of Iași, Romania

## MATERIAL AND METHODS

The site, with a surface of 22,081.0 m<sup>2</sup>, is located in the central area, CP – the central area located inside the protection perimeter of urban architectural and historical values.

The current location has two sub-areas:

- The Assembly of National Theater of Iași, located in the northern part of the assembly, delimited by the Cuza Vodă street to north, Dancu street to east, I.C. Brătianu street to west and Agatha Bârsecu street to south;
- Theater Park Esplanade (towards the Metropolitan Cathedral), a square delimited by the street Agatha Bârsecu to north, sidewalk alley of the Metropolitan Cathedral Hotel and Roset Roznovanu Palace to east, Ștefan cel Mare și Sfânt boulevard to south and I.C. Brătianu street to west. Although the two sub-areas were formed in various stages, they currently form a unitary assembly, valorizing a major urban axis, Metropolitan Cathedral – National Theater, perpendicular to the route of Union Square – Palace of Culture axis, extending the interest elements into the deep urban network.

The current configuration is the result of the urban evolution of the city, of transformation of last two centuries.

The northern front of Cuza Vodă street (former Golia) was finished at the beginning of the 19<sup>th</sup> century by erecting the Balș House and Notre Dame de Sion Institute. The crossroad between Cuza Voda street and Gh. I. Brătianu street, former Marzescu and previously Majilor, defined the City Hall Square, here, in 1888, the statue of Miron Costin being erected. On the other side of the location, towards east, it was located since 1541 the Dancu Monastery until its demolition in 1903 and the arrangement of a “fir garden”.<sup>2</sup>

After the completion of the National Theater in 1896, according to the project of architects Fellner and Helmer, the statue of Vasile Alecsandri was erected in front, in 1908, by replacing the statue of Gheorghe Asachi.

The area between Stefan cel Mare street, I. C. Brătianu (Mârzescu) street, Roznovanu Palace and Agatha Bârsecu street (former May 9<sup>th</sup> street, previously Capitan Paun street), after the Second World War, had a stable configuration with dwellings developed on two, three floors, with public spaces at ground floor. On the northern side, towards Agatha Bârsecu street, to the extension of Roznovanu Palace outhouses, it is located the Pomul Verde garden, the first theater of Yiddish language, organized by the poet Avram Golfaden (Sutu, 1923).

The ample works of urban systematization in the 70s made possible the dream of Mayor Vasile Pogor and its counselors, in 1892, when they approved the location of the National Theater with its main façade towards the Metropolitan Cathedral, imagining one of the major axis of the central area.

## RESULTS AND DISCUSSIONS

**The historical site of the National Theater.** This site was formed in two stages. The first stage starts in 1888 and ends in 1908 – by decommissioning the constructions of Cuza Voda, Dancu, Agatha Bârsecu and I.C. Brătianu streets and by erecting the statue of Miron Costin, followed by the construction of the Theater and of the Power Plant, the creation of the small square in front of the Theater and finalizing by replacing, in this small square, the statue of Gheorghe Asachi, in 1908, with the one of Vasile Alecsandri.

The second stage can be considered the period between 1955 and 1967 when, towards north, in the former garden of the Theater, extensions of the building were made with necessary spaces for the good operation of the Opera who had its offices here (Mitican, 2002). Beginning with 1997, together with the building site for the restoration works of the Theater building, the north-east side of the park was decommissioned from the public circuit. In 2003, on the north-west side, a provisional construction is carried out, “The Cube”, designated as a hall for experimental shows, necessary as restoration works were carried out for the main hall. A controversial architectural presence, this Cube completely alters what was left of the Theater Park, organized in classical style, with alignments of trees and shrubs, alleys with benches, green lawns, flower borders (Ciobanasu, 2011).

The esplanade location is delimited by the Agatha Barseanu street to north-east, access alley to the Hotel of Metropolitan Cathedral and the Roset-Roznovanu Palace to south-east, by I.C. Bratianu street to north-west, and by the Stefan cel Mare si Sfanta street to south-west, the site being arranged between 1971-1985 by arh. Gh. Cheptea.

The park is defined by a central area supporting the urban axis between the Metropolitan Cathedral and the National Theater. On the right and left of this compositional axis, there are placed fountains, alleys, vegetal masses.

The presence of the I.C. Bratianu street and of the access alley to the Hotel of Metropolitan Cathedral, in its extension being located the lateral façade of the Roznovanu Palace – Iasi Municipality City Hall, parallel to this axis, has led to the placement of some belt type vegetal masses on the two long sides mainly consisting of sycamores delimiting the territory and separating it from adjacent areas. Compared to this high floor, the size of the trees decreases towards the center, contributing to a good visibility between the two monuments. By placing these two vegetal masses, the view is directed towards the two architectural presences, shielding the less important directions.

The time passing and the action of climate but also human factors determined the state of this park as not being at the expected quality level as we may notice significant damage to pavements, stone panels of fountains, supporting walls, statue pedestals, stairs or to urban furniture made up of lighting units, benches, trash cans, as well as the improper exhibition ways of the works of plastic artists.

**Description of the investment.** The major compositional principle remains the urban axis formed by the National Theater and the Metropolitan Cathedral which all other elements are subordinated to. Moreover, in order to fully value the presence of many historical and architectural monuments, a second significant urban axis was proposed formed by the Bals House – the office of “George Enescu” University of Arts together with the Philharmonic building on one hand and the Old Metropolitan Cathedral – Saint George Church on the other end of the axis, as an expression of the existing urban continuum.

Although considered as a unitary system, with elements of compositional continuity, for an easier presentation, we shall independently present the two sub-areas.

**The historical site of the National Theater.** The arrangement of this area with a surface of 12,045 sq. m. comprises the free land or the land to be cleared by decommissioning the provisional building, e.g. The Cube, the former park around the National Theater and the small square in front of this monument.

In the area towards north-east, between the Theater, Bals – Notre Dame de Sion House, the following are proposed:

- transformation in pedestrian space of the current Cuza Voda street and removal of visual barriers such as kiosks, advertising panels that hinder the northern façade of the Theater. We are suggesting to build a pedestrian square around the statue of Miron Costin and taking this statue out of the auto vehicle traffic context, connecting this objective to the pedestrian surfaces of Theater and surrounding green area.

- It is provided the delimitation of the neighboring area of the National Theater by remaking the époque grating. The grating should be doubled by a hedge made of *Buxus sempervirens*. In the same alignment, groups of *Betula verucosa* and *Acer platanoides* were placed, trees with a certain transparency favorable for perceiving the vis-à-vis built front;

- remaking the green area after the model of Notre Dame de Sion garden of the old photographs. Within this garden, the busts of General Berthelot, composer Alexandru Flechtenmacher, actress Aglae Pruteanu are to be reinstated. The access to the garden should be made from Cuza Voda street and from the small square created around the state of Miron Costin. Besides the oak of Berthelot, groups of *Ulmus pendula*, *Salix matsudana* and *Prunus pissardii* should be planted that, by size, texture and color of foliage should create harmonious compositional elements. The shrubs complementing the green mass should be groups of *Spireea vanhouttei*, *Hibiscus syriacus*, *Philadelphus coronarius*, *Piracanta coccinea* and *Chaenomelles japonica*;

- carrying out a parking lot for the theater's personnel with 18 place out of which two for disabled persons. The access in this parking lot should be carried out from Dancu street and should be monitored;

In the eastern part of the Theatr, towards Dancu street, we are having in view the following:

- continuation of the époque enclosure of Theater Garden. This garden should be remade over the public underground arrangements which are currently in the area, so that these should not be visible anymore (water tanks for fire prevention, generator room, fire pump room). The area of the northern façade of the "Power Plant" should be reconsidered by remaking the pedestrian lanes and adding green areas. In this area, a second parking lot for the theater's personnel should be also provided with a capacity of 16 places out of which two for disabled persons. In the near vicinity of the Theater, parallel with the south-east facades, similar to the alignment to the small square from the main entrance,

bordered arrangements of *Buxus sempervirens* should be installed in concordance with the eclectic decorative elements of the building. The space between these areas and the parking areas should be freely arranged with tree groups of various sizes creating a green background which hides the service area. The valuable species were kept adding groups of *Robinia pseudocacia*, *Fraxinus pendula*, *Sophora japonica*, *Prunus hisakura*, *Tilia tomentosa*, hedges of *Carpinus betulus*, near the parking lots, as well as groups of shrubs such as *Juniperus horizontalis*, *Syringa vulgaris* and *Yucca filamentosa*.

In the western part of the theater, the following are proposed:

- decommissioning of the “Cube” building and in its location an unconventional playground should be installed in open space, under the elevation of the National Theater pedestal, so that its façade to be visible both from I.C. Bratianu street and from the Cuza Voda street. A major, pedestrian alley should mark an urban axis towards Bals House. Along this alley, an area with the busts of writers could be remade. This area should be public, not delimited by an enclosure or limitative plantations to I.C. Bratianu street and should allow access to the unconventional playground and to the National Theater (side entrances). At the limit of street sidewalk, towards the park, an alignment of *Tilia tomentosa* should be created, the axis-alley being flanked by a row of *Taxus baccata* that is mowed in a pyramid shape, giving rhythm to the route. Similar to the south-east and north-east façade, it should have bordered alignments of *Buxus sempervirens* and *Mahonia aquifolia*, in classical inspired compositions.

The small square in front of the Theater should be remade as follows:

- remaking the shape and the platform finishing after the époque photographs and drawings;

- the two ground floors, divided by ellipse, near the statue of the poet Vasile Alecsandri should include flower arabesques while the prolonged ground floors near the access platforms should be marked by alignments of *Buxus sempervirens*;

- The Theater’s small square should be only for people underlining the pedestrian character of the area between this small square and the Theater Park Esplanade. The pedestrian feature should be carried out by decommissioning the parking lots and the road traffic and building an underground parking lot under the Theater’s Square (Ciobanasu, 2011).

**National Theater Park Esplanade.** This area has a ground surface of 10,036 square meters. The major axis is to be kept, increasing its size by installing in the middle area of two longitudinal developed fountains and in the center of gravity of the composition, this axis should intersect with a transversal platform on which a large fountain was erected, crossed by two footbridges. This composition is within the limits of a spirally developed alley intersecting all other secondary alleys. The land configurations observes the existence of the three platforms at decreasing elevations from the Theater Small Square towards the Stefan cel Mare boulevard, the connection between different elevations carrying out by ramps and stairs. The rehabilitation works

suggest a composition freeing the central axis of any disturbing vegetal presence, allowing at the same time good visibility along the newly proposed axis between the Bals House and the Old Metropolitan Cathedral. It was also suggested freeing the side façade of Roznovanu Palace – City Hall, so that to be easily viewed from the park entrance as well as from walking along the boulevard. The idea of placing the two vegetal masses to the side limits, I.C. Bratianu street, respectively the access alley of the Hotel of Metropolitan Cathedral, is still valid by preserving the sycamore alignments towards the street and installing another one on the south-east side towards the hotel. Regarding the plantation assembly, a special attention was given to the homogenous groups with species of similar characteristics such as size, foliage, aspect, so that to prevail on the contrasting groups since great variety alters the idea unity. Another concern was that of integrating the parking lots arranged at park level, proposing associations of shrubs and lianas decreasing the impact of constructions compared to the vegetation. For the alignments near the streets, *Platanus hybrida* was planted together with *Acer platanoides*, *Tilia tomentosa* or accents of *Catalpa bignonioides*. Among the category of second and third size species, we may mention *Betula verucosa*, *Prunus pissardii*, *Prunus cerasifera*, *Malus floribunda*, *Albizzia julibrissima*. Among the conifers we can find species of *Abies concolor*, *Larix decidua*, *Picea pungens*, *Taxus baccata* and *Thuja occidentalis*. The used shrubs are both of the fallen leaves groups as well as of the perennial leaves group such as *Berberis tundbergi*, *Buxus sempervirens*, *Juniperus horizontalis*, *Forsythia suspensa*, *Hibiscus syriacus*, *Spirea vanhouttei*, *Chaenomelles japonica*, *Piracanta coccinea*, *Philadelphius coronarius*, *Mahonia aquifolium*, *Yucca filamentosa*, *Tamarix tetandra*, *Cotinus coggygia*. An important surface of the proposed arrangements represents the grassed lawns that allow free sight, underlining both the trees and shrubs framing them as well as the isolated silhouettes. The grass carpets have figurative valences enhancing the varied coloring of the wood vegetation, varied by season, and intensify by contrast the chromatics of the floral arrangements. This green background is another unifier element of the park (Ciobanasu, 2011).

## CONCLUSIONS

The modernisation and rehabilitation of the National Theater public park is a prerequisite for the development of Iasi's historical centre. The proposed rehabilitation solution try to solve the current and future specific functional needs in the conditions of clearing the stylistic constraints, suggesting a unitary system with an important esthetical function of creating a harmonious urban landscape.

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# CREATIVE DESIGN IN THE AGRICULTURAL LANDSCAPE

## DESIGN CREATIV IN PEISAJUL AGRICOL

**DASCALU Doina Mira**<sup>1</sup>  
e-mail: doinamira@yahoo.com

**Abstract.** *At first sight, cultivated agricultural environment does not provide a convenient ambience to landscape design. However, some landscape creations, located right in the middle of farmland fields, succeed to prove the virtues of such pioneering facilities. Important psycho-physical effects are created by combining both design manners for philosophic-romantic-rural and modern-ecologic-sustainable atmosphere.*

**Key words:** agricultural environment, landscape design, effects

**Rezumat.** *La prima vedere, mediul agricol cultivat nu pare a oferi o ambianță propice amenajărilor peisagistice. Câteva creații speciale, amplasate chiar în mijlocul unor culturi agricole, demonstrează virtuțile acestui tip de amenajări deschizătoare de drumuri. Efectele psiho-fizice sunt create prin ambientări care îmbină atât atmosfera filosofic-romantic-rurală, cât și cea modern-ecologica-durabila.*

**Cuvinte cheie:** peisaj agricol, design peisager, efecte

## INTRODUCTION

At first sight, cultivated agricultural environment does not provide a convenient ambience to landscape design. In the history of landscape architectural creations there weren't many compositions related to agricultural terrains features or working with the agricultural environment elements.

Not even today, from the landscape architecture point of view, the subject of virtues and qualities of the agricultural landscape didn't arouse the interest of many landscape designers.

Why this absence of interest regarding the agricultural environment?

However, in the past few decades, in the context of the emphasis of global pollution, some daring landscape designers approach the beauty of features combined with the utilitarian particularities of the agricultural landscape.

Some landscape creations, located right in the middle of farmland fields, succeed to prove the virtues of such pioneering facilities. Important psycho-physical effects are created by combining both romantic-rural and modern atmosphere.

We don't talk about the well known utilitarian decorative plantations, but about creations, working with large agricultural plots, that tend towards a visionary symbolism, with important sustainable, economic and educative results (Dascalu Doina Mira, 2006).

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<sup>1</sup> University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

## MATERIAL AND METHOD

Studying the existent few cases, we can observe two tendencies:

1. one that underlines a philosophical-aesthetical symbolism;
2. other one underlines how saving, productive and also full of many aesthetic qualities, the agricultural landscape can be. I selected and analysed two interesting case studies, in order to prove that is possible to promote the aesthetic aspect of agricultural plantations, through judicious design proposals, also with multifunctional and sustainable effects on multiple levels: economic-social, educative-cultural, hygienic-sanitary.

## RESULTS AND DISCUSSIONS

Some of the most important aesthetic qualities of the agricultural landscape are very visible at a big territorial scale in the general aerial images. There are geometrical combinations of forms and colours, with a big power of impressing positively the human mind, soul and awareness, emanating the majestic deep and innocent beauty of our Mother Earth. First we should remind that since the 70's the Austrian artist Hundertwasser underlined how not to waste the urban terrain, obtaining agricultural plantations in a builded urban environment. In his visionary lay-out and sketches we can observe, at urban scale, the geometrical beauty of his roof-cultivated plots, terraces with different colours and features. Garden-roofs are nowadays an already used sustainable solution, but not in the idea of roof-agricultural plots. In the context of ecological disasters that threaten the planet, the idea of working with agricultural plots as morphologic elements of landscape architecture is not a very useful but saving solution that should be promoted and applied at urban and territorial level. (Dascalu Doina Mira, 2006)

**The first case** we want to present is of the designer Fernando Caruncho who works in a philosophical manner in the rural context. (Taylor G., Cooper G., 2000)



**Fig.1-** Fernando Caruncho, Catalonia Garden. Geometric cuts in the crops, bordered with rhythmic alignment of cypress and olives Photo <http://www.telegraph.co.uk/gardening/gardenstovisit/7692561/Fernando-Carunchos-geometric-gardens.html>

Two gardens in the middle of some crops are located in Catalonia (fig. 1) and Galicia (fig. 2). He is working on limited surfaces trying an original mixture between ancient agriculture and formal contemporary gardens.

His design style combine profound simplicity with a subtle sophistication, prouving how the wheat, olive trees, cypresses, water and grass can be the primordial elements of a beautiful landscape composition, full of calm, peace, majesty and dignity.



**Fig. 2** - Fernando Caruncho-Galicia Garden.

Armonious cypress alignment on the mountain background

<http://www.telegraph.co.uk/gardening/gardenstovisit/7692561/Fernando-Carunchos-geometric-gardens.html>

His concept seems to have almost theoretical impact, with its deep philosophical-aesthetical symbolism, but I feel that the beauty of this kind of landscape design can signal the debut of a new style with a major useful and sustainable impact. Caruncho doesn't work on large scale, but on limited surfaces, valorising the details and the subtle beauty of ordinary agricultural elements.

**The second case** (Krauel Jacobo, 2006) approach large agricultural surfaces and will explain better the meaning of my study ideas. On the other part of the world, in China, another kind of design tries to raise the collective awareness about a new agricultural landscape design solutions. In 2002, the Shenyang City in North of China commissioned some architects to create a big Architecture University campus, located downtown.

Due to a high interest for architecture in China, the project of the university extended, creating congestion and overcrowding its urban location of downtown. After much deliberation, the principal designer, Kongjian Yu, decided the best solution was to move the campus to a bigger location, unfortunately in the agricultural suburbs. The designers fight with important difficulties of site conditions and budgetary limitations.

The new site for the proposed campus had agricultural use – it was a rice field, known for a high quality, due to the cool climate and its longer growing

season. The soil quality was good and a viable agricultural irrigation system was still in place. It was important not to destroy all these values.

The budget was small: only about one US dollars per square meter was allocated for landscaping.

The timeline was short: the university required the design to be developed and implemented within one year.



**Fig. 3 - Architecture University Campus Shenyang China. Study platform for students in the middle of the crops. Photo: Kongjian Yu, Chao Yang**  
<http://www.asla.org/awards/2005/05winners/090.html>

As China moves towards modernization, the landscape architects must address issues of food production and sustainable land use, two of the biggest current issues on nowadays China's horizon. The concept of the landscape design tried to use rice-native plants and rice plots as design elements, in order to keep the landscape productivity, while also fulfilling a new role as an environment for learning (fig. 3. fig. 4).



**Fig. 4 - Architecture University Campus Shenyang China. Study platform detail.**  
Photo: Kongjian Yu, Chao Yang <http://www.asla.org/awards/2005/05winners/090.html>

An important goal of the landscape project was also to raise the awareness of land and farming amongst architecture students, who usually want to become city dwellers. In addition, the university designers try to demonstrate how inexpensive and productive, but also with many aesthetic qualities, the agricultural landscape can become, through a careful design and management, usable space as well.



Fig. 5 - Architecture University Campus Shenyang China. Photo: Kongjian Yu, Chao Yang  
<http://www.asla.org/awards/2005/05winners/090.html>

Major features of this design :

- the productive campus rice paddy was designed to be a composition with small open platforms for relaxation, all along many walkways spanning the landscape; in this way the area kept its complete functionality as rice paddy, with its own system of irrigation (fig. 5).
- other native crops were kept also, such as buckwheat growing in rotation across the campus, annually.
- the productive and aesthetic aspects of this agricultural landscape involved both students and faculty teachers into a dialogue of sustainable territorial development and food production.

The rice produced on this campus continues to be harvested and distributed "Golden Rice", serving both as a keepsake for visitors of the university, also as an economical resource and source of identity for the campus. The most important fact: the distribution of this "Golden Rice" raised the awareness of this new landscape design solutions, that could both continue old, yet crucial uses, such as food production, while supporting new uses, such as the education of students.

## CONCLUSIONS

1. Landscape compositions *into and with* functioning agricultural plots might constitute a new sustainable design that allows the process of agriculture to

become transparent and accessible to all. Relaxation and education of the people became part of a productive landscape. The farming processes can potentially become educative and productive laboratory for all interested.

2. On the background of the nowadays dreadful crisis, I want to underline that this work with agricultural plots as morphological design elements of landscape architecture compositions, should be promoted and applied on a large scale, due to their important sustainable, ecologic and economic impact.

3. The productive and aesthetic aspects of this landscape design will involve all the people into a dialogue of sustainable urban and territorial development. (Shri Mataji Nirmala Devi, 1995)

4. In my opinion, in the context of the emphasis of global pollution and food crisis, a combination between these few initiatives might initiate a new stylistic wave in the landscape design, with a very important impact on the level of collective awareness, with positive issues of food production, sustainable land use and aesthetical-educative features.

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# THE IMPORTANCE OF LANDSCAPE POLICIES IN THE TERRITORIAL DEVELOPMENT

## IMPORTANȚA POLITICILOR DE PEISAJ ÎN DEZVOLTAREA TERITORIALĂ

**FABIAN Claudia<sup>1</sup>, DOBRESCU E.<sup>2</sup>**

e-mail: klaudiafabian@gmail.com

**Abstract.** *Landscape in recent years has become an important element of territorial policies. This has a priority role in the functional adaptation and harmonization of the European Landscape Convention with national legislation. The landscape holds the role of an identity symbol through its mark of authenticity that leaves on the region and on the local territory. Using landscape as a motor of territorial development led to the adoption of policies to conserve and value the diversity of landscapes. These new policies will influence the remarkable landscape as well as the ordinary landscapes apparently without an evident aesthetic loading. Finally, this study aims to identify both the preservation policy as well as valorization policy of landscape in all his complexity and opens new possibilities on local level.*

**Key words:** landscape policies, the European Landscape Convention, landscape identity, territorial development

**Rezumat.** *Peisajul a devenit în ultimii ani elementul vedetă al politicilor teritoriale. Acesta are un rol prioritar în contextul adaptării și armonizării Convenției Europene a Peisajului cu legislația națională. Peisajul deține rolul unui simbol identitar prin amprenta de autenticitate pe care o lasă asupra regiunilor și a teritoriului local. Utilizarea peisajului ca motor de dezvoltare teritorială a dus la adoptarea de politici care să conserve și să valorifice diversitatea peisajelor. Aceste noi politici vor influența atât peisajele remarcabile, cât și cele banale și aparent lipsite de o încărcătură estetică evidentă. În final acest studiu își propune să identifice atât politicile de prezervare, cât și cele de valorizare a peisajului în toată complexitatea sa și să deschidă noi posibilități de abordare la nivel local.*

**Cuvinte cheie:** politici de peisaj, Convenția Europeană a peisajului, peisaj identitar, dezvoltare teritorială

### INTRODUCTION

The use of landscape in the current practice of European territorial policy is due to its entering into public debate and the use of regional policies (Debarbieux, 2007).

With the adoption of the European Landscape Convention (ELC), it got a new possibility of expression at the level of territorial development policy. This document established a common European framework (Donadieu and Perigord,

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<sup>1</sup> Alexandru Ioan Cuza" University of Iasi, Romania

<sup>2</sup> University of Agricultural Sciences and Veterinary Medicine of Bucharest, Romania

2005) for the development of landscape, without attempting its uniformity. Landscape enjoys the recognition of its importance at European level and opens new opportunities to express their territories. Considering it an expression of local identity gives the possibility of valorization. The landscape opens new ways of expression for local stakeholders. Creating a unique document to establish the European landscape policies is the recognition of landscapes diversity, but also the guidance for the subscribing States of the Convention to adopt national policies which thus introduce the landscape in their legislation. Through these actions the population and their environment are brought into the center of public life (Donadieu and Perigord, 2005). Nowadays the landscape helps to achieve the political construction of the new European territories in which democratic values and principles of sustainable development are placed in the center of discussions.

## **MATERIAL AND METHOD**

This article examines the impact that the landscape policies have on areas and innovative principles on which they intend to put these policies into practice. As a signatory country of the ELP, Romania must adopt the commitments of this document. Considered as an important component of Europe's cultural and natural heritage, the landscape has a coagulator role for the territorial cohesion policy (Council of Europe, 2006).

Therefore using the European examples of applying public policies at different territorial scales from national to local level will be a support in the development of their national policies. Raising population's awareness regarding the landscape and its closeness are two important elements which lead to valorization and to progress of a territory.

Highlighting the role of landscape and landscape policies in a territory are benefic in a context where there must be taken important steps towards adopting and implementing the ELC as a signatory country.

ELC is one of the democratic ways through which local stakeholders can be included through direct participation in landscape transformations (Auzanneau, 2001). Using European documentation which have the landscape for example as a motor for regional development can become an advantage in the enunciation of new internal regional policy and is a first step towards involving the population in developing their own living space.

## **RESULTS AND DISCUSSIONS**

The year 2000 brought a change in the landscape approach in terms of rules and conventions. Until then UNESCO was the sole organism which protected and organized a policy of conservation of sites with remarkable natural and cultural landscapes at European and global level. Since 2000, the ELC adoption has opened a new opportunity for European landscape to be appreciated, protected and especially preserved (Rössler, 2001). This occurred due to economic and social changes that led to the trivialization, transformation and degradation of landscapes (Dejeant-Pons, 2001). For Signatories States, the Convention legislates the juridical role of landscape and harmonizes economic law with cultural and social law (Dejeant-Pons, 2001).



The creation of common European policy regarding the landscape was determined by the desire for affirmation of a European identity, and ELC emerged as a result to the awareness of the framework of life deterioration and loss of local image. It is thus supported the desire to live in a non-degraded environment, which has a certain quality of life without restraining the development of economic environment. It gives a life framework to ensure a sustainable development of territories (Council of Europe, 2006), but also economic and social development.

Using the landscape in spatial policies showed a higher closeness of living space by population, in which the development projects were more easily accepted (Davodeau, 2005). With the acceptance of major regional projects due to the landscape, the actions become more credibility at local level.

Adopting ELC showed that the actions on the development and protection of the territories are more readily applicable and the territories management is evolving. Now there is a moving from planning field of gardens and parks to a higher level of planning as the new area of application of landscape projects (Davodeau, 2005). We can illustrate here the creation of *Atlas of Flemish landscape*, a first step towards identifying the patrimonial landscape and shaping future development scenarios of landscape. The first form of Flemish landscape atlas was elaborated in 2001 and established three types of contemporary Flemish landscape characteristics (Antrop and Van Eetvelde, 2008). These were classified according to importance and socio-cultural impact in: patrimonial landscapes (traditional landscapes, rural landscapes and scenery, parks and gardens historic landmark), urban landscapes and the third category of the processed agricultural landscapes. In 2005 this document was improved, but it has remained a perfectible instrument through the need to include the entire Flemish landscape. The landscape atlas is an instrument subjected to continuous transformation in the context of rapid territorial dynamics as that of Flanders. Although it did not integrate all the principles of the ELC, this document was quickly used for Territorial Planning policies and patrimonial policies (Antrop and Van Eetvelde, 2008).

The landscape policies support the treating in a consistent and global form of the territory. This leads to the strengthening of links between each society and the space they live in, by reinforcement of natural and cultural values of each territory. Landscapes are considered a richness of the European territory exactly through their natural and cultural diversity. Maintaining and protecting the rich European landscape represents the wishes of unity in diversity through the protection of cultural and natural heritage of Europe (Council of Europe, 2006). Landscape policies enrich the European Spatial Planning goals by strengthening socio-economic cohesion, because the landscape is actually the result of a fortunate combination between richness and variety of natural and traditional cultural elements specific to each European territory basis. By defining a coherent European landscape, this becomes an inestimable potential stock for sustainable development planning, in accordance with the directories principles adopted at the

CEMAT meeting in Hanover in 2000 (Council of Europe, 2006). Landscape policies are thus awareness tools for different local stakeholders in a form of concerted management planning (Dubois, 2009).

The strengths of landscape policies are the multidisciplinary involvement and the participatory character through which local actors are recruited for their application. Through active participation of population and administrative actors the landscape was able to democratize the management of life framework (Dubois, 2009).

The democratic dimension of the landscape is highlighted through the possibility of involving citizens in the selection of landscapes considered valuable as well as their active participation in decision-making regarding the landscape transformations, from management to its protection and evolution. Introducing these actions with regard to landscape is one of ELC's innovative components, as it calls for creation of mechanisms for protection and management at all decision-making levels from local to European level and leaves open the way for all actors to express on the landscape, from experts to ordinary citizens (Fairclough, 2002). Through democratization of landscape a change was made in the accessibility field: landscape no longer belongs to an elite, it can now be approached by the simple citizens.

Introduction of landscape policies in the development planning as active elements revealed three basic characteristics that are expressed through keywords: protection, management, planning of the landscapes. Each of these terms is clearly defined.

"Landscape protection" means the preservation of significant elements which are recognized as patrimonial values created by nature or made by human intervention (Ouériat S., 2006). As example is the case of Switzerland, which following the modification of legislation to protect nature and landscape in 2007, introduced in the federal legislation the concept of Regional Nature Park (Gerber et al., 2007). This instrument created based on the French model recognizes the importance of landscape as part of economic impulse made by tourism, and especially it recognizes the value and importance of landscape preservation. In Romania, an action of protecting the landscape is the creation of the Natural parks, as specific instruments to protect the natural areas with remarkable flora and fauna as well as specific cultural elements of the protected space. The role of a Natural park in Romania is to maintain in its perimeter the natural and cultural elements in a form slightly altered by accelerated economic activities of the territory.

"Landscape management" will describe the actions of transforming the landscape, trying to define the concept of sustainable coagulant evolution of social, economic and environmental actions in the aspects of a territory (Ouériat, 2006). Maintaining the principles of sustainable development in landscape management, a relevant example may be given by rapprochement the banks of the river Rhone in the French city of Lyon. This project succeeded to bring closer the residents to the river banks, which were until then inaccessible, through the

transformation of urban parking in a green promenade. The stakes of the project was to improve quality of life and sustainable conversion of the urban landscape.

"Landscape planning" describes instead in an active form the changes and landscape evolution covered in the action of restoring, rehabilitation and innovation of landscape (Ouériat, 2006). As an example we mention the rehabilitation of Carbonia city of Sardinia, Italy. The concern for conservation of cultural heritage, and restoring of the urban center determined its choosing as an example of good practice. The project was awarded by the Council of Europe with the 2010-2011 prizes for landscape.

Following the project development, called Carbonia Landscape Machine, it achieved the harmonious combination between urban rehabilitation through enhancement of architectural, socio-economic and environmental heritage. This project followed closely the landscape spatial principles by restoring and innovate transformation of the city landscape of Carbonia, but in the spirit of sustainable development. The project enjoyed large public participation, involving different social groups in the success of the project, who were attracted and sensitized by information and knowledge campaigns. Thus the use of landscape policies involved in managing and preserving territories causes a major asset in applying regional development policies with direct impact on social and economic environment. By applying management, conservation and protection policies, the decision at regional and local level is made transparent. Involving local actors will bring about a democratic and participatory framework life by local government. So, we find at European level the different models of landscape integration into public policies, but also legislative difficulties which delay the adoption of Convention principles. The Romanian legislation does not give an independent definition of the landscape, but this is found mentioned in the legislation and urban planning, environmental legislation or legislation dealing with the heritage problem. Because it is not legislated, the landscape fails to make its place in the territorial projects. For now the role of environmental and natural landscape prevails through its involvement in various types of protected areas, but the cultural role and the importance of traditional image are not yet fully exploited.

## CONCLUSIONS

1. The importance of implementing the landscape policies at the local level comes as a counterbalance to territorial development based only on infrastructure development and increasingly present intervention of the anthropogenic factor into territory.

2. Highlight the value of a landscape and awareness of landscape leads to a greater ownership of the people and more involvement in the protection and controlled progress of the landscape. The participation in the landscape transformations is thus democratized. Residents are aware of their role in their planning projects. As a result, a greater sensitivity to their environment is developed.

3. Maintaining the sustainability of the territory and taking into account the management / preservation / landscape planning.

4. Finding local identity offers new ways of development with the help of landscape, so this leaves the conservation area and becomes an innovative element in land use.

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# THE IMPACT OF LANDSCAPE DESIGN ON URBAN AREA. CASE STUDY OF IASI

## IMPACTUL AMENAJĂRILOR PEISAGISTICE ASUPRA SPAȚIULUI URBAN. STUDIUL DE CAZ MUNICIPIUL IAȘI

**GRIGOROVSKI M.<sup>1</sup>, GHEORGHÎĂ Carmina<sup>1</sup>**

e-mail: mirceag1@yahoo.com

**Abstract.** *This study aims to be a synthesis of case studies of small landscape improvements in the city of Iasi and highlights the quality factors of these urban improvements under the following criteria: urban, morphological, structuring, plant usage and identity, while examining the impact of these criteria on public space and the urban image.*

**Keywords:** landscape design, urban area, the impact of landscap design

**Rezumat.** *Studiul se consideră a fi o sinteză a studiilor de caz asupra amenajărilor peisagistice din municipiul Iași și evidențiază factorii de calitate ce aparțin acestor amenajări după criterii urbane, morfologice, structurante, vegetale, identitare, analizând totodată impactul acestor criterii asupra spațiului public și asupra imaginii urbane.*

**Cuvinte cheie:** amenajări peisagere, spațiu urban, impactul amenajărilor peisagistice

### INTRODUCTION

The image of the city and, by default, measuring the quality of life, depend largely on public space, which plays a major role and polymorphous. Between spatial conformity and the needs of the inhabitants, appears the question: should we change the city in order to improve the quality of life or vice versa? However, the two processes follow and mold each other - the history of cities has pointed out that spatial transformation accompanies spatial development and back.

The town charm derives from the wide range of activities characteristic of each one individually. Filtering and analyzing nature through art, a viewer ennobles the urban space (sometimes disqualified by default) discovering the beautiful encrypted discourse (most often studied) of urban objects (Grigorovski and Răchieru, 2011).

Specific places, constructed or landscape designed, natural, or artificial, the public space of a city have, depending on their players, different meanings. Thus, the practitioners of built space (architects, planners, geographers, engineers and landscape architects) approach these urban and architectural fragments as trump cards of a city, as major structural basic elements of a built environment. For residents and users, the public space of the cities represents meeting places, landmarks, which plays a major role in the social cohesion of the individual.

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<sup>1</sup> "Gheorghe Asachi" Technical University of Iași, Romania

Historians, sociologists, environmentalists are oriented on the information and messages that these spaces send, facts regarding the history, culture, mentality and the level of economic and technological development, the quality of life and the values that define society over time. (Țurlea, 2008)

In present time, the most important aspect of urban life is cities practicability; its bearability as a public space. Thinking about public space does not involve speaking about its ancient meanings of this urban space, the agora, a concept that cannot be revived, but which can be rehabilitated based on the needs of the contemporary man. The city is not only man-made landscape, but also heritage, tradition, culture. Everyday life, sound, light and darkness, vegetal, air, humans, water, sky - all these spaces and landscapes are assembled in a continuous motion and in continuous evolution, in landscapes that we perceive consciously or unconsciously. (Grigorovschi and Răchieru, 2011) Improving the attractiveness of public spaces under current conditions depends on quality, vitality, and security.

According to EU directives and legislation (Emergency Ordinance 114/2007), our country must provide 26 hectares of green areas per inhabitant in each locality, by the end of 2013. The norm of 26 m<sup>2</sup> / capita is the minimum accepted by the European Union while the World Health Organization recommends a 50 m<sup>2</sup> / resident. Currently, the average green area per capita in Romania is 9 m<sup>2</sup>, of which 5 m<sup>2</sup> in urban areas. However, Stockholm is 70 m<sup>2</sup> / resident, Vienna is 70 m<sup>2</sup> / resident, in London 64 m<sup>2</sup> / resident, 31 m<sup>2</sup> / resident in Warsaw and in Bucharest is 2.5 m<sup>2</sup> / resident. In Iasi, in 2010, according to data provided the Environmental Protection Agency is approximately 20 hectares of green space per inhabitant.

## MATERIAL AND METHODS

This study aims to be a synthesis of studies conducted with the students of The "G.M. Cantacuzino" Faculty of Architecture, Iasi, fifth year, in Landscape discipline.

The work carried out with the students had first to identify determinant items of the quality of spaces and landscaping, and their classification by categories of quality factors, following various grounds: urbanity, morphology, structuring, vegetable, identity, etc. The stakes of the study was to analyze the impact of these criteria on public space and on urban image.

The landscape to be observed, small or medium, are located in the center of the town or in its immediate vicinity, in areas with different predominate functionality (university area, residential area, cultural area, protected area, etc.), thereby ensuring the character of generality of the results.

The study areas mentioned above were observed during October 2011 – May 2012. The base of the study was mainly periodic visual observation. The data were collected and processed following a series of quality factors of landscape design, divided in criteria.

The case studies have been carried out at the following locations:

- the front of the B Building belonging to The "Al. I. Cuza" University;
- the area defined by The "Voievozi" Park – The Student's Cultural House – The Children's Palace;

- the garden of "St. Spiridon" Hospital; the area defined by The Palace of Culture – "Luceafărul" Theater;
- the area near Blvd. Țuțora and "Splai Bahlui Right Bank" Street junction from Podu Roș;
- the junction area between "Splai Bahlui Left Bank" Street with "Gh. Ghibănescu" Street.

First phase study involved designing an evaluation grid for landscape design and urban spaces. Second phase of the study required a proper analysis of the pilot areas mentioned above, by applying the evaluation grid developed in the previous phase. Thus, we determined the impact of urban criteria, structuring, morphological, and identity, vegetable, mineral, water, etc. on public space and on urban image. The analysis of landscape design and urban areas according to established quality criteria was made by allocating to each criterion one of three impact values: low, medium, high. Phase III forced students on drafting solutions to revitalize and redevelop studied areas, in order to eliminate as much as possible the specific malfunctions of studied areas and increase the quality of these spaces.

## **RESULTS AND DISCUSSIONS**

As result of the first phase of this study, an evaluation scale upon the current state of the case-study areas was established. Thus, quality factors of landscape design were grouped into, elements of the same category as subordinate of a criterion. The established criteria are: urban; structuring; morphological; identity; vegetal; aquatic; mineral; green space management; diurnal-nocturnal ratio; the psychological effect.

The urban criteria applied to study area seek especially its location within the city, heading to the main traffic routes, to the historic city center, to the downtown area. At the level of relations established with the existing neighborhoods we attempted to determine the relationship with neighboring urban functions (dominant / subdominant / complementary) and establish urban texture space (the ratio between the built and green space) and visual impact that prints the urban image, the overall image. Also in this category are included the specific landmarks of the analyzed area, that turns the stud- area into a landmark in itself and the versatility of functions, the possibility of using the area by urban actors.

The structural criteria evaluates elements of space composition (axes - real, virtual, directory paths, delimiting vegetal elements, mineral elements), the size of studied site, parceling, accessibility, existing course (the existence of a logical route, ergonomic) and relationship to other spaces green.

Morphological components follow the relationship between morphological elements (mineral, vegetable, aquatic), but also the morphology the parties that make up the whole and the relationships established between its parts (contrast, similarity, subordination, equality, unity, complementarity, harmony, etc.).

The identity of a place is conferred on its specific activities that it can accommodate and the presence of urban elements that facilitates certain activities. Identity criterion aims to determine and establish symbol, which individualize space. (fig. 1, 2, 3)

Vegetal criterion quantifies the adaptability and diversity of present plant species depending on the season, also analyzing the complexity of current vegetal composition (color, size, dynamics, durability, etc.).

The mineral criterion identify the extent to which the ground level, through sloping, uneven or its flatness, soil types and their possible recovery and artificial interventions can significantly improve the quality of public space.

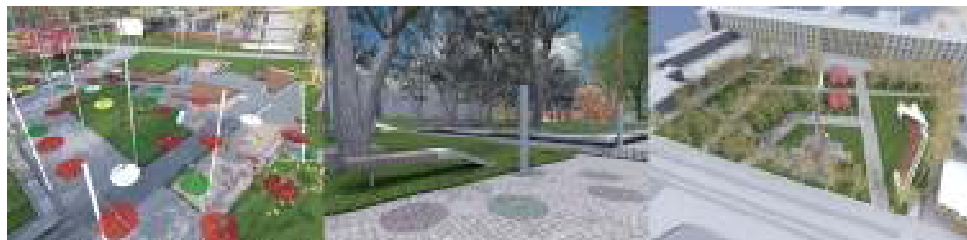
An important role in analyzing the impact of landscaping on public space and on the urban image is held by the presence of water, in its dynamic or static form of natural or manufactured element. The study tends to modify the image of the landscaped space by including water assisted by intensity of light (day/night, season) (fig. 3, 5, 6).

Determining the daytime /night ratio aimed at identifying specific elements that define different area depending on time of day.

The economic efficiency criterion establish the level of influence which it green space management has by management understanding costs of maintenance, of rehabilitation / development, waste management and public contribution to the welfare area. An important factor of this category is the the possibility of introducing profitable activities.

Public space corresponds to needs and structural strength related to community, to all urban actors involved in shaping it, to culture, political determination, the social and community needs. (Grigorovschi and Răchieru, 2011)

In general, the proposals for improving the urban image of the area and the overall visual impact have sought to create new compositions of the space, compositions that allows the materialization of attractive pathways, with one or more axes of composition sustained centers of interest grouped by their importance. (fig. 1, 3, 4, 5, 6). It was also noted that the introduction of temporary or permanent structures, structures that can accommodate different cultural and social activities (film screenings, multimedia events) and alternating events that take place day and night (fig. 2, ), which implies providing the space lights and lasers games (fig. 1, 4, ), increase considerably the quality of landscaping, and therefore the public space and the urban image.



**Fig. 1** - The front of the B Building belonging to The "Al. I. Cuza" University - proposal

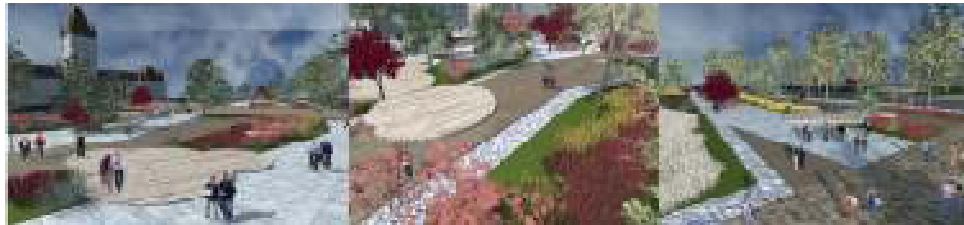




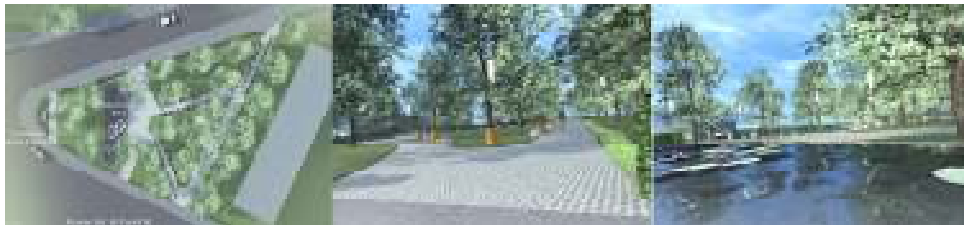
**Fig. 2** - The area defined by The “Voievozi” Park – The Student’s Cultural House – The Children’s Palace – proposal



**Fig. 3** - The garden of “St. Spiridon” Hospital – proposal



**Fig. 4** - The area defined by The Palace of Culture – “Lucefărul” Theater; - proposal



**Fig. 5** - The area near Blvd. Țuțora and “Splai Bahlui Right Bank” Street junction from Podu Roș – proposal



**Fig. 6** - The junction area between “Splai Bahlui Left Bank” Street with “Gh. Ghibănescu” Street - proposal

## CONCLUSIONS

The notion of place, space, living framework for, shape, define the landscape of urban life and constitutes the same number of elements that interact and continually seek to achieve a balance of forces acting to define the landscape and scenery in the city where we evolve as actors. (Grigorovschi and Răchieru, 2011). The impact of each criterion on the quality of public space landscape improvements of Iasi has virtually the same value. There are no predominant quality criteria, the quality of space and landscape improvements is determined by the value of all high quality factors, combined, and aggregated in a spatially homogeneous system that generates the overall quality of space, uniqueness, attractiveness, and "genius loci."

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# EPHEMERAL SPATIAL STRUCTURES IN PARKS AND GARDENS – PERCEPTION AND MEANING

## STRUCTURI SPAȚIALE EFEMERE ÎN AMENAJAREA PARCURILOR ȘI GRĂDINILOR – PERCEPȚIE ȘI SEMNIFICAȚIE

NICA R.M.<sup>1</sup>, PETROVICI Liliana-Mihaela<sup>1</sup>  
e-mail:rmnica@gmail.com

**Abstract:** *Ephemeral spatial structures located in public spaces such as squares, gardens and urban parks may constitute complementary compositional elements in the contemporary landscaping. These temporary and reversible interventions on the landscape are counting on the psychological and perceptive impact and on an amplification of social contact, without creating, from the viewer, an instinctual denial of the novelty. Ephemeral spatial structures, which are simultaneously architectural and artistic, are not perceived as aggressions or alterations of the familiar space, but rather as urban furnishings that mark a certain event in the daily city life. In the case of the outdoor spaces design, these objects with a temporary existence are conceived as an integral part of the landscaping creation. Being detached from constraints of the perennial, these objects offer the opportunity of a new sensorial experience of the space.*

**Key words:** temporary landscape design, ephemeral urban furnishing, artistic and architectural installation, reversible interventions on natural environment, psychological and perceptive

**Rezumat:** *Structurile spațiale efemere amplasate în spații publice precum piețe, grădini sau parcuri urbane pot constitui elemente compoziționale complementare în amenajările peisagistice contemporane. Aceste intervenții temporare și reversibile asupra peisajului mizează pe impactul psihologic și perceptiv, pe o potențare a contactului social, fără a atrage din partea individului o reacție instinctivă de respingere a noului. Instalațiile spațiale efemere, ce pot fi atât arhitecturale cât și artistice, nu sunt percepute ca agresiuni sau alterari ale unui spațiu deja familiar, ci mai degrabă ca obiecte de mobilier urban care marchează un anumit eveniment în viața cotidiană. În cazul amenajării spațiilor exterioare, aceste obiecte cu o prezență temporară sunt concepute ca parte integrantă a creației peisagistice. Fiind desprinse de constrangerile permanentului, acestea oferă prilejul unei experiențe senzoriale inedite a spațiului.*

**Cuvinte cheie:** amenajări peisagere temporare, obiecte de mobilier urban efemere, instalație artistică și arhitecturală, intervenții reversibile asupra mediului natural, impact psihologic și perceptiv.

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<sup>1</sup> “Gheorghe Asachi” Technical University of Iași, Romania

## INTRODUCTION

Through ephemeral spatial structures we understand that eventful and conceptual component of landscape design, with the single purpose of marking of a moment and the transmission of a message or a concept. As in the case of conceptual art, the ephemeral landscape design does not create a transient object, but an event.

In the city's general context of the built and natural environment, the transient term is synonymous with impermanent, reversible and temporary. The ephemeral, in this case, includes an understated comparison with the human existence time and not with the historical time. And so it is realised a reference to the conventional life expectation of a contemporary spatial structure and less to timeless existence assimilated to the ancient temples and gothic cathedrals.

Much like the ephemerae, insects that live as adults just one day, the ephemeral is an action, a phenomenon, a creation or an event with the duration limited to a single day. It is a short, fugitive, impermanent or unstable presence, an unstable and temporary phenomenon, that perpetually modifies and transforms.

## MATERIAL AND METHODS

Through human action (interaction, movement, event) a spatial structure overcomes the stage of aesthetic object and becomes an urban object with a temporary presence. Every object has a temporal stance, a temporary existence, but the transient implies a different standing towards the time, related to the everyday life. A fraction of the built environment life expectancy (determined by the materials sustainability and the moral usage of the functional structure) in the case of ephemeral designs is replaced by a precise predetermined life cycle.

## RESULTS AND DISCUSSIONS

The ephemeral intervention on the environment does not represent an authoritarian or intrusive gesture. These objects are not perceived as aggressions or alteration of an already familiar space, but rather as urban furniture objects that mark a certain event in the city life. Similar to art, the ephemeral structures placed in parks and gardens can, thus, count on the psychological and perceptive impact and on a revival of social contact, without generating an instinctive gesture of denial from the viewer.

Transient constructions, detached from the permanent constraints, are the occasion for a complete sensorial experience of space. Gaston Bachelard mentions that the space perception is not just empirical, but is possible even thought means of emotional memory. The space is perceived through the memory filters and thus, personalized.

In defining the way these objects are perceived and interpreted an essential factor is the *objects scale*, in this case the *smallness*. Usually these interventions are at a human scale, they interact in the same time with a relative small number of individuals. The reduced dimensions caused, mainly by economical and technical factors, lead to a more intimate relation between

landscape, architecture and individual. "The constructed mass and the reduction of the intervention scale generate physical and psychological safeness, social contact and communication, closeness (...)" according to Jane Jacobs. (Jacob, 1961)

The ephemeral landscaping intervention does not have just a function (rest, reunion, signal), but it transfers to the individual through a visual representation, an emotional state and intellectual stimulates him towards an introspection (fig. 1) One can speak about the object physical and mental representation, of the physic and mental projected image:"To perceive means to make an ephemeral adjustment, interesting and local (...) of two images, one supported by the substance, the other one crossed by the conscience that lasts and that is reported to I."(Wunenburger, 2004)



**Fig. 1 - Serpentine Gallery Pavilion, Londra, UK, 2009, Architects: SANAA**  
Photo credits: © Iwan Baan, [www.archdaily.com](http://www.archdaily.com)

Placed in a garden or a park, an ephemeral pavilion does not belong to a place, but is effectively perceived in relation to a certain place, a public space. It is a nomad object of whose presence does not leave visible traces, but only on an ideational level. We refer to the "some species of places set outside any place, even if they are in an effective way, located." (Foucault, 1994).

The transient spatial structures used in landscaping design live only through the direct relationship with the individual, meantime other attributes such as durability, stability, comfort and integration in context pass in a secondary plan. The intervention in public space of ephemeral constructions is not a leaning towards the superficial, consumerism or a denial of historical values, but an occasion for a complete sensorial challenge – sight, hearing, smell, and tactile sense.

Set in public gardens and parks, these *architectural* and *artistic installations* become a dynamic factor of the designed space. They become a rest point, a landmark in a much wider landscape path. The space defined is permeable, the line between the inside and the outside is very thin and in most



**Fig. 2** - The installation called *A path in the forest* is a suspended bridge, sustained exclusively from the trees trunks. An event is created, a surprise in the landscape path. The intervention is reversible and does not aggressively intervene in the natural habitat of the forest. Tallinn, Estonia, 2011, autor: Tetsuo Kondo, Photo credits: © thecoolhunter.net

cases there is no obvious demarcation between the exterior and the interior of the spatial structure. The natural environment keeps its continuity and coherence, the interpenetration with the protected space cancels the distinction between nature and human ephemeral intervention (fig. 2).

Another surprising aspect arises from an analysis of these objects, the one of the public-private dichotomy. Although by definition these installations are public spaces, the experience offered to the viewer is usually intimate. The transmitted sensation can be unusual for a public space – physical and mental safeness, social contact and closeness. The outcome is a semi-public space (or semi-private), a *heterotopia* that brings together two different worlds. The focus is on the annulment of the traditional perceptive conventions: inside-outside, private-public, present-past antinomies.

The transient existence is present even on the level of the used materials. The focus on the sudden experience and on the impression of the moment leads to the usage of uncommon materials in landscaping design. Having in consideration that durability, tightness and hydro-thermal insulation are not relevant criteria; the materiality concept can be reconsidered. The ephemeral character of these interventions is highlighted even through the limited durability of the used materials – cardboard, canvas or – in an unusual way for a construction – vegetation. The Romanian pavilion for the International Expo from Hanover in 2000, whose envelope was entirely realized from a live *green reticule* fits perfect in this trend.

Limited time expectancy in exploitation implies a judicious usage of construction materials – the usage in small amounts and of few types of materials in the same project, the obtaining of a maximum spatial result with a minimum of resources, the use of local recycled materials. The transient existence of an architectural installation contrasts with its extensive design process (fig. 3).



**Fig. 3** - *The garden of knowledge* is a temporary artistic installation realised in Quebec - Canada from 40 000 books. The unusual material defines walls, benches and floors. Authors: 100 Landschaftsarchitektur; Photo credits: © Thilo Folkerts, [www.archdaily.com](http://www.archdaily.com)

The Serpentine Art Gallery from Kensington Gardens –London gives every year, to well known architects, the opportunity to design and build a three months lasting pavilion on the lawn in front of the building.

In 2011 Peter Zumthor created on this occasion, from the concept of *hortus conclusus* (lat. closed garden), a garden in the medieval convents, a contemplative room, a garden within a garden. The outcome is an outside space, although deeply private, detached from the London verve (fig. 4).

The garden effect it is obtained through this enclosing (the fence, the enclosure wall, the closing from the neighborhoods) and through the privateness and the psychological safeness thus obtained. The spatial structure is the background for the interior lighted garden. The access from the garden is realized through a transitory labyrinth-like space, a narrow and oppressive lobby. The entrances from both directions are detached which implies a spatial transition before entering the interior. The garden is a self-centered space that invites the passersby to a moment of contemplation and offers an inedited perception of the space. A black, austere, prism-like volume, without scale and a functional implied form - this is the exterior of the construction. This image, intentionally retained, is in the same time intriguing.

The inside tilted roof, the proportions and the relating to the near gardens spaces created by Peter Zumthor, suggest a subtle allusion towards the two characteristic elements of Ancient Rome – the *atrium* and the *impluvium*. These

two elements were defined in the ancient architecture as the center of the house to which the other parietal displaced room related. They represent the architectural elements that mark the vertical axis in the relation with the divinity.

Zumthor creates the modern *atrium* of whose exterior offers a visual stimulus to the viewer, a mental challenge, for once entered in the interior space to discover a visual and a spiritual balance. The color contrast created by the black painted walls and the large shadow of the roof eaves cuts of a piece of the



**Fig. 4** - Exterior and interior image of the pavilion created by Peter Zumthor for *Serpentine Gallery Pavilion*. London, UK, 2011  
Photo credits: © Walter Herfst, [www.archdaily.com](http://www.archdaily.com)

sky lightness. This is a project set exclusively on the revaluating and the stimulation of the public space defined by the urban garden and the actual art gallery.

## CONCLUSIONS

1. The transient spatial structures potential, used in the urban gardens and parks landscaping, is represented by the playful and innovative spatial experiments, but also by the possibility of testing of unused before in the case of built environment materials and constructive techniques. Ecological constructive systems, economically unprofitable, set in an early stage of development and yet impossible to use in common practice, can be the occasion for experimental constructions.

2. Ephemeral spatial structures are considered a complementary step in the permanent architecture and the traditional landscaping design, of whose value they enhance. In the contemporary landscaping design the vanguard theories and concepts can take shape, without restrains of the permanent.

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# NATURAL AND ARTIFICIAL MATERIALS IN LANDSCAPE DESIGN - AESTHETIC AND MEANING -

## MATERIALE NATURALE ȘI ARTIFICIALE ÎN ARHITECTURA PEISAGERĂ - EXPRESIE ȘI SEMNIFICAȚIE

*PETROVICI Liliana-Mihaela*<sup>1</sup>, *NICA R.M.*<sup>1</sup>

e-mail: liliana.petrovici@yahoo.com

**Abstract.** *The landscape art explores quite diverse the aesthetical qualities of the finishing materials, so that they gain multiple meanings and poetic qualities. The natural and artificial materials, creatively used and adapted to context, bring aesthetical and semantic variety in architecture of parks and gardens. Natural materials, such as wood, stone or brick, - symbolize tradition and rootedness; they have preciousity and nobleness, warmth and vitality. The stone signifies power, perennially and prestige. The wood is the symbol of universal substance; it gives the feeling of intimacy and protection. The water and vegetation create vitality and beauty, harmonizing the relationship between the nature and manmade environment; they are a symbol of regeneration. Artificial materials such as metal, glass, concrete, plastic, symbolize the human control, the control of reason on the environment. The metal – signifies flexibility and concentration. The concrete, a malleable artificial stone, means lastingness and hardness; it can generate a large variety of spaces and forms.*

**Key words:** natural and artificial materials, plastic and semantic effects, symbol, meaning, identity, diversity, landscape design

**Rezumat.** *Calitățile estetice ale materialelor de finisaj sunt explorate foarte divers în design-ul peisager, astfel încât dobândesc multiple sensuri și calități poetice. Materialele noi sau tradiționale, utilizate creativ și adaptat la context, aduc diversitate semantică și estetică în arhitectura parcurilor și grădinilor. Materialele naturale – lemnul, piatra, cărămida – simbolizează tradiție și înrădăcinare, au prețiozitate și noblețe, vitalitate și căldură. Piatra exprimă putere, perenitate și prestigiu; lemnul semnifică substanța universală, conferă sentimentul de intimitate și protecție. Apa și vegetația aduc vitalitate și frumusețe, armonizează relația dintre mediul natural și mediul creat de om, sunt un simbol al regenerării. Materialele artificiale - metalul, sticla, betonul, plasticul simbolizează controlul uman, al rațiunii, asupra mediului. Metalul semnifică flexibilitatea și concentrarea. Sticla transparentă sau translucidă are capacitatea de a absorbi, de a reflecta sau de a lăsa lumina să pătrundă, astfel încât oferă senzații variate și schimbătoare. Betonul transmite ideea de trăinicie și duritate; este o piatră artificială „maleabilă” ce poate genera o mare varietate de forme spațiale.*

**Cuvinte cheie:** materiale naturale și artificiale, efecte plastice și semantice, simbol, semnificație, identitate, arhitectură peisageră

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<sup>1</sup> “Gheorghe Asachi” Technical University of Iași, Romania

## INTRODUCTION

The finishing materials have the role to enhance the aesthetic qualities of the landscaping; they transmit ideas, feelings, and create certain ambiances. They communicate a large variety of sensations such as tactile sensations – of warmth or coldness, thinness or hardness, olfactory sensations and visual sensations – depending on texture, color and the way of absorbing or reflecting light.

## MATERIAL AND METHODS

The landscape art explores quite diverse the aesthetical attributes of natural and artificial materials, so that they gain multiple meanings and poetic qualities. “Materials can be made to glitter and vibrate”, states Peter Zumthor, in his concern to reach the complete expressivity of finishing materials, to discover their aesthetical valences by adapting them to the context of creation (Zumthor, 2006).

## RESULTS AND DISCUSSIONS

**Natural materials**, such as wood, stone or brick, signify tradition and rootedness. They have vitality and warmth, nobleness and preciousness. They get the simple beauty of the things created by nature.

The raw and unpolished materials generate organic landscaping that seems to be pure creation of nature. The treated and processed materials are a symbol of a harmonious relation between man, technology and nature.

**Wood** – contains a superhuman wisdom and science, it is the symbol of universal substance (Eseev, 2001). In Catholic religion, wood is the symbol of the cross.



**Fig. 1** - Norwegian Wild Reindeer Centre Pavilion, Hjerkind, landscape architects Snøhetta Oslo AS the inner wooden shell shaped by means of computer aided design and advanced processing technologies

The wood generates the feeling of warmth and protection; it emphasizes the role of shelter that some landscaping elements have (fig. 1). It is

gentle to touch and it has material plasticity, being easily to process in various ways. The wood is a modern and traditional material; it can be adapted to the new technologies and to the contemporary requirements and styles in landscape art.

**The stone** symbolizes power, perennially and prestige, beautifully aging as the ruins (Vlad Gaivoronschi, 2003). The stone creates order and stability, is characterized by mass, weight and power, by “ease, naturalness and elegance, beauty and simplicity” (Werner Blaser, 2003). The stone beautifies and ennobles the landscape environment of urban parks and gardens and is a powerful element of local identity (fig. 2).



**Fig. 2** - Stone River, New York, landscape artist Jon Piasecki

- made of row and polished stone extracted from the site, this landscape work creates an organic image and provides the feeling of merging with nature

Besides wood and stone, **water** and **vegetation** also represent natural “materials” of landscape art compositions (fig. 3). They create vitality and beauty, harmonizing the relation between the natural environment and the manmade environment. The water and the vegetation have aesthetic, symbolic and ecologic roles.



**Fig. 3** - Sunnylands Center and Gardens / The Office of James Burnett , Frederick Fisher + Partners, California – in the arid area of Sonoran desert, water and vegetation used as landscape design compositional elements generate a powerful semantic impact

Water is a symbol of purity, wisdom, grace and virtue; it is a source of bodily and spiritual regeneration (Chevalier, 2009). Water is one of the four essential elements of which the universe is created. It is the origin of life, the primordial substance, from which all forms arise and where they come back by regression.

Both in static and dynamic states, water as compositional element in landscape art has the property of reflection and audio-visual effects, which confers energy, space and vitality. The artesian wells and basins bring psychological benefits, are point of attraction, places of communion, sources of strength and regeneration.

**Artificial materials - concrete, metal, glass, plastics** – „put into work, they oppose the natural, the aleatory, and symbolize the human control, the control of reason on the environment ... these materials do not know – are not allowed to age in the way the stone, wood, crude or fired earth do” (Ioan Augustin, 2007).

**The concrete** is an artificial stone inspiring lastingness and hardness. “Easy to mould in amorphous and fluid forms” (Zaha Hadid, 2004), the concrete has generated, during the modern and contemporary times, new forms and novel spatial and constructive solutions (fig. 4).



**Fig. 4** - Negev Monument, Beersheva, Israel, 1968, landscape architect Dani Karavan  
The concrete – an artificial stone that can generate fluid, organic shapes

Although apparently an impersonal material without aesthetical qualities, the concrete can communicate a wide variety of visual impressions, depending on the way of processing. Creatively and ingeniously treated, it can prove plastic valences and a specific expressiveness. Tadao Ando exploited in its works “the power”, but also “the sensitivity” of the concrete (fig. 5). Realizing that its aspect and qualities depend on the formwork, the way of consolidation and casting in forms, he transformed it into a mild and sensitive material, approaching in texture and beauty to wood and paper.



**Fig. 5** - Awaji Yumebutai, 1995, architect Tadao Ando - the concrete turned into a mild and sensitive material, approaching in texture and beauty to wood and paper

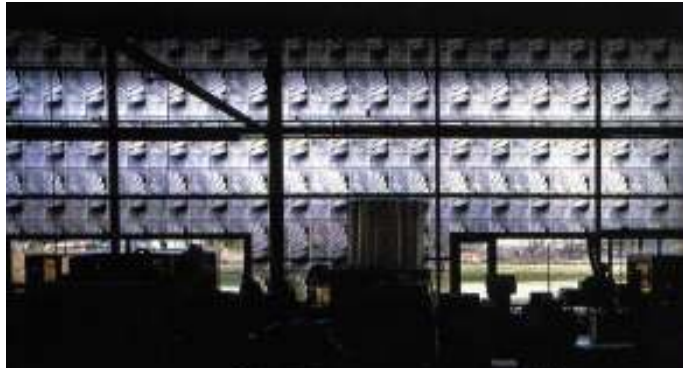
**The metal** – signifies flexibility and concentration. The steel buildings of modern period represented a symbol of technological progress, the metallic structure allowing the evolution of buildings at a higher scale. The metal has brightness, coldness and hardness; in combination with warm natural materials, it can create particular semantic and plastic contrasts. Certain metal types – such as titanium, copper – used as finishing materials, have remarkable plastic qualities, confer an air of preciousity. The artist Anish Kapoor use the reflection and mirroring effects of the metal in order to enhance the artistic expression of the landscaping (fig. 6, 7).



**Fig. 6,7** - Metallic sculptures - Non-object and C-Curve, Londra, 2007, artist Anish Kapoor

**The glass** - by transparency, translucence and capacity of absorbing or reflecting light, it offers various and changing sensations. The glass facilitates the communication between indoor and outdoor space – garden, court -, and the relation with the nature or with the light.

By applying serigraphy with motifs inspired by nature, the glass gain new and interesting effects in the works of the architects Herzog & de Meuron (fig. 8).



**Fig. 8** - Ricola Warehouse, Mullhouse-Brunstatt, 1995, architects Herzog & de Meuron - new aesthetic and semantic effect for the glass serigraphed with nature inspired motifs

## CONCLUSIONS

The contemporary technology opens up new opportunities in the materials processing and the aesthetics of the landscaping. Both the natural and artificial materials, used creatively, not only as aesthetic meaning, but also in the scope of transmitting ideas and concepts, used coherently and adapted to the context of the landscape creation, bring a large aesthetic and semantic diversity in the designed environment, becoming an important resource of identity.

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Fig. 8. © Hisao Suzuki, Peter Gossel & Gabriele Leuthauser - *Architecture in the 20th century*, Taschen, Koln, 2005, p. 541

# TEMPORARY CONSTRUCTIONS AND LANDSCAPE RECOVERY AFTER DISASTERS

## CONSTRUCȚII TEMPORARE ȘI REABILITAREA PEISAJULUI DUPĂ DEZASTRE

PURCARU A.<sup>1</sup>, PURCARU (GRECU) Codrina<sup>1</sup>

e-mail: codrusa@yahoo.com

**Abstract.** *Over time, there have been many examples of natural disasters and beyond, from which entire communities were destroyed and people left homeless had to receive help from authorities. This paper focuses on just this kind of temporary building to house victims, and to what extent these types of construction can contribute to the landscape recovery.*

**Key words:** natural disasters, temporary buildings, landscape recovery.

**Rezumat.** *De-a lungul timpului s-au înregistrat nenumărate exemple de dezastre naturale și nu numai, în urma cărora comunități întregi au fost distruse, iar oamenii rămași fără locuințe au fost nevoiți să primească ajutor de la autorități. Această lucrare analizează tocmai acest tip de construcții temporare pentru adăpostirea sinistraților, și în ce măsură aceste tipuri de construcții contribuie la reabilitarea peisajului înconjurător.*

**Cuvinte cheie:** dezastre naturale, construcții temporare, reabilitarea peisajului.

### INTRODUCTION

This paper proposes an analysis of how technical (constructive) response and the opportunities of natural sites that can compete at a favorable resolution, while identifying key risk generating situations in the current territorial planning and management strategies for emergency situations.

### MATERIAL AND METHODS

Recent years have brought many situations where natural disasters (especially floods) have put Romania in front of some disturbing facts: systems and site improvements designed for risk reduction underwent a continuous degradation, with devastating effects on the occurrence of unwanted "events".

An assessment of the building fund generated by disadvantaged sections of society (both rural and urban outskirts), shows the presence of another major risk to our country: earthquakes.

After such occurrence, the authorities are put in a position to intervene as quickly, primarily to reduce the destructive effects and then to shelter people who are at risk after partial or total destruction of homes.

In this segment, are generated enormous costs related to the necessity of investing resources in building temporary shelters and from the disruption of normal economic activities (people are put for a certain period in a position to reorganize life).

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<sup>1</sup> "Gheorghe Asachi" Technical University of Iași, Romania

This economic and social turmoil fully justifies an approach in developing a constructive system with reasonable cost-performance ratios and analyzing the opportunities generated by landscape recovery with all its components in parallel with the technical and administrative approach.

## RESULTS AND DISCUSSIONS

Literature that addresses the issue of disaster mitigation strategies and their effects identified three types of events, each with its specificities: a first category of events with natural causes, a second with technological reasons (which cover the hazardous materials and waste) and a last category, which has become increasingly global presence, the terrorism.

An analysis of frequent disasters in our country lead us to channel the discussion on natural disasters, terrorist threats and technological events, although generates dramatic effects are less likely.

A variety of unpleasant events, generally described as natural phenomena, can affect a community. Problems arise when these natural phenomena occur in areas inhabited by men: flood plains, in areas exposed to strong winds generating winter snowstorm in proximity of earthquake faults, unstable slopes or in dry areas with increased risk of forest fires.

For an event to be classified as a disaster should have a major impact on community life. Any event on catastrophic proportions affecting unpopulated areas cannot be termed as disaster. These events disrupt the social life in the communities affected, causing resident's relocation and interruption of activities in schools, hospitals and in the administrative area. Are affected in many cases vital infrastructure: water supply, electricity and communications.

Earthquakes, as historical records show, are events that have a major destructive potential.

Interdisciplinary researches done on earthquakes conclude that damages that occur depend on several independent factors:

- characteristics and structure of the seismographic fault;
- earthquake size, evaluated as magnitude;
- frequency of large earthquakes with large magnitude;
- proximity to the epicenter of a community occupying a built environment, served by infrastructure;
- attenuation of seismic waves in relation with the distance from the epicenter;
- specific conditions of sites where ground vibrations can amplify certain frequencies;
- resistance of buildings and infrastructure affected by the earthquake. (Stoltman, 2007)

"Flooding" is only part of the relationship between water and human activities. The term is used to describe the presence of water in territories that are usually dry.



Floods are part of natural processes that are perceived to be catastrophic only in relation to human activities. There is also the reverse this statement: periodic flooding that is taking place in many parts of the world, produces abundance due to the nutrient rich silt deposits.

For a great part of the world population, floods are important events with annual repetition.

Floods are essential to ecological balance in many areas; this phenomenon is a natural part of the hydrological regime of any river.

Floods can be caused by tides or storms at sea, by rivers or streams, because of sewers overflow in urban areas, by the sudden melting of snow, or because of accidents that generate collapse of dams or dikes.

Flooding can occur due to weather conditions developed far away from flooded areas.

In Romania, we are witnessing in recent years disasters due primarily to the development of settlements in flood plains. Other risks for the population are the degradation of hydrotechnical works, massive deforestation and slow response of authorities in the evacuation of those exposed.

Urban planning took into account more recently real estate pressures exerted by population than the need to protect life and property, allowing built development of constructions on flood plains towns.

A particular situation is represented by snowstorms that succeed to block entire regions.

Beyond the lack of organization of authorities taking action to maintain circulation even on main routes of communication, we have seen recently a serious problem: the disappearance of forest belts in the plains lead to the endangering of villages with disruption of normal life for weeks on end.

When this phenomenon becomes apparent, as with the case of floods, the participation of landscape in generating a livable habitat becomes important (stabilizing the banks of major rivers and by setting snow away from settlements).

The first forest protective curtains were made in 1880 in Ialomița. Then in 1884 were planted in southern Oltenia protection curtains with a view to avoid desertification over a length of 95 km with a width of 1-3 km, the *Acacia* species being used.

The effect of these forest curtains was positive in the sense that agricultural crops in that area were much higher.

Until 1937 there was no protective curtains planted, but in that year, this activity was resumed, so in 1957 there were already 9,500 hectares.

In 1969 according to a Decision of the Council of Ministers, it begins the deforestation of protection curtains, in order to create farmland. In the same year, after clearing these curtains, summer winds raised and deposited a thick layer of sand, emphasizing the trend of desertification. Of the existing 9,500 hectares in the period, 1969 to 1980 remained only 900 hectares. (Racolta, 2012)

Only in 2002, the Romanian Parliament adopted Law 289 of May 15, which sought primarily to identify areas where forest belts are required, establishment, management and how to finance this work.

After this brief overview of general issues related to defining risk factors, we will mention that in our country the most common approaches for housing people after disasters generate responses in the area of building "low cost" houses. The idea of creating temporary shelter always generated anguish among both public and especially among those affected.

The problem with the attempts for sustainable development after disasters is that in many cases people are overwhelmed by the seriousness of the situation and the urgency of solving the basic needs for food and shelter. (Phillips, 2009)

Housing is the major problem to be addressed in a process of reconstruction after a disaster. Homes are often the biggest investment that most families can afford, representing approximately 70% of urban area buildings. Housing destruction has a profound influence both on economically and psychosocial level, as few owners can afford the luxury of paying insurance to entirely cover such damage.

The psychological state of those involved is actually worsened by this mechanism: despite the focused economic effort, during post disaster recovery people are getting poor living conditions in tent camps or in public institutions that shelter them.

This research tries an optimization of the administering authority response for such situations by highlighting all the components participating in the phenomenon.

Significant resources are estimate to be allocated in a post disaster situation, with repetition every few years, fact that reinforces the need for judicious planning.

Any response in the area of temporarily housing people exposed in the way of disaster, will certainly take into account both the opportunities "free input" of energy and favorable site conditions. In the light of emerging needs in post-disaster situations, it becomes important to define the term "landscape" beyond the design requirements in terms of sustainable development.

"Landscape" in this context will be defined as that part of the habitat that complements recovery approach for suitable housing conditions with specific contributions: enhancing quality of life, cultural value, ecological value, economic value.

**The impact of the "landscape" factor in disaster recovery economy of resources and processes.** A very popular concept behind the disaster recovery strategies is sustainability. Brenda Phillips identified six principles underlying this concept: building a consensus through a participatory process of recovery, quality of life, economic vitality, equity and environmental quality; prevention to create a disaster resistant. Interesting from our point of view will be the extent to which "landscape" is involved in these processes.

### *Participatory processes*

Putting in discussion a large number of issues will trigger community involvement in decision-making. Expression of these wishes will generate more understanding from the community for unpopular decisions. Those who manage resources for recovery will have to build this "fund of sympathy" on community attachment to the "cultural landscape".

### *Quality of life*

As opposite to the more subtle concept of life quality, pressure to rebuild is felt stronger by most. Including these concerns in the reconstruction effort will generate attachment to the urban landscape and for local identity. Places where we live matters more for each of us than an abstract idea about an "ideal city".

### *Economic vitality*

Economic activities that have survived a disaster are generally activities that were successful and before the disruptive event. Reactivation of economic activities is vital for generating an increased dynamic of community recovery after a disaster, thru fees that are paid and by revenue generated in the community. The resulting economic value of restoring the landscape will be put in balance with the victims need for sheltering. Therefore it is necessary global thinking that can use resources towards a sustainable development of affected human habitat.

### *Equity*

Although not all have the same chance at recovery, a strategy aimed at sustainability, should consider setting up premises for an equitable solutions to problems that arise following a disaster. Noting that the social dynamics is a generator of cultural landscape, a holistic approach should take into account the reverse mechanism: landscape recovery with all its components can compete with other factors to restore social and cultural attachments.

### *Environmental quality*

Post-disaster recovery provides an opportunity to start protecting natural resources and even change how we interact with the environment. Reconstruction can address problems that could not find answered before such as creation of new parks with permeable surfaces to increase water retention, thus avoiding overflows and flooding of those areas.

### *Disaster resistance*

By incorporating preventive measures in the recovery, process after a disaster can reduce the impact of future catastrophic events on communities. There are two forms of disaster mitigation: structural prevention (as addressing anthropogenic structures: buildings, dams, the proportion of surfaces with high water retention to waterproof areas), and non-structural preventive measures

consisting of insurances, alarm systems for those exposed, educational programs and "planning".

## CONCLUSIONS

As a final word, landscape rehabilitation in parallel with housing and facilities rehabilitation after disasters should be a basic component of coherent regional planning and prevention of losses both in economic and social area. It is also especially important the consolidation of the public confidence in the responsiveness of the authorities.

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# NEW SYSTEMS FOR BUILDINGS CONFORT

## NOI SISTEME PENTRU ASIGURAREA CONFORTULUI ÎN CLĂDIRI

*PURCARU (GRECU) Codrina<sup>1</sup>, PURCARU A.<sup>1</sup>*  
e-mail: codrusa@yahoo.com

**Abstract.** *Comfort in buildings is determined by several factors among which we mention the state of thermal comfort, sound, smell, sight and touch, not to mention the psychological or environmental factors. In the beginning of the millennium the world is facing many challenges more or less serious. One of the challenges in buildings domain is just finding the perfect balance between achieving the safety, health and comfort and of course ensuring energy efficiency according to the driving sustainable development. This paper examines some constructive systems that can meet the conditions of hygiene, comfort and efficient energy, and underline the solution of combining two already known and widely used systems: the ventilated facades and Canadian or Provençal well. The vegetation enhance the ambiental effect of this sistem and it may be located within interior ventilated facades or to outdoor of buildings envelope.*

**Key words:** thermal comfort, natural ventilation, Canadian well, vertical gardens

**Rezumat.** *Confortul în clădiri este determinat de o serie de factori între care enumerăm starea de confort termic, acustic, olfactiv, vizual și tactil, ca să nu mai amintim de factorii psihologici sau ambientali. În acest început de mileniu lumea se confruntă cu o multitudine de provocări mai mult sau mai puțin grave. Una dintre provocările domeniului construcțiilor este însăși găsirea echilibrului perfect între realizarea condițiilor de siguranță, igienă și confort concomitent cu asigurarea unei eficiențe energetice în acord cu principiile Dezvoltării Durabile. Lucrarea de față analizează câteva sisteme constructive care pot satisface condițiile de igienă, confort și eficiență energetică și indică o soluție în combinarea a două sisteme deja cunoscute și larg utilizate: fațada ventilată și puțul canadian sau provençal. La potențarea efectelor de ambientare, va contribui și vegetația, care poate fi amplasată spațial în cadrul sistemului nou creat, în interior sau în exterior.*

**Cuvinte cheie:** confort termic, ventilare naturală, puț canadian, grădini verticale

### INTRODUCTION

This paper will highlight aspects of comfort parameters in buildings and natural ventilation of buildings, also new methods and technologies applied to meet sustainable development in construction: safety, durability, hygiene, and comfort and energy efficiency.

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<sup>1</sup> "Gheorghe Asachi" Technical University of Iași, Romania

## MATERIAL AND METHODS

Humans can get feeling of comfort depending on several factors and a rather limited range of values of temperature and humidity. Natural ventilation of buildings, indispensable for indoor air quality and comfort, requires some energy for heating /cooling of fresh air. Natural ventilation of buildings can be achieved either by opening windows or channels or special towers for ventilation, where air is circulated through the temperature and pressure differences between inside and outside the building and the air's natural tendency to climb, leaving cooler air to enter the building on the lower level. One of the applications of natural ventilation, efficient in saving energy for preheating / precooling air ventilation, is the Canadian (Provençal) well. In principle, this system requires the introduction of air into the building through a channel placed at a certain depth in the soil, to raise the temperature in cold season, and for cooling during the warm season.

## RESULTS AND DISCUSSIONS

Below, we illustrate, according to the author's previous work (Purcaru, 2011, a), several examples of energy efficient buildings that use multiple methods and technologies for conservation and green energy production, meeting all conditions hygiene and comfort of international public buildings. It also presents the results of an experiment on natural ventilation in buildings and construction elements, made in the Technical University "Gheorghe Asachi" of Iasi, Faculty of Civil Engineering and Services.

Building for offices and training facilities to tax consulting in Stuttgart, Germany (Hindrichs and Klaus, 2007) is near the city center and business centers zone, at the intersection of two major traffic arteries, being visible from all directions.

Building envelope was designed to meet all customer requirements and location: the office is very well lit and naturally ventilated, but still not exposed to noise and pollution inherent to a dense traffic area. So we opted for a double glass façade, naturally ventilated through a heat tunnel, located in the basement, with the sectional area of  $1 \text{ m}^2$ , the length of 500 m, and the walls 20 cm thick concrete to store the heat of air in summer, and then releasing it in winter (fig. 1). Outside air is introduced into the building pre-cooled or pre-heated according to season, during its passage through that tunnel and then entered in the ventilated façade through vertical pipes. Stack effect of air promotes its distribution at each level of the building.



**Fig. 1-** Thermal labyrinth in horizontal section (left) and ventilated façade section (right), (Hindrichs and Klaus, 2007).

"Solar XXI" is one of high energy efficiency buildings, built in Lisbon, Portugal and operational in 2006. According to this building project is based on several concepts that promote energy efficiency in buildings, such as good insulation outside the building, the orientation most commonly occupied areas to the south, proper shading of the windows of these spaces, photovoltaic panels, underground piping system for precooling / preheating air needed in building ventilation (fig. 2), lighting and natural ventilation of the building.



**Fig. 2** – Detail in the plan (left) and section (draфта) of underground piping system for building ventilation and air conditioning, (Oliveira Pano and Gonsalves, 2011)

"Energon" is the largest building in Europe constructed according to passive house standards whose project started in a competition held in 2000 (Faigle, 2005). In the next year, they began the construction of the building, and finalized after a year. This building has five levels distributed around an atrium of the same height (fig. 3, left), covered with glass panels and integrate in its structure all the techniques and most efficient solutions to save and recover heat and power and also the green energy production techniques (photovoltaic panels integrated into roof).



**Fig. 3** – Overview of the EnerGon building exterior (left), natural ventilation circuit diagram of the last level (middle), air vents absorption system for underground wells (right), (Faigle B.,2005), (Lindemann, 2007)

Among these techniques show the following (Faigle, 2005; Lindemann, 2007): special prefabricated curtain walling façades curve shape, having a high thermal insulation, both in the opaque and glazed area; glass-covered central atrium acts as a distributor of fresh air in offices, air conditioning with an active thermal concrete core (fig. 3, middle); heating is done by a biomass boiler in proportion of 80%, but also by heat recovery devices for cooled IT area and kitchen area; cooling due to 40 geothermal wells, each one with 100m depth (fig. 3, right).

During the first year of operation they have done researches to evaluate energy efficiency and building costs, technical equipment, but also comfort, highlighting the efficiency and performance of the building, compared with other buildings in the same category, but not use technologies mentioned above.

In our country has made a first step in developing research programs in passive building technologies, currently being built by the Polytechnic University of Bucharest, an passive house experimental building equipped with Canadian well. Monitoring behavior of this building after its using, it will provide a very useful material for the improvement and generalization of the system.

On Technical University of Iași, in the author's doctoral research is done an experiment that simulated a small-scale natural ventilation of a ventilated façade channel coupled to a Canadian well. This experiment followed the potential energy of the ground, but at the same time avoiding adverse aspects of this system. Typically, Canadian shaft introduced the pre-cooled or pre-heated air directly into the room, including the possible infiltration of radon, odors, fungus, spores that grow in underground pipes system.



**Fig. 4** - Overview of experimental model of a Canadian well pipe coupled to ventilated facade channel (right) detail of Canadian well model immersed in water (left)

The operating principle of the model (fig. 4) is based on the criterion of similarity Grashof, which says that the report between buoyancy force and friction force is constant. Thus, compliance the conditions of equation (1)

$$(1) \quad (\Delta T L^3)_{\text{prototype}} = (\Delta T L^3)_{\text{model}},$$

where  $\Delta T$  is temperature difference and  $L$  is the length, which provides to the model conditions similar to reality, would require, in addition to geometric similarity (the model is 1:5 scale) to work with much larger temperature differences than those from nature and would not be acceptable in the laboratory ( $1000^\circ\text{C}$ ). According to the previous proposal in Department of Civil Engineering, (Radu et al., 2008) it may proceed backwards, applying small temperature differences to model-scale and inferring temperature differences much lower to natural scale.

Following measurements (temperature and air velocity in ventilated channel, in coil, in water and in lab) concluded that the air circulation through the Canadian well-ventilated façade is provided only by the temperature difference that occurs in reality on ventilated façade channel direct sunlighted in summer. In winter, heat trapped in the canal, inside the building, can generate air circulation

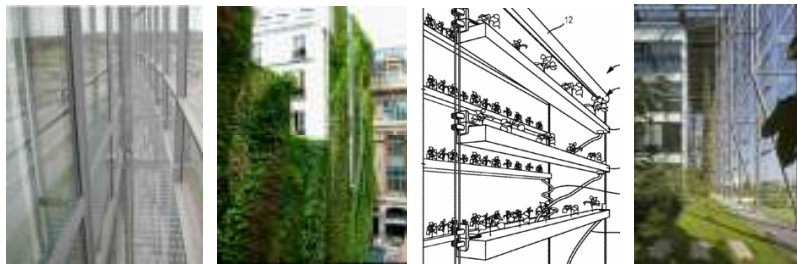


coil absorbs heat underground without a fan, as it appears in a Canadian well functioning documentation.

All this pre-heating / pre-cooling air systems in a ventilated façade can also be used to give maximum value in winter, to the exterior vertical garden made by one famous botanist, Patrick Blanc (fig. 5b) mainly for aesthetic reasons, and to improve indoor air quality in crowded urban spaces or outdoors (Blank, 2012).

Mentioned is the fact that there are specialized plants that can eliminate certain types of pollutants and placing them in a ventilated double skin façade (fig. 5a, d) can significantly improve air quality in the building. For example, according to (Purcaru, 2011, b) for a single day in an office, a species of ivy is able to remove 90% of benzol content and released by tobacco smoke, synthetic fibers, or dyes and plastics. Aloe vera, banana, spider plants and philodendron are effective against the agents of formaldehyde from insulation foam and particle board. Trichlorethylene in paints and glues is best removed with chrysanthemums and gerberas.

Speaking of these vertical gardens are not neglected energy efficiency qualities of buildings: protects against heat in summer and from cold winter. If these vertical gardens or other plant vertically growth systems as shown in the drawing system patented by Adams (Adams et al., 2012) (fig. 5c) would be placed between the glass walls of a naturally ventilated double skin façades with help of one Canadian well, the results of energy efficiency, air quality and ventilation thus generated would be much improved. But energy efficiency results not only of this proposal can be confirmed by further research conducted for this purpose.



**Fig. 5** – In order from left to right are: a. Picture inside of a ventilated double skin façades (Blomsberg, 2007), b. Image of vertical garden attached to a parisian building turbot (Blanc, 2012), c. Detail of greenhouse with vertical plant growth system (Adams et al., 2012), d. Vertical gardens between two walls of glass (Marani, 2011)

## CONCLUSIONS

In conclusion, natural ventilation in buildings, presented as complex system of Canadian well and ventilated façade, along with all methods and green technologies, including the presence and role of plants on blind walls or between glass façades can be harmoniously integrated in the design of complex buildings and their surrounding environment.

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**Consilier editorial:**

**Vasile VÎNTU**

**Tehnoredactori:**

**Liliana ROTARU  
Liliana Elena CHELARIU**

**Corectori:**

**Lucia DRAGHIA  
Liliana ROTARU  
Gică GRĂDINARIU**

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**Vasile VÎNTU**

**Technical Editors:**

**Liliana ROTARU  
Liliana Elena CHELARIU**

**Readers:**

**Lucia DRAGHIA  
Liliana ROTARU  
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