ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

UNIVERSITATEA DE ŞTIINȚE AGRICOLE
ŞI MEDICINĂ VETERINARĂ
“ION IONESCU DE LA BRAD” IAŞI

LUCRĂRI ŞTIINȚIFICE

Vol. 57

NR. 1

SERIA HORTICULTURĂ

EDITURA “ION IONESCU DE LA BRAD”

IAŞI 2014

ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

“Ion Ionescu de la Brad” Publishing House Iaşi
ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

CONTENT

CUPRINS

CHAOS CONTROL OF CHAOTIC CHEMICAL SYSTEMS

CONTROLUL SISTEMELOR CHIMICE HAOTICE

OANCEA Servilia[1], OANCEA A.V.[2], GROSU I.[3]
e-mail: liaoancea@yahoo.com

Abstract. *Chemical systems can exhibit chaotic behaviour and this fact is very important for chemical processes and for biological structures. From this point of view the control of these phenomena have a great practical impact despite the fact that it is very dificult; this is the reason the theoretical models are useful in these situations. The control using these models can give the informations about the selfcontrol inside the biological structures where the behaviour of the dynamic systems is realized by a feedback mechanism. The main aim of this paper is to study the synchronization of two chemical chaotic systems proposed by Samardzija and which is based on 9 rections and 3 intermediary species, using the adaptive feedback method of control. The transient time until synchronization depends on initial conditions of two systems, the strength and the number of the controllers.*
Key words: *chaotic chemical system, chaos control*

Rezumat. *Sistemele chimice pot avea comportare haotică şi acest fat este foarte important pentru procesele chimice şi structurile biologice. Din acest punct de vedere controlul acestor fenomene are un mare impact in ciuda faptului că este foarte dificilş acesta este motivul pentru care modelele teoretice sunt utile în aceste situaţii. Contolul sistemelor pe baza acestor modele poate da informaţii despre autocontrolul din structurile biologice unde comportarea sistemelor dinamice se realizează printr-un mecanism de feedback. Scopul principal al acestei lucrări este de a studia sincronizarea a două sisteme chimice propuse de Samardzija care se bazează pe 9 reacţii şi 3 specii intermediare, folosind o metodă de control de tip feedback. Timpul după care se obţine sincronizarea depinde de condiţiile initiale ale celor doua sisteme si de intensitatea controler-ului.*
Cuvinte cheie: *sistem chimic haotic, controlul haosului*

INTRODUCTION

Chemical reaction systems have become one of the favorite domains to study nonlinear systems, both experimentally and theoretically. These systems can exhibit chaotic behaviour and this fact is very important for chemical processes and for biological structures. From this point of view the deliberate control of these phenomena have a great practical impact despite the fact that it is very dificult; this is the reason the theoretical models are useful in these situations. In

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
[2] Erasmus Mundus Advanced Spectroscopy in Chemistry Master, Bologna University, Italy
[3] "Al. I. Cuza" University of Iasi, Romania

addition, the control using these models can give the informations about the selfcontrol inside the biological structures where the behaviour of the dynamic systems is realized by a feedback mechanism. Over the last decade, there has been considerable progress in generalizing the concept of synchronization to include the case of coupled chaotic oscillators especially from technical reasons. When the complete synchronization is achieved, the states of both systems become practically identical, while their dynamics in time remains chaotic. Many examples of synchronization have been documented in the literature, but currently theoretical understanding of the phenomena lags behind experimental studies (Grosu, 1997; Grosu et al., 2008; Lerescu et al., 2004; Lerescu et al., 2006; Oancea et al., 2009; Oancea et al., 2011). The main aim of this paper is to study the synchronization of two chemical chaotic systems based on the adaptive feedback method of control. One of these chemical models was proposed by Samardzija and it is based on 9 rections and 3 intermediary species.

THEORY

The model proposed by Samardzija represents some chemical reactions and its mechanism consists in the following elementary steps (Wang and Chen, 2010):

1. $A_1 + X + 2Z \xleftrightarrow{k_1} X + 3Z$
2. $X + Y \xleftrightarrow{k_2} 2Y$
3. $A_2 + X + Z \xleftrightarrow{k_3} Z + P_1$
4. $A_3 + X + Z \xleftrightarrow{k_4} X + P_2$
5. $A_4 + 2Y \xleftrightarrow{k_5} 3Y$
6. $2Z \xleftrightarrow{k_6} P_3$
7. $A_5 + X \xleftrightarrow{k_7} 2X$
8. $Y \xleftrightarrow{k_8} P_4$
9. $A_6 + Z \xleftrightarrow{k_9} 2Z$

The time evolution of the intermediary species X, Y , and Z is given by a nonlinear system of equations:

$$\begin{aligned}
\frac{dx_1}{dt} &= -k_2 x_1 x_2 - k_3 x_1 x_3 + k_7 x_1 \\
\frac{dx_2}{dt} &= k_2 x_1 x_2 + k_5 x_2^{\,2} - k_8 x_2 \\
\frac{dx_3}{dt} &= k_1 x_1 x_3^{\,2} - k_4 x_1 x_3 - 2k_6 x_3^{\,2} + k_9 x_3
\end{aligned} \tag{1}$$

This system has a chaotic behaviour, for the following constants:

$k_1 = 1$ $k_2 = 2$ $k_3 = 1.5$ $k_4 = 20$ $k_5 = 0.8$

k_6=12.85 k_7 = 45 k_9=514.2

1. Chaotic dynamics of chemical system

Coosing k_8 as a control parameter, we can know dynamics of this system.

For k_8 =50 the strange attractor for this system is given in the figure 1.

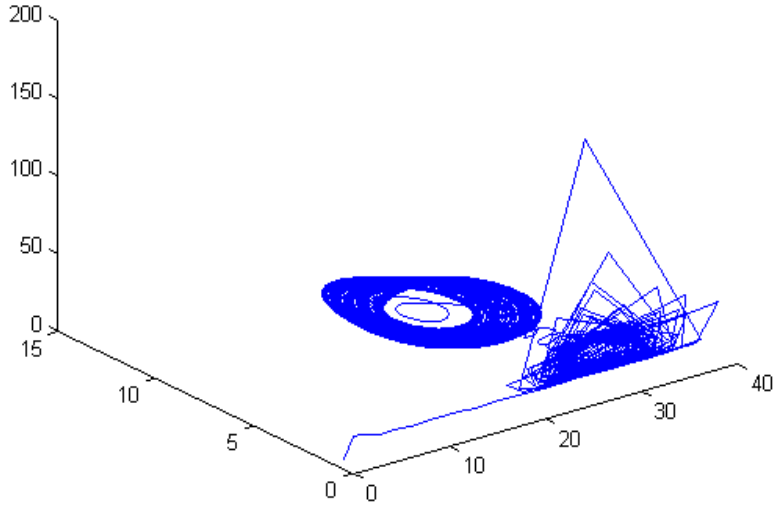
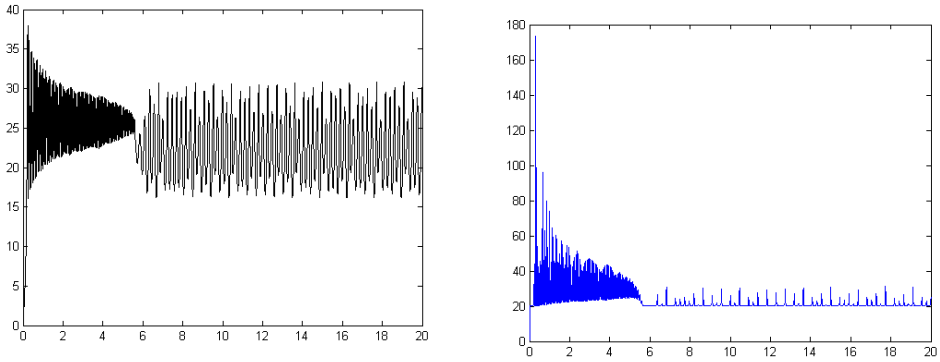


Fig. 1 – Phase portrait of (x_3, x_1, x_2,) for system (1) with initial conditions 1 1 1

The dynamics of the this chaotic chemical system is given in figure 2.



a

Fig. 2 – a-x_1(t); b- x_3(t) for k_8 =50;

The chaotic behavior is sustained by Lyapunov exponents from figure 3.

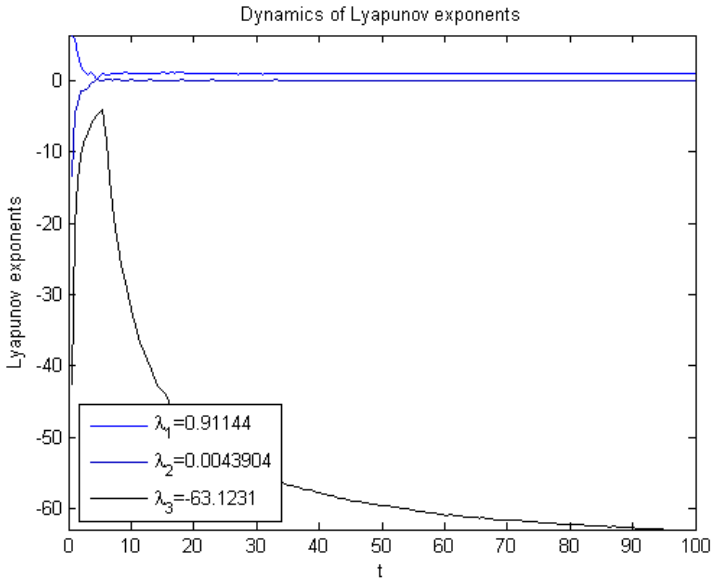


Fig. 3 – The Lyapunov exponents

This system is very sensitive to initial condition. Then we choose k_8 =43

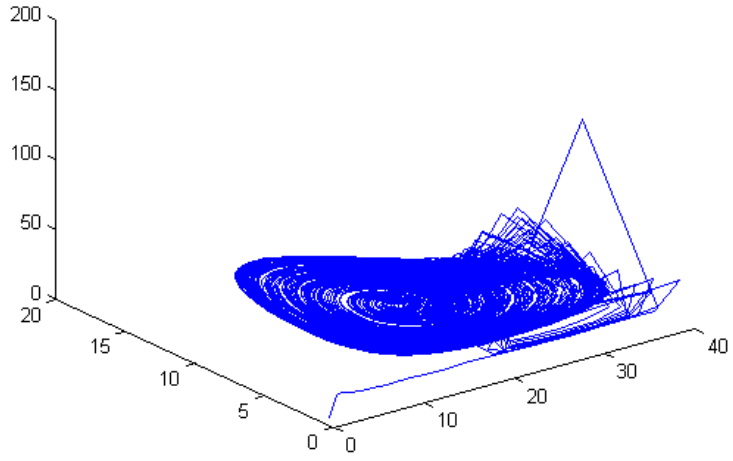
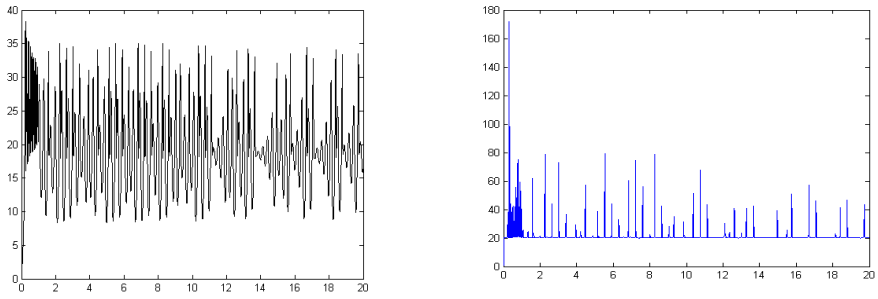


Fig. 4 – 3D attractor (x_3, x_1, x_2) for k_8 =43

Figure 5 shows the changes of the variable x and z with time for k_8 =43.



a b

Fig. 5 – a-x_1(t); b- x_3(t) for k_8 =43;

2. Synchronization of two chaotic systems

To synchronize two identical chemical syststems we followed the method proposed by Guo et al. [8], Hu and Xu [9], based on Lyapunov-Lasalle theory. Let be a chaotic non-autonomous system:

$$\dot{x} = f(x,t) \quad \text{where } x = (x_1, x_2 \ldots)^T \in R^n$$

is the state vector of the system and $f = (f_1, f_2 ..)^T \in R^n$ is the non-linear vector field of the system, which is considered as a driving system.

For any $x = (x_1, x_2 \ldots)^T \in R^n$ and $y = (y_1, y_2 \ldots)^T \in R^n$

there exists a positive constant l such that:

$$\left| f(x,t) - f(y,t) \right| \le l \max \left| x_i - x_j \right| \quad \text{i, j=1,2...n}$$

The slave system will be: $\dot{y} = f(y,t) + z(z_1, z_2, \ldots)$ where $z(z_1, z_2, \ldots)$ is the controller. If the error vector is e = y - x, the objective of synchronization is to make

$$\lim \left\| e(t) \right\| \to 0$$

$$t \to +\infty$$

The controller is of the form: $z_i = \varepsilon_i (y_i - x_i)$ and $\dot{\varepsilon}_i = -\gamma_i e_i^2$, i=1,2...n

and γ_i , i = 1,2,. . . , n are arbitrary positive constants.

RESULTS AND DISCUSSION

According this method of synchronization, the slave system for this chemical system will be:

$$\frac{dy_1}{dt} = -2y_1y_2 - 1.5y_1y_3 + 45y_1 + z_1(y_1 - x_1)$$

$$\frac{dy_2}{dt} = 2y_1y_2 + 0.8{y_2}^2 - k_8y_2 + z_2(y_2 - x_2)$$

$$\frac{dy_3}{dt} = y_1{y_3}^2 - 20y_1y_3 - 25.7{y_3}^2 + 514.2y_3 + z_3(y_3 - x_3) \qquad (2)$$

and for the control strength:

$$\dot{z}_1 = -(y_1 - x_1)^2$$
$$\dot{z}_2 = -(y_2 - x_2)^2$$
$$\dot{z}_3 = -(y_3 - x_3)^2 \qquad (3)$$

Figures 6-9 demonstrate the syncronization of the two chemical systems.

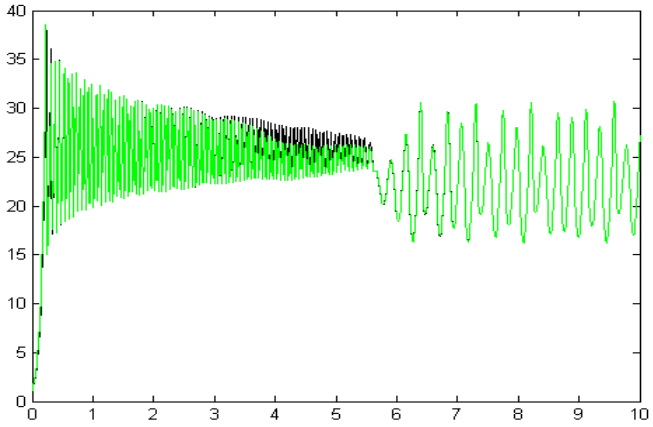


Fig. 6 – x1(t)- black y1(t)- green[$x_1(0)$=1, $x_2(0)$=1, $x_3(0)$=1; $y_1(0)$ =1.1; $y_2(0)$ = 1.1 $y_3(0)$ = 1.1; $z_1(0)$ =1; $z_2(0)$ = 1 ; $z_3(0)$ =1]

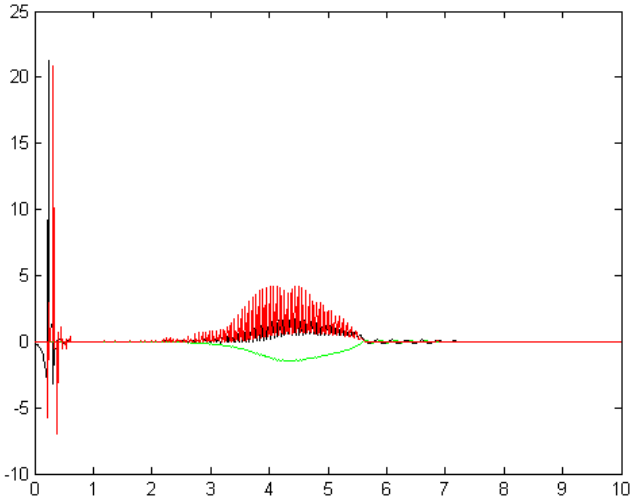
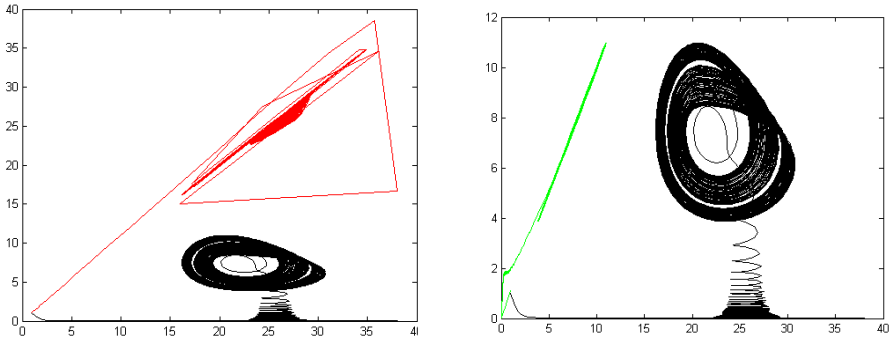


Fig. 7 – Synchronization errors between master and slave systems [$x_1(0)$=1, $x_2(0)$=1, $x_3(0)$=1; $y_1(0)$ =1.1; $y_2(0)$ = 1.1 $y_3(0)$ = 1.1; $z_1(0)$ =1; $z_2(0)$ = 1 ; $z_3(0)$ =1]



a b

Fig. 8 – Phase portrait of a) :(x_1, x_2-black) and (x_1,y_1-red); b): (x_1, x_2-black) and (x_2,y_2-green) for two systems [$x_1(0)$=1, $x_2(0)$=1, $x_3(0)$=1; $y_1(0)$ =1.1; $y_2(0)$ = 1.1 $y_3(0)$ = 1.1; $z_1(0)$ =1; $z_2(0)$ = 1 ; $z_3(0)$ =1]

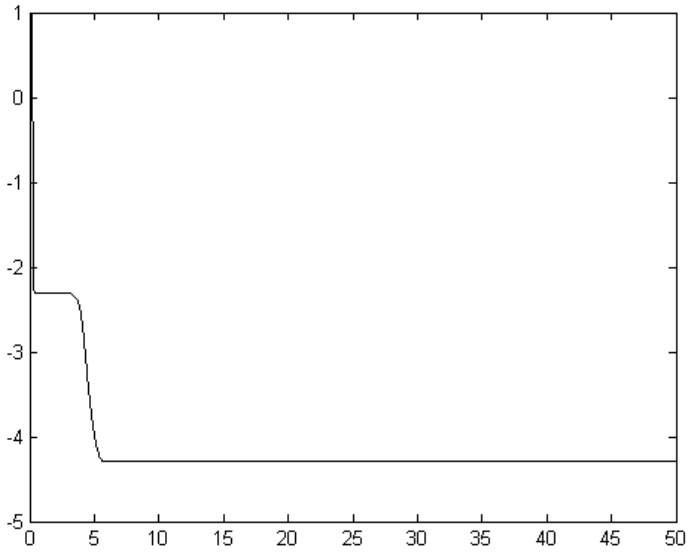


Fig. 8 – The control strength z_1[$x_1(0)$=1, $x_2(0)$=1, $x_3(0)$=1; $y_1(0)$ =1.1; $y_2(0)$ = 1.1 $y_3(0)$ = 1.1; $z_1(0)$ =1; $z_2(0)$ = 1 ; $z_3(0)$ =1]

Debin Huang (2005), by testing the chaotic systems including the Lorenz system, Rossler system, Chua's circuit, and the Sprott's collection of the simplest chaotic flows found that we can use a single controller to achieve identical synchronization of a three-dimensional system (for Lorenz system this is possible only we add the controller in the second equation).

For these systems we achieved the synchronization if one controller is applied only in the first or in the second equation (fig. 9).

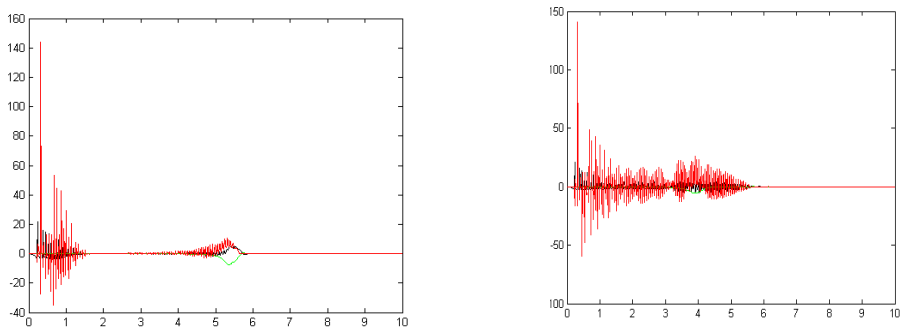


Fig. 9 – Synchronization errors between master and slave for chemical systems with one controller [$x_1(0)=1$, $x_2(0)=1$, $x_3(0)=1$; $y_1(0)=1.1$; $y_2(0)=1.1$ $y_3(0)=1.1$; a- $z_1(0)=1$; b- [$x_1(0)=1$, $x_2(0)=1$, $x_3(0)=1$; $y_1(0)=1.5$; $y_2(0)=1.5$ $y_3(0)=1.5$; $z_2(0)=1$]

CONCLUSIONS

In this work we analized the dynamics of the Samardzija system wich is based on 9 rections and 3 intermediary species and we realized the synchronization of two systems using an adaptive feedback method. The transient time until synchronization depends on initial conditions of two systems, the strength of the controllers and their number. Then we can control this chemical system in accordance with recent debates of Wang and Chen (2010) about full global synchronization and partial synchronization in a system of two or three coupled chemical chaotic oscillators.

REFERENCES

1. **Grosu I., 1997** -*Robust Synchronization*, Phys. Rev. 56, pp. 3709-3712
2. **Grosu I., Padmanaban E., Roy P. K., Dana S. K., 2008** - *Designing Coupling for Synchronization and Amplification of Chaos*, Phys Rev Lett 100, 234102, pp. 1-4
3. **Lerescu A.I., Constandache N., Oancea S., Grosu I., 2004** - *Collection of master-slave synchronized chaotic systems*, Chaos Soliton Fract., 22(3), pp. 599-604
4. **Lerescu A.I., Oancea S., Grosu I., 2006 -** *Collection of Mutually Synchronized Chaotic Systems,* Physics Letters A, 352, pp. 222-228.
5. **Oancea S., Grosu F., Lazar A., Grosu I., 2009 -** *Master–slave synchronization of Lorenz systems using a single controller*, Chaos, Solitons and Fractals, 41, pp. 2575-2580
6. **Oancea S., Grosu I., Oancea A.V., 2011 -** *Biological control based on the synchronization of Lotka-Volterra systems with four competitive species*, Rom. J. Biophys, 21(1), pp. 17-26
7. **Wang J.W., Chen A.M., 2010 -** *Partial synchronization in coupled chemical chaotic oscillators*, Journal of Computational and Applied Mathematics, 233, pp. 1897-1904
8. **Guo W., Chen S., Zhou H., 2009 -** *A simple adaptive-feedback controller for chaos Synchronization*, Chaos, Solitons and Fractals, 39, pp. 316–321
9. **Hu M., Yang Y., Xu Z., Guo L., 2008 -** *Hybrid projective synchronization in a chaotic complex nonlinear system*, Mathematics and Computer in Simulation, 79, pp. 449-457
10. **Huang D., 2005 -** *Simple adaptive-feedback controller for identical chaos synchronization*, Phys. Rev. E, 71, 037203

SYNTHESIS OF SULFOCHLORIDE DERIVATIVES OF THE ARYL OXYALKYL CARBOXYLIC ACIDS AS INTERMEDIATES IN OBTAINING COMPOUNDS WITH BIOLOGICAL POTENTIAL

SINTEZA SULFOCLORURILOR ACIZILOR ARIL-OXIALCHIL CARBOXILICI CA INTERMEDIARI ÎN OBȚINEREA UNOR COMPUȘI CU POTENȚIAL BIOLOGIC

TROFIN Alina[1], ONISCU C.[2], UNGUREANU Elena[1]
e-mail**:** atrofin@uaiasi.ro

Abstract. *Aryl - oxyalkyl carboxylic acids and their derivatives are compounds with high biologic potential, having various pharmacological properties or auxin - type growth regulator action. The pharmacological tests determined that the presence of substituted or unsubstituted sulphonamidic groups in the phenoxyacetic derivatives confers them a toxicity which in most cases is negligible; they also have high bioavailability and can be used as effective growth stimulants for various plant species at low concentrations. Since the R_1, R_2 - substituted phenoxyacetic acid esters and their sulfochlorides are intermediates used in the synthesis, this paper presents the general scheme of the process for the chlorosulfonation of the considered aryl - oxyalkyl carboxylic esters, the mechanism of the reaction, the obtaining method and the sulfochlorides yields for the methyl esters of fenil-1,2-dioxiacetic, fenil-1,3-dioxiacetic şi fenil-1,4-dioxiacetic acids in the reaction with the chlorosulfonic acid.*
Key words: *phenoxyacetic, methyl esters, sulphochloride, growth regulator*

Rezumat. *Acizii aril-oxialchil carboxilici şi derivaţii lor fac parte dintre compuşii chimici cu un potenţial biologic ridicat, având diverse proprietăţi farmacologice sau regulatoare de creştere de tip auxinic. Prin teste farmacologice efectuate asupra compuşilor sintetizaţi s-a determinat că prezenţa grupărilor sulfonamidice, substituite sau nesubstituite, în moleculele derivaţilor fenoxiacetici conferă produselor finale o toxicitate de cele mai multe ori neglijabilă; de asemenea, au o biodisponibilitate ridicată şi pot fi folosite ca produse stimulatoare de creştere eficiente pentru diverse specii de plante, în concentraţii mici. Deoarece atât esterii acizilor fenoxiacetici R_1,R_2-substituiţi cât şi sulfoclorurile acestora sunt intermediari folosiţi în sinteză, în această lucrare au fost realizate studii referitoare la schema generală a procesului de clorosulfonare pentru esterii aril-oxialchil carboxilici luaţi în considerare, mecanismul de reacţie, metoda de obţinere şi randamentele în sulfocloruri pentru esterilor metilici ai acizilor fenil-1,2-dioxiacetic, fenil-1,3-dioxiacetic şi fenil-1,4-dioxiacetic în reacţia cu acidul clorsulfonic.*
Cuvinte cheie: *fenoxiacetic, esteri metilici, sulfoclorură, regulator creştere*

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
[2] "Gh. Asachi" Technical University of Iasi, Romania

The aryl - oxyalkyl carboxylic acids and their derivatives are chemical compounds with high biologic potential, having various pharmacological properties. Numerous compounds from this class enter into the composition of drugs with different use:

- the radical of the 2,3-dichloro phenoxyacetic acid is contained in the structures of two diuretic drugs: *Tricrinafen* and *Edecrin*;

- the radical of the 2,6-dichloro phenoxyacetic acid enters in the structure of *Lofexidine*, with central antihypertensive action;

- various anti-inflammatory drugs have phenoxyacetic or α-phenoxypropionic structure: *Ibufenac* (derives from phenoxyacetic acid), *Paduden* (derives from α-phenoxypropionic acid), *Aclofenac*, *Fenclofenac*, *Fenprofen*, *Percluson*;

- A number of products with antibacterial activity have in their structure phenoxyacetic radicals (*Penicillin V*), α-phenoxypropionyl (*Feneticiline*) and α-phenoxybutyryl (*Propiciline*);

- *Meclofenoxat* with a psycho energizing action is a derivative of p-chloro phenoxyacetic acid, as well as Iproclozide, used as an analeptic for the thymus (Oniscu, 1988);

- a number of derivatives of phenoxyisobutyric acid have lipid-lowering properties: *Beclobrat*, *Clofibrat*, *Fenofibrat*, *Teofibrat*;

Some phenoxyalkyl carboxylic derivatives have remarkable applications in agriculture, like the acids: 2,4- dichloro phenoxyacetic (2,4-D acid), 2,4,5-trichloro phenoxyacetic (2,4,5-T acid) with selective herbicide action and α-(2,4,5-trichloro phenoxy)-propionic acid (2,4,5-TP acid) used as selective herbicide in corn and cotton cultures.

Used in small doses, these products have growth regulating action. In case of exceeding the effective dose, phenoxyacetic acids turn into defoliants. For example, the 1 : 1 mixture between 2,4,5-T acid and 2,4-D acid butyl esters was used by U.S.A. as defoliant in the Vietnam War, under the name of "orange agent" (Neamtu and Irimie, 1991).

The α-(2-methyl, 4-chloro phenoxy)-propionic acid (2M-4CP acid) destroys weeds resistant to other herbicides, being used in cereal crops protection.

The α-(2,4-dichloro phenoxy)-propionic acid (2,4-DP acid) stimulates fruit growth and prevents their fall before harvest (Comanita et all., 1986).

A series of γ-phenoxybutyric acids are highly selective herbicides: γ-(2,4-dichloro phenoxy)-butyric acid (2,4-DB acid) or γ-(2-methyl, 4-chloro phenoxy)-butyric acid (MCPB acid) etc.

C. Oniscu and coworkers synthesized a series of esters, amides and hydrazides of the phenoxyalkyl carboxylic acids, as well as derivatives of the phenoxyalkyl carboxylic acids with sulphonamide group (Oniscu, 1968; Botez and Oniscu, 1972), which represent a new class of growth stimulators. From this class of compounds, two substances with auxinic action were tested in sugar beet, carrots, grapevine, and roses cultures, with remarkable results, allowing the

product 2-sulphonamide, 4-chloro phenoxy-acetic acid's approval as sugar beet crop growth stimulator under the name of ASFAC.

Pharmacological tests performed on other novel compounds synthesized by the same group determined that their toxicity is almost zero; also they have a high bioavailability and remarkable neurostimulation, antidepressants and anticonvulsants properties (Nigovic et all., 1996).

A particularly valuable compound obtained from research in the class of phenoxyalkyl carboxylic sulphonamides derivatives is dimethylaminoethyl hydrochloride ester of the 2-chloro-4-sulphonyl dimethylamido phenoxyacetic acid (Romener), an efficient metabolic regulator of nerve cell, energizer, antidepressant and antipsychotic.

MATERIAL AND METHOD

The general scheme for obtaining the derivatives of aryl- oxyalkyl carboxylic sulphonamides comprises the following steps:

- R-phenoxyacetic acids obtained from the corresponding phenols by condensation with monochloroacetic acid in an alkaline solution (NaOH);
- obtaining the methyl, ethyl etc. esters of these acids;
- esters' chlorosulfonation;
- chlorosulfonated esters' condensation with ammonia, substituted amines or other compounds with amino groups (Dumitrascu, 1998)

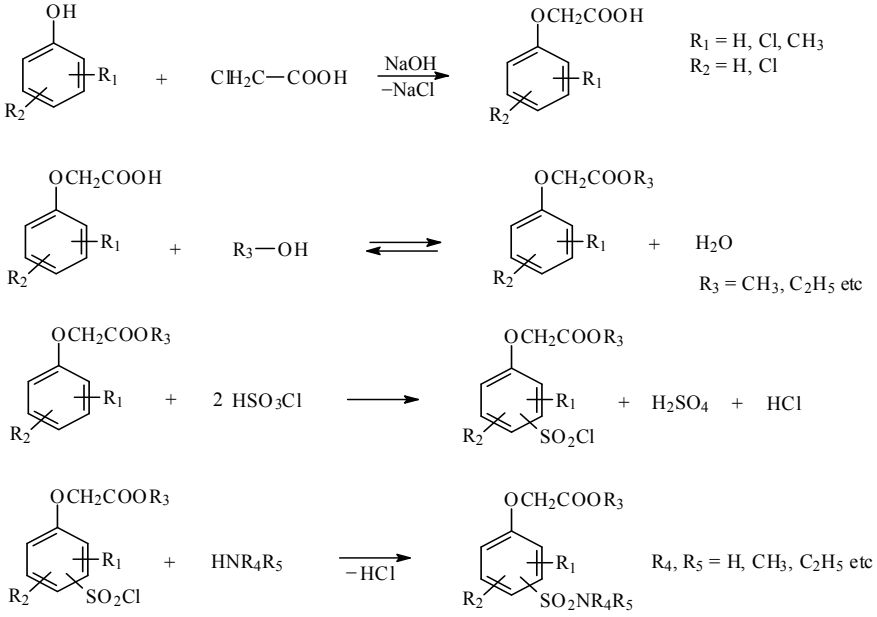


Fig. 1 - The reaction scheme for obtaining sulphonyl amido-phenoxyacetic derivatives

The same steps of the general scheme for obtaining the derivatives of aryl oxyalkyl carboxylic acids sulphonamides are followed also to synthesize compounds

containing in their structure two oxyacetic groups grafted in various positions of the aromatic nucleus.

Since the R1, R2-substituted phenoxyacetic acid esters and their sulfochlorides are intermediates used in the synthesis of all the compounds prepared by the sequence of reactions described above, studies were conducted on obtaining them.

Chlorosulfonation reaction mechanism, according to the literature (7), is shown in the diagram below (fig. 2) indicating that in the first step takes place the sulphonation with SO_3 and in the next step the sulphonic acid is converted to sulphochloride.

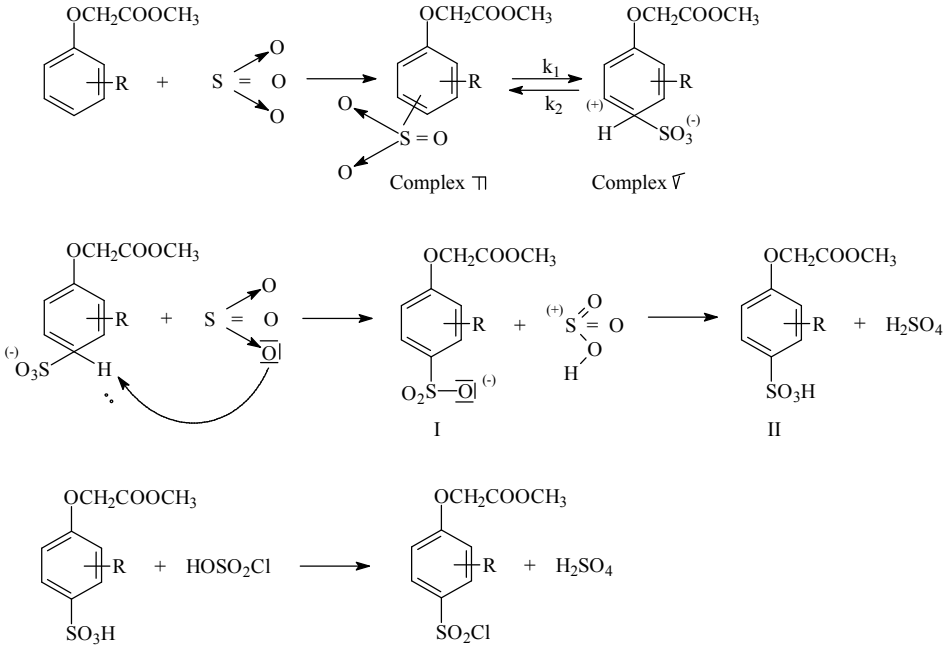


Fig. 2 - Chlorosulfonation reaction mechanism

Obtaining the sulfochlorides of the phenoxyacetic and phenyl-dioxyacetic acids methyl esters was performed according to the scheme shown in figure 1.

The general procedure for preparation is as follows:

- over 0.7 moles of chlorosulfonic acid cooled to 0 - 50°C are added in small portions, with continuous stirring, 0.1 moles methyl ester, so that the temperature does not exceed 5^0C;

- after the addition of the ester, the reaction mixture is maintained at the same temperature 30 - 40 min and then the temperature is raised to values specific to the type of the ester, maintaining the temperature for 90 - 100 minutes, when the formation of the sulphochloride takes place;

- finally, the mixture is cooled to 5 - 6^0C and poured into a mixture of water and ice, under vigorous stirring, to destroy the unreacted chlorosulfonic acid and to precipitate the sulphochloride;

- the obtained sulfochloride is filtered, water washed until pH = 6.5, is recrystallized from a mixture of water - acetone (2: 1 volume ratio) or benzene, then is dried at temperatures $\leq 40^0$C.

RESULTS AND DISCUSSIONS

According to the literature data on the chlorosulphonation of the aryl-oxyacetic acids (Oniscu, 1968), the chlorosulphonation of the phenyl dioxyacetic esters with chlorosulphonic acid is a process of pseudo equilibrium which can be kinetically described by the equation:

$$\frac{C_{SCl}}{C_{E_S}} = \frac{k_1}{k_2} \frac{C_{E_0}(M-1) - C_{SCl}}{C_{C_0} + C_{SCl}} \qquad (5)$$

in which: C_{SCl}=momentary concentration in sulphochloride (moles/l); C_{Es} = momentary concentration in sulphonic ester (moles/l); C_{Eo} = initial concentration of the ester (moles/l); C_{Co}=initial concentration of the sulfuric acid in the chlorosulphonic acid; M=the ratio between the concentration of the chlorosulphonic acid and the ester's concentration; k_1=rate constant for the formation of sulphochloride (l/moll·h); k_2=rate constant for the transformation of sulphochloride into sulphonic acid, determined by the sulfuric acid (l/mol · h).

Also, the literature (Oniscu, 1968) states that the molar ratio between the aryl-oxyalkyl carboxylic esters and the chlorosulfonic acid in the process of chlorosulphonation is 1:7. Based on these data, we initially set the optimal value in the chlorosulphonation process of the phenyl dioxyacetic esters of 1: 6. Under these circumstances, we observed the influence of reaction temperature on the chlorosulphonation of the phenyl dioxyacetic esters.

The chlorosulphonation of the ***phenyl-1,2-dioxyacetic acid's methyl ester*** was done by treating 0.6 mole of chlorosulphonic acid to 0.1 moles ester at a temperature of 0 - 5^0C. After merging the reactants, we raised the temperature at different values and then maintained it for one hour. Finally, the reaction mixture was diluted with ice + water mixture when the sulphochloride ester is separated.

We worked with temperature between 15 – 35^0C, and the obtained results are presented in table 1.

Table1.

Temperature (^{0}C)	15	20	25	30	35
η (%)	55	75	86	94	88

The chlorosulphonation of the ***phenyl-1,3-dioxyacetic acid's methyl ester*** followed the same steps described above, with the completion of the reaction at temperatures in the range of 10 – 25^0C. The obtained results are presented in table 2.

Table 2.

Temperature (^{0}C)	10	15	20	25
η (%)	60	85	92	89

The chlorosulphonation of the ***phenyl-1,4-dioxyacetic acid's methyl ester*** was also carried out under the conditions shown above and the completion of the reaction was carried out at temperatures between 35 – 60^0C, for one hour. The obtained results are presented in table 3.

Table 3.

Temperature (^{0}C)	35	40	45	50	60
η (%)	32	60	72	80	75

Representing graphically using the coordinates η - t^0C, the data presented in the tables above shows that for each case there is an optimum temperature range (fig. 3).

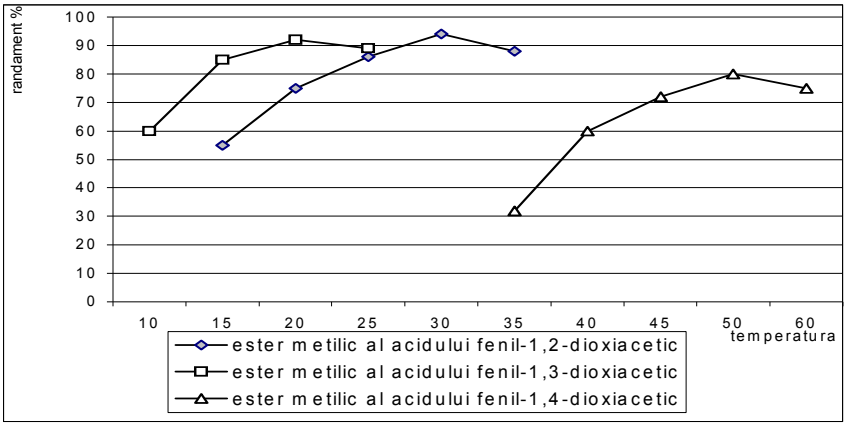


Fig. 3 - The variation of efficiency with temperature in the chlorosulphonation process

The minimum reaction temperature, of approximately 20^0C, is noted for the phenyl-1,3-dioxyacetic ester, fact explained by the concurring orientation of the two oxyacetic acid groups existing in the phenyl ring.

CONCLUSIONS

1. The molar ratio between the reactants in the chlorosulphonation process was established at 1:6 (dioxyacetic ester:chlorosulphonic acid);

2. The optimum reaction temperatures for the three obtained esters were: 30^0C for the phenyl-1,2-dioxyacetic acid's methyl ester when we obtained the best efficiency value – 94%, 20^0C for the phenyl-1,3-dioxyacetic acid's methyl ester when we obtained the highest efficiency value of 92% and 50^0C for the phenyl-1,4-dioxyacetic acid's methyl ester when we obtained 80% efficiency.

REFERENCES

1. Botez Gh., Oniscu C.,**1972** - *Brevet RO nr. 56123*

2. Comăniță Eugenia, Șoldea Camelia, Dumitrescu Elena, **1986** - *Chimia și tehnologia pesticidelor*. Editura Tehnică, București.

3. Dumitrașcu Adina,**1998** – *Teză de doctorat*, Univ. Tehnică "Gh. Asachi" Iași

4. Neamțu G., Irimie F., **1991** - *Fitoregulatori de creștere – Aspecte biochimice și fiziologice,* Editura Ceres, București.

5. Nigovic B., Kojik-Prodic Biserka, Antolic S., Tomic Sanja, Puntarec V., and Cohen J.D., **1996** - *Structural Studies on Monohalogenated Derivatives of the Phytohormone Indole-3-acetic Acid(Auxin)*, Acta Cryst., B52, 332-343.

6. Oniscu C., **1968** - *Cinetica reacției de clorosulfonare a esterilor acizilor clor-fenoxiacetici,* Bul. Instit. Polit. Iași, 8 (XIX), 231

7. Oniscu C., **1988** – *Chimia și tehnologia medicamentelor*, Ed. Tehnică, București

PYRROLOPYRIDAZINE DERIVATIVES SUBSTITUED WITH FLUOR: SYNTHESIS AND FLUORESCENT PROPRIETIES

DERIVAŢI PIROLOPIRIDAZINICI SUBSTITUIŢI CU FLUOR: SINTEZĂ ŞI STUDIUL PROPRIETĂŢILOR FLUORESCENTE

TUCALIUC Roxana Angela [1], TRINCĂ Carmen Lucia[1], MANGALAGIU I.[2]
e-mail: roxanatucaliuc@yahoo.com

Abstract. *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, in classical conditions and using microwave irradiation. For 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. *Derivaţii 1,2-diazinici sunt compuşi cu proprietăţi deosebite în medicină (anti-HIV, medicamente antivirale şi împotriva cancerului, proprietăţi antibacteriene şi antifungice), cu proprietăţi opto-electronice (compuşi cu proprietăţi de cristale lichide şi produse derivate foarte fluorescente: senzori şi biosenzori materiale electroluminiscente, lasere) şi în agricultură (compuşi cu activitate erbicidă şi stimulatori în creşterea şi dezvoltarea plantelor). Reacţiile de cicloadiţie 1,3-dipolare sunt cea mai accesibilă metodă în sinteza derivaţilor pirolopiridazinici, în condiţii clasice şi sub acţiunea microundelor. Pentru derivaţii sintetizaţi au fost înregistrate spectrele de absobţie şi emisie, în etanol, cloroform şi ciclohexan la temperatura camerei.*
Cuvinte cheie*: derivaţi pirolopiridazinici, fluorescenţă, cicloadiţii 3+2 dipolare.*

INTRODUCTION

1,2-diazines are reviewed in literature for their applications: compounds with different biological activities (anticancer, antituberculosis, antimicrobial, antihypertensive etc.), opto-electronics properties (fluorescent derivatives used as sensors and biosensors, electroluminescent materials, lasers and other semiconductor devices) and compounds with liquid crystal properties (Mangalagiu, 2011). Herbicidal activity and grow up factor for plants are also reviewed (Mitsumori et al., 2005; Valeur, 2002).

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
[2] "Al. I. Cuza" University of Iasi, Romania

In a preliminary communication (Zbancioc et al., 2006; Butnariu et al., 2009; Tucaliuc et al., 2013) is presented the synthesis and spectral analysis of pyrrolopyridazine derivatives. The reaction pathway involves, in the most frequent cases, a Huisgen [3+2] dipolar cycloaddition of ylides to dipolarophiles (activated alkenes and alkynes).

However, this strategy has some disadvantages: lack of control over stereo- and regioselectivity, long reaction times, high energy consumption, and sometimes, low yields.

During the last few decades microwave irradiation (MW) has become an increasingly valuable tool in organic chemistry, since it offers a versatile and facile pathway in a variety of syntheses.

Furthermore, interphase transfer catalysis reactions under MW conditions have the great advantage of using small amounts of, or even no organic solvents ('solvent free'), such reactions are more environmentally friendly and generate less side products (Van der Eycken et al., 2006; Loupy, 2002).

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 and 2. The preparation of all derivatives (**9a, 9b', 9b'', 9c, 10a, 10b, 10c)** involves two steps: initially N-alkylation of the pyridazine (**1**), fig. 1, 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 and alkynes nonsymmetrical substitued: ethyl 4,4,4-trifluorocrotonate and ethyl 4,4,4-trifluorobutinoate), fig. 2.

When the dipolarophile was ethyl 4,4,4-trifluorocrotonate (*trans*-isomer, nonsymmetrically diactivated olefine) the reactions involved additional stereo and regiochemical problems, in one therm chorochemistry (Epiotis, 1978). While for ylides **8a** and **8c** the reaction occur chorospecifically, for ylide **8b** (R = Cl) they occur choroselectively**,** after flash chromatography and crystallization from an appropriate solvent, we recovered an inseparable mixture of two regisomers (**9b'**and **9b''**, 1:1)**.**

The reaction with ethyl 4,4,4-trifluorobutinoate leads to the aromatised pyrrolopyridazine **10a-c**. Aromatisation of the initially hyrogenated diazine **iii** occurs spontaneously and could be explained by oxidative dehydrogenation.

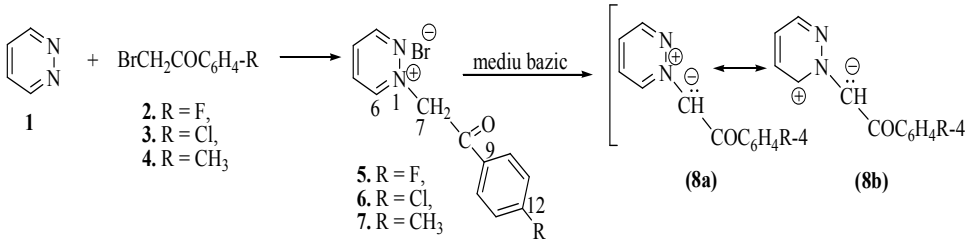


Fig. 1 - N-alkylation of the pyridazine.

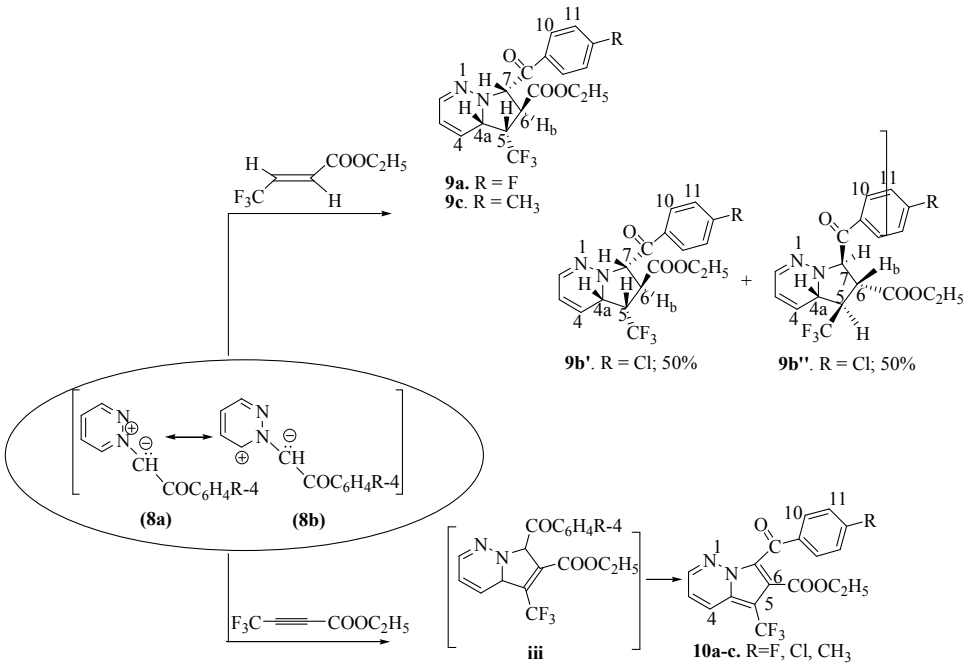


Fig. 2 - 3 +2 dipolar cycloaddition of diazinium ylides.

MW assisted reactions were carried out using a monomod reactor (STAR-2, CHEM corporation, USA). Table 1 lists the optimized conditions, under MW and classical heating. Using MW irradiation, in liquid phase, the best results were obtained applying a constant irradiation power (25% of the full power of the magnetron, 50 W) and varying the temperature ("power control").

Attention was then focused on interphase transfer catalysis reactions. In this study, the solid phase was a mixture of potassium fluoride and N-(p-R-phenacyl)-pyridazinium bromides; the liquid phase consisted of dipolarophiles dissolved in trioctyl-methyl-ammonium chloride–Aliquat 336 (a tensioactive compound that acts as transfer catalyst). The resultant biphasic system is subjected to the action of microwaves using the monomode reactor at 50 W. The best results have been obtained by applying a constant temperature and varying the irradiation power („temperature control").

We presume that the MW heating approach is more effective in [3+2] dipolar cycloaddition reactions due to two factors: the mode of action under MW irradiation and the structure of the ylide intermediate.

It is well known that the magnetic field component of MW radiation is responsible for the dielectric heating effect. The greater the dipole moment of the molecule, the larger the effect of the MW energy will be. The ylides having a 1,2-dipolar structure are excellent dipoles and, therefore, the efficiency of MW heating increases considerably when compared with classical heating.

The results listed in table 1, show the efficiency of the MW irradiation in comparison with the classical heating: the yields were increased in some cases, and the amount of solvent required was reduced.

Table 1

Cycloaddition reactions of pyridazinium ylides with activated alkenes and alkynes under microwave heating and classical conditions

Compd.	Classical		Microwaves			
	Reaction time/min	Yield %	Liquid phase		Interphasic transfer catalysis (KF-Aliquat)	
			Reaction time/min	Yield %	Reaction time/min	Yield %
9a	180	14	5	10	15	-
9b'+9b''	180	16	5	11	15	-
9c	180	9	5	7	15	-
10a	180	38	5	59	15	56
10b	180	41	5	59	15	52
10c	180	46	5	68	15	58

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 1H NMR and 13C 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 spectrophotometerin 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.

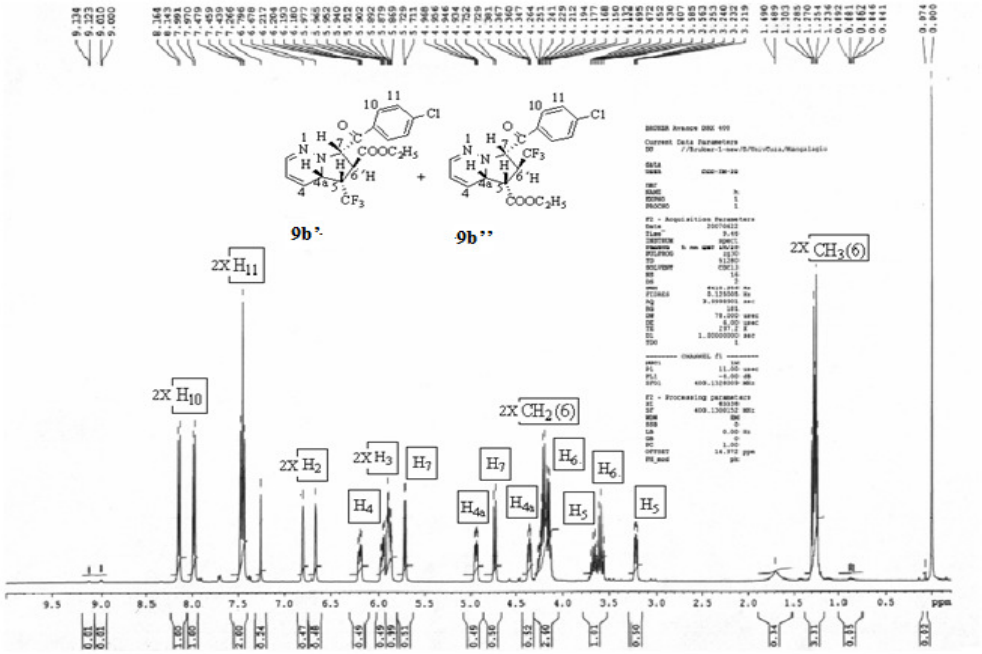


Fig. 3 - 1H-NMR spectrum for compounds **9b'+9b''**.

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 λ_{ex} = 365 nm and λ_{em} = 410–460 nm.

Relative quantum yields were determined by using anthracene in ethanol (= 0,27 at 25° C) (Parker, 1986). Although, compounds are relatively similar in molecular structure, exhibit clear differences in their experimental absorption and emission spectra, as summarised in table 2.

Table 2

λmax (nm) of absorption spectra and relative quantum yields (%) of piridazine derivatives

Comp.	Fluorescence (λ_{max}, nm) (quantum yield %)			Absorption (λ_{max}, nm)		
	Etanol	Cloroform	Ciclohexan	Etanol	Cloroform	Ciclohexan
9a	420	416	Insolubile	315	320	Insolubile
9b'+9b''	418	414	Insolubile	314	322	Insolubile
9c	430	424	Insolubile	318	320	Insolubile
10a	450	447	Insolubile	330	327	Insolubile
10b	452	449	Insolubile	332	325	Insolubile
10c	456	453	Insolubile	332	331	Insolubile

RESULTS AND DISCUSSIONS

The results listed in table 1 show the efficiency of the MW irradiation in comparison with the classical heating: the yields were increased in some cases, and the amount of solvent required was reduced.

As shown in table 2, the componds are blue emitters (λ_{max} of fluorescence around 420-456 nm, λ_{max} of absorption around 320-331 nm) and have low quantum yield.

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

If pyrroloderivatides were fully aromatised, 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 piridazine ring, , both in liquid phase and interphasic transfer catalysis.

2. The microwaves induceds a remarkable acceleration of the [3+2] dipolar cycloaddition reaction of pyridazinium ylides to activated alkene and alkyne and allowed a general and facile method for the preparation of pyrrolopyridazine derivatives.

3. Stereo-, regio- and chorochemistry of the cycloadditions were studied.

4. The compounds obtained and tested posses fluorescent proprieties (λ_{max} of fluorescence is around 420-456 nm, λ_{max} of absorption is around 320-331 nm).

5. A certain influence of the substituents concerning absorption and fluorescent properties were observed: the subtituent from the position 5 being important for fluorescence.

RFERENCES

1. **Butnariu R., Mangalagiu I., 2009 -** *New pyridazine derivatives: Synthesis, chemistry and biological activity*. Bioorg. Med. Chem., 174, pp. 2823-2829.
2. **Epiotis N.D., 1978 -** *Theory of Organic Reactions*. Springer: Berlin, p. 34.
3. **Loupy A., 2002 -** *Microwaves in Organic Synthesis*, Wiley, Weinheim, Germany.
4. **Mangalagiu I. I., 2011 –** *Recent Achievements in the Chemistry of 1,2-Diazines,* Curr. Org. Chem., 15, pp. 730-752.
5. **Mitsumori T., Craig I. M., Martini, I. B. Schwartz B. J., Wudl F., 2005 -** Macromolecules, 38, pp. 4698–4704.
6. **Parker C. A., 1986 -** *Photoluminescence of Solutions*, Elsevier, Amsterdam.
7. **Tucaliuc R., Cotea V. V., Niculaua M., Tuchilus C., Mantu D., Mangalagiu I. I., 2013 -** *New pyridazine-fluorine derivatives: Synthesis, chemistry and biological activity. Part II*. European Journal of Medicinal Chemistry, 67, pp. 367-372.
8. **Valeur B., 2002 -** *Molecular Fluorescence*, WileyVCH, Weinheim.
9. **Van der Eycken E., Kappe O., 2006 -** *Microwave-Assisted Synthesis of Heterocycles*, Springer-Verlag, Berlin, Heidelberg, Germany.
10. **Zbancioc G., Mangalagiu I., 2006-** *Microwave-Assisted Synthesis of Highly Fluorescent Pyrrolopyridazine Derivatives,* Synlett, 5, pp. 804-806.
11. **Zbancioc G., Mangalagiu I., 2010 –** *Pyrrolodiazine derivatives as blue organic luminophores: synthesis and properties,* Tetrahedron, 66, pp. 278-282.

TECHNIQUES FOR ESTABLISH OPTIMAL VALUES OF DIMENSIONING VARIABLES USED IN CORRECT PRINTING OF THE TECHNICAL DRAWING

TEHNICI DE STABILIRE A VALORILOR OPTIME ALE VARIABILELOR DE COTARE PENTRU IMPRIMAREA CORECTĂ A PLANȘELOR DE DESEN TEHNIC

SLONOVSCHI A.[1], PRUNĂ L.[1]
e-mail: andreislonovschi@yahoo.com

Abstract. *The achievement, with the computer graphics help, by using AutoCAD software, of the technical drawings is apparently a well-known issue by many of users. But the multitude of stages that lead, finally to the printed form of a technical drawing, starting with the loading and establishing the properties of the layers and ending with the printing process, makes this action to be not an easy task. From practical experience, the authors know the fact that between all the stages required to obtain the printed form of a drawing the phases that may pose the most problems are in number of three. The first is the one that refers to the choosing a standard format for printing. The second takes into account the case when the users can not represent or represent incorrectly, on the same format, multiple representations using different scales. Finally, the third concerns the situation in that the users can't indicate the representation scale. Taking to account these inconvenient, the authors have developed a working way that comes to support of users and facilitate the understanding process of the stages listed above.*
Key words: *technical drawing, dimensioning, representation scale, format, CAD*

Rezumat. *Realizarea asistată de calculator, utilizând programul AutoCAD, ale planşelor de desen tehnic este un domeniu aparent pe deplin cunoscut de mulți utilizatori. Dar multitudinea de etape ce conduc, în cele din urmă la forma tipărită a unui desen tehnic, începând cu încărcarea şi stabilirea proprietățile straturilor şi terminând cu procesul de imprimare, face ca această acțiune să nu fie o sarcină ușoară. Din experiența practică, autorii cunosc faptul că dintre toate etapele necesare pentru a obține forma tipărită a unui desen, fazele care pot prezenta cele mai multe probleme sunt în număr a trei. Prima este cea care se referă la alegerea unui format standard pentru tipărire. Cea de a doua ia în considerare cazul în care utilizatorii nu pot reprezenta sau reprezintă în mod incorect, pe acelaşi format, mai multe reprezentări folosind scări diferite. În cele din urmă, a treia se referă la situația în care utilizatorii nu pot indica scara de reprezentare. Luând în considerare aceste inconveniente, autorii au dezvoltat un mod de lucru care vine în sprijinul utilizatorilor şi facilitează procesul de înțelegere a etapelor enumerate mai sus.*
Cuvinte cheie: *desen tehnic, cotare, scară de reprezentare, format, CAD*

[1]"Gh. Asachi" Technical University of Iaşi, Romania

INTRODUCTION

Realisation assisted by computer, by using the computer program AutoCAD, of the technical drawings is an area where "everyone knows" because any graphical representation is essentially a conglomeration of line segments, rectangles, convex polygons, circles, arcs, ellipses and so on, and if the one who achieves the drawing knows both AutoCAD and the rules of the civil or industrial technical drawing, he may easily obtain the desired drawing.

It is known the fact that the achievement, by the classical method, using the technical drawing instruments (ruler, compass, pencil), of the technical drawings assume:

- choosing of a standard format of representation;
- establishment of one or more representation scales;
- reducing all distances to the appropriate scales;
- fitting to page of graphical representations;
- representation of the elements;
- achievement dimensioning.

Realisation assisted by computer, by using the computer program AutoCAD of the technical drawings, is totally different face to classical way and, if one of stages is not known or fully understand, the risk that the drawing be incorrectly is high.

From experience, the authors established that, during the time of technical drawing achievement, when AutoCAD is used, from the multitude of stages, the great majority of users:

- does not set correctly, depending on the used unit measure for the drawing, the unit measure for the standardised formats;
- does not fit correctly the graphical representation into printing format;
- cannot represent or represents incorrectly, on the same drawing, more graphical representations realised to different scales;
- cannot specify the adopted scale or scales.

Taking to account these inconvenient, the authors developed a strategy of work that comes to support the AutoCAD users and facilitate the understanding of stages listed above.

MATERIAL AND METHOD

The working mode proposed by the authors is designed as a flowchart and for the correct performance of all steps listed above AutoCAD users simply must carefully follow the logical scheme and to execute the steps outlined.

In order to verify the effectiveness of the logical scheme the authors have proposed to test it on several types of representations, both assembly drawings and details drawings, because in this way it is possible to cover a large range of representation scales (Table 1) and standard formats (Table 2). (Slonovschi A., Prună L., 2010, 2013).

Table 1

The representation scales commonly used and areas for their use

Areas of use of the representation scales	Commonly used representation scales
Construction details	1:1, 1:2, 1:5, 1:10, 1:20
Working drawings	1:20, 1:50, 1:100
Site plan	1:200, 1:500
Site layout plan	1:1000, 1:2000

Table 2

The standard layouts and theirs size according to adopted unit of measure, millimetre, centimetre, and meter

The standardized formats	Sizes		
	millimetre (mm)	centimetre (cm)	meter (m)
A_4	210 x 297	21 x 29.7	0.21 x 0.297
A_3	420 x 297	42 x 29.7	0.42 x 0.297
A_2	594 x 420	59.4 x 42	0.594 x 0.42
A_1	841 x 594	84.1 x 59.4	0.841 x 0.594
A_0	1189 x 841	118.9 x 84.1	1.189 x 0.841

Testing was done on a group of 27 students.

Each student received the next two AutoCAD files (*.dwg):

- metal pole S_1; representation and basic details and capital.
- plan formwork - reinforcement plan GS_2.

The general characteristics of the two drawings are: all representations are made on 1:1 scale, there isn't any dimension placed on drawing and isn't established any standard format.

Regarding on the first drawing:

- was realized in millimetres;
- the representation of the pole does not change and does not scales;
- basic and capital details does not change but must be done to a bigger scale face to the existing one.

Regarding to the second drawing:

- was realized in centimetres;
- the representation of the beam does not change and does not scales;
- the sections through the beam does not change but must be represented to a bigger scale than the existing one.

The logical schema shown in figure 1 was made in such way that, in both cases, the students to resolve the next requirements:

- to establish, according to the units of measure used to the realization of the drawing, the units of measure for the standardized formats;
- to optimally fit, to a standardized format, the assembly, the metal pole S_1 and the plan formwork - reinforcement plan GS_2;
- to specify the adopted format;
- to specify the standardized representation scale that was adopted for the assembly;

to establish for details (basic details and capital and the sections through the beam) an optimum standardized scale;

- scaling the joining details with a value of scale chosen in such way that to the print, the details to be represented to the scale established to the previous step;
- to verify, in both situations, if the assembly and the details drawing are optimal framed to the chosen standardized format;
- in case that, the represented elements exceeded the chosen format, the students must adopt a new format or they must modify the representation scale of the details until all are fit within chosen format.

Because the logical schema is complex, the authors took the decision to note the boxes with numbers and letters and to explain separately the content of these.

Figure 1 represents the first part of logical schema through that the users are directed to individual branches in that all reports are done according to the adopted units of measure, millimeter, centimeter or meter.

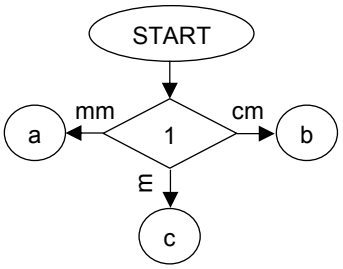


Fig. 1 - The first part of the logical schema

The components of this part of logical schema are:

- 1 – units of measure in that the drawing was made;
  - ✓ a – the branch in that all settings and values are according to millimetre;
  - ✓ b – the branch in that all settings and values are according to centimetre;
  - ✓ c – the branch in that all settings and values are according to meter.

If the branch "a" is continued the logical schema looks like in figure 2.

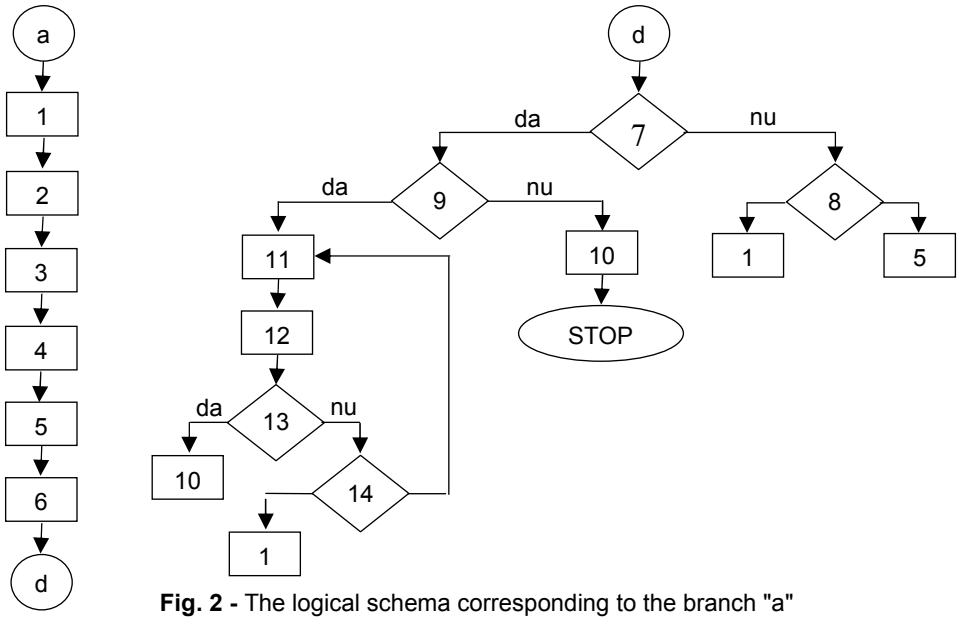


Fig. 2 - The logical schema corresponding to the branch "a"

The components from branch "a" of the logical schema are:

- 1 – one of formats A_4, A_3, A_2, A_1, A_0 are adopted;
- 2 – whatever format the title block has the sizes 185 x 40 (mm);
- 3 – the format and the title block are represented, using the command **RECTANGLE** with the sizes chosen previously;
- 4 – The representation scales can be reported to the values 1, 2, 5 or multiples with 10, above unit or below par of these numbers, thus:
  - ✓1:1;
  - ✓2:1, 5:1, 10:1;
  - ✓1:2, 1:5, 1:10;
  - ✓1:20. 1:50, 1:100, etc.
- 5 – will adopt a scale;
- 6 – the scale of the format and title block is modified using SCALE command, with a value inversely proportional of representation scale value, presented in step 3, thus:
  - ✓ if the adopted scale is 1:1 the scaling factor has value 1;
  - ✓ if the adopted scale is 1:n the scaling factor has value n;
  - ✓ if the adopted scale is n:1 the scaling factor has value 1/n.
- 7 – shall be determined whether the format adopted in step 1 and scaled in step 6 best fits in the representation received;
- 8 – it changes the adopted format in step 1 or the chosen scale in step 5;
- 9 – the drawing contains representations that could be realized at different scales?;
- 10 – it continues with the dimensions settings realization and with the dimensioning of the drawing;
- 11 – shall be determined a scale for realization of the details , according with table 3:

Table 3

The schema of determining of the representation scales

The representation scale of the assembly, adopted in step 5	The possible representation scale of the detail	Ratio of scales	The possible representation scale of the detail
1:100	1:50	100 / 50 = 2	2
	1:20	100 / 20 = 5	5
	1:10	100 / 10 = 10	10
1:50	1:20	50 / 20 = 2.5	2.5
	1:10	50 / 10 = 5	5
	1:5	50 / 5 = 10	10

- 12 – it scales detail/details with a value of the scale factor according to the ratio between the representation scale of the assembly and desired representation scale of detail/details;
- 13 – shall be determined if, both plane of assembly and detail/details scaled are optimally fit in the adopted format at step 1;
- 14 – it changes the representation format adopted at step 1 or the chosen representation scale of the detail at stage 11.

The logical schema corresponding to the branch "b" (drawing realized in centimetres) is different from the logical schema corresponding to the branch "a" only by the first two steps, because both the format sizes and the title block sizes must be

given in centimetres.

The logical schema corresponding to the branch "c" (drawing realized in meters) differs from the logical schema corresponding to the branch "a" only by the first two steps, because both the format sizes and title block sizes must be given in meter.

RESULTS AND DISCUSSIONS

As a result of the application by the students in the two groups of the logical schema, were consisted the following:

- a rate of 85.19% can follow the logical schema and:
  - ✓ can establish correctly the standardized printing format and the unit of measure used for specifying the sizes of the format;
  - ✓ can represent correctly, on the same drawing, multiple representations realized to different scales;
  - ✓ can indicate the representation scale or scales adopted in order to achieve the representations of the assembly and detail.
- a rate of 14.81% can follow the logical schema and:
  - ✓ can correctly establish the printing standardized format of the drawing and the unit of measure used for specify the sizes of the format;
  - ✓ can indicate the adopted scale in order to achieve the assembly representations;

The students from the second group (14,81%) cannot understand why is necessary to represent, on the same drawing, multiple realisations at different scales. From the discussions with them, the authors, have deduced that the students don't know the technical drawing rules regarding to the representation, on the same drawing, of multiple drawings realized at different scales and the advantages given by this type of representations.

Following a brief presentation of the rules and advantages and this small percentage of students was able to correctly represent, on the same drawing, multiple representations at different scales.

CONCLUSIONS

1. The logical scheme proposed by the authors is an indispensable tool for those who want to realize, correctly and rapidly, a series of stages that precede the printing process of any drawing;

2. The breakdown on the types of units of measure (millimetre, centimetre, and meter) presents the advantage that the logical schema can include a diverse array of types of representation and may be successfully used by any student or specialist that wishes to correctly print a technical drawing.

REFERENCES

1. Slonovschi A., Prună, Antonescu I., 2010 – *Desen tehnic pentru construcții.* Ed. PIM, Iași, pp. 22, 27-32, 35-36.

2. Slonovschi., A., Prună L., 2013 - *The International Conference on Engineering Graphic and Design.* Timișoara 13 - 15 June 2013, pp. 27-30.

USING MOODLE TO COLLECT AND ANALIZE THE STUDENT FEEDBACK FORMS FOR TEACHER EVALUATION

UTILIZAREA MOODLE PENTRU COLECTAREA ŞI ANALIZAREA FIŞELOR DE EVALUARE A CADRELOR DIDACTICE

CĂLIN M.[1], CHIRUȚĂ C.[1], TRINCĂ Lucia Carmen[1]
e-mail: mcalin@uaiasi.ro

Abstract. *At the end of each academic semester, the students from USAMV Iasi are asked to fill in a standard assessment sheet to state their opinion on the quality of teaching performed by every teacher they had classes with. The paper proposes the use of the Management Information System capabilities of the Moodle e-learning environment to colect and analize these feedback sheets. This would lead to saving materials, time, and subsequent processing effort.*
Keywords: *Moodle, e-learning, feedback*

Rezumat. *La fiecare sfârşit de semestru, studenții USAMV Iași sunt rugați să completeze câte o fișă de evaluare pentru fiecare cadru didactic cu care au avut ore în semestrul respectiv. În prezent, acest lucru se face folosind formulare de hârtie. Lucrarea propune utilizarea capabilităților de management al informațiilor ale mediului de e learning Moodle pentru colectarea și centralizarea in format electronic a acestor fișe de evaluare. Acest lucru ar duce la economie de materiale, de timp și de efort ulterior pentru procesarea și analiza informațiilor primite.*
Cuvinte cheie: *Moodle, e-learning, feedback*

INTRODUCTION

At the end of each academic semester, the students from USAMV Iasi are asked to fill in a standard assessment sheet to state their opinion on the quality of teaching performed by every teacher they had classes with. Presently, this is done using paper forms that are subsequently processed.

Using the Management Information System capabilities that Moodle has can be used to collect and process such forms in electronic form.

PROPOSED APPROACH

In the beginning of the 2012-2013 academic year, a Moodle e-learning platform was installed and put into use at The University of Agricultural Sciences and Veterinary Medicine of Iasi. Its end users are the university students who benefit from the courses developed by their teachers. More than 4200 users are now registered: the students in the bachelor cycle, and the respective teaching staff.

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

Moodle has good Management Information System capabilities (Hollowell, 2011) that allows using it to colect and analize these feedback sheets in electronic form. This would lead to saving materials, time, and subsequent processing effort.

The general idea is that in Moodle 2.1 the teacher can build, among other activities that he includes within a course, an activity that asks students to state their oppinions on different matters, for example on the level of the course material, or on the quality of the teaching activity. Such an activity is (of course) called *feedback*. It consists of a series of questions that are included within a form. There are several (Rice, 2011) types of questions that can be included: multiple choice (rated or not rated), numeric answers, short text answers etc.

A feedback can be signed or anonymous as the teacher decides when building the activity.

This is how feedbacks work. Students are asked to execute the feedback activity in a certain period of time. Each student fills in the electronic form and return it to the teacher. When the completion time is over, the teacher will look over the received answers. He also has the possibility to view statistiscs that Moodle automatically performs on the set of answers. It is also possible for the teacher to export these statistics in Excel format for further processing.

Following is **the proposed approach** for solving the teaching evaluation task. We will use personas to describe the process. These personas are:

- *Admin* – the Moodle platform administrator
- *Head of Department* – the head of the teaching department in the university where the evaluation takes place
- *Teacher* – a member of the the respective department
- *Students* – the students that had classes with Teacher

The staring point is the building of a template course, say **Evaluation 0**. Admin creates and saves it for further utilisation in several copies. This course contains a single activity: a feedback. This is built accordig to the calssical paper form used to evaluate teaching activity within the university. Figure 1 shows a section of the feedback form (in Romanian). All the question showed in this section are of the type *multiple choice – single answer*. There are also (Rice, 2011; https://docs.moodle.org/21/en/Main_page) other types of questions included in the form: numeric answers for sating levels of satisfaction, and text answers for making personal suggestions.

Using the **Evaluation 0** course, *Admin* will create a course regarding *Teacher*, say **Evaluation *Teacher***. The *Students* would be later enrolled in this course. But also *Admin* sets this course to be managed by *Head of Department*, not by *Teacher*. In other words, through the **Evaluation *Teacher*** course *Head of Department* will ask *Students* for their opinion about *Teacher*.

Admin will create (Büchner, 2011) a separate course for each *Teacher* in the department, and all of these courses will be managed by *Head of Department*.

The students enrolment to the evaluation courses should be made by , but is likely that he will ask Admin for some help in this matter. To accomplish the

enrolments, *Admin* will need to receive from *Head of Department* the groups of *Students* corresponding to each *Teacher*. had classes with must possess this information.

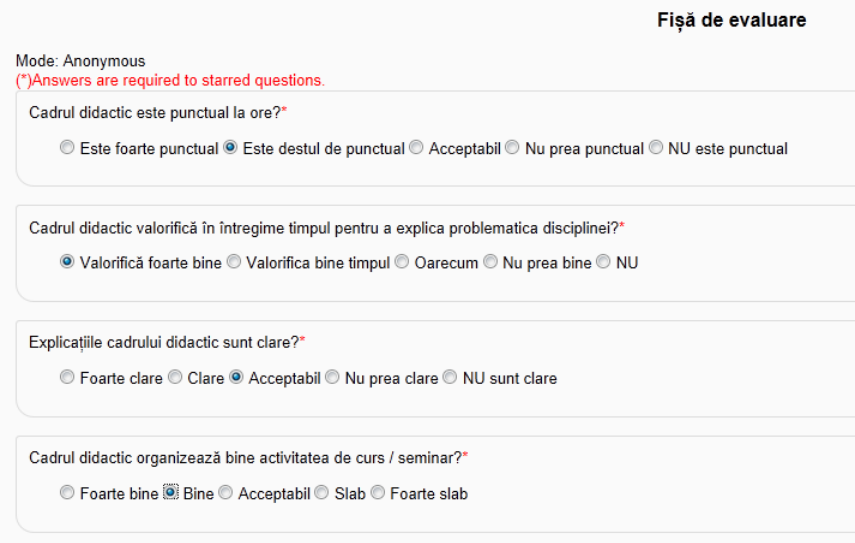


Fig. 1 - A section of the feedback form (in Romanian)

Putting into operation the evaluation process means to ask students to fill in the evaluation forms. Generally speaking, a student that logs in to the e-learning site will only see the list of courses that he is enroled in. Among these, he will see one **Evaluation *Teacher*** course for each *Teacher* he had classes with. These courses will become visible within a certain period of time which is set by *Head of Department* who manages them. In this period of time all the students should access and perform the feedback activities.

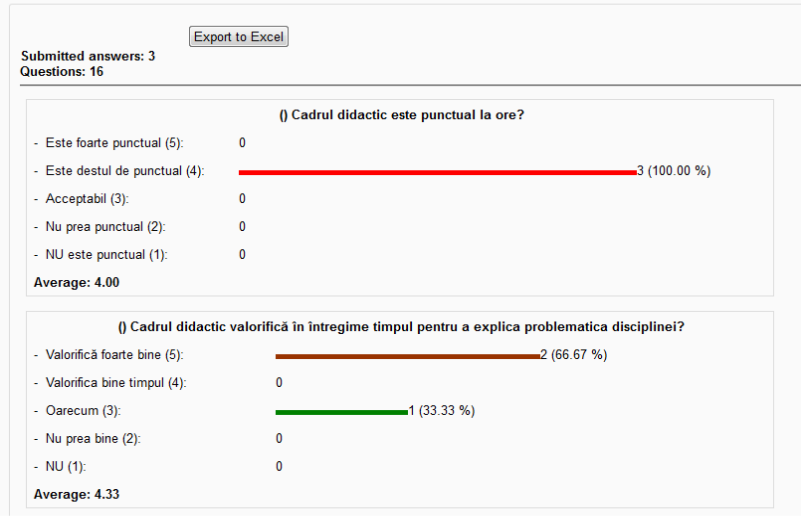


Fig. 2 - Statistics performed on the collected answers

After the closing of the evaluation interval, *Head of Department* will process the feedback for each member of the department. To this effect, he will enter (as course manager) every **Evaluation *Teacher*** course and perform the available statistics. He can also export in Excel those statistics. Figure 2 shows an example of such statistics performed with fictive test data.

CONCLUSIONS

A procedure was proposed for collecting the assessment forms that state the students opinion on the quality of teaching. This procedure involves using the Management Information System capabilities of the Moodle e-leasrning environment.

The first real world application of the described procedure is planned for the end of the first semester of the university year 2014-2015 in the Department of Sciences of The University of Agricultural Sciences of Iasi.

REFERENCES

1. **Büchner A., 2011** - *Moodle 2 Administration*. Packt Publishing Ltd. ISBN 978-1-84951-604-4
2. **Hollowell J., 2011** - *Moodle as a Curriculum and Information Management System.* Packt Publishing Ltd. ISBN 978-1-849513-22-7
3. **Rice W., 2011** - *Moodle 2.0 E-Learning Course Development*. Packt Publishing Ltd. ISBN 978-1-849515-26-9
4. ******* - *MoodleDocumentation* - https://docs.moodle.org/21/en/Main_page

GENETIC DIVERSITY BY LOCAL VARIETY OF PEANUT BASED ON ISOFLAVONES, TOTAL FAT, AND UNSATURATED FATTY ACID CONTENT CHARACTERS

DIVERSITATEA GENETICĂ A UNOR VARIETĂȚI LOCALE DE ARAHIDE, PE BAZA CONȚINUTULUI CARACTERISTIC DE IZOFLAVONE, GRĂSIMI ŞI ACIZI GRAŞI NESATURAȚI

WANGET Sesilia Anita[1], ROSTINI Neni [2], KARUNIAWAN Agung[2]
e-mail: sesilia_wanget@yahoo.com

Abstract. *Genetic diversity of peanut germ plasm is important for plant breeder in making decision regarding selection and method of plant breeding. The data of isoflavones, fats, and unsaturated fatty acid characters were used to estimated genetic diversity. This study was expected to provide information of peanut breeding in the future. The result of this study showed that the genetic diversity of 22 peanut accesions was narrow.*
Keywords*: genetic diversity, accession, peanut, unsaturated fatty acid, isoflavone*

Rezumat*. Diversitatea genetică a germoplasmei arahidelor este importantă pentru amelioratori în ceea ce priveşte metodele de selecţie şi cultivare. Conţinutul de izoflavone, grăsimi şi acizi graşi nesaturaţi a fost folosit pentru a estima diversitatea genetică. Studiul a avut ca scop obţinerea de informaţii referitoare la cultivarea şi ameliorarea arahidelor. Rezultatele studiului arată ca distribuţia diversităţii genetice a celor 22 de cultivare este îngustă.*
Cuvinte cheie*: diversitate genetică, cultivar, arahide, acizi nesaturati graşi, izoflavone.*

INTRODUCTION

Peanut crop is the second most important food crops after soybean. The composition of peanut seeds of economically important are 12-33% carbohydrate, 20-30% protein, and lipid / fat 40-50% (Salisbury and Ross, 1991) as well as mineral deposits such as Calcium, Chloride, Ferro, Magnesium, Phosphorus, Potassium and Sulfur (Sudjadi, 2001). Potential content contained in this placing peanuts as a highly nutritious food crops.

High oil content in peanuts is economically desirable characteristics. Peanut plants known to contain a variety of fatty acid compounds. The presence of fatty acids serve as a source of high energy, so that the peanuts are classified as food crops. The main components include the bean seed protein and fat (Baker, 2002). Fatty acids containing high energy (produce more ATP).

In America and Europe, is known for its peanut oil content containing unsaturated fatty acids (omega 3 and omega 6). In Indonesia, a study of omega 3,

[1] Fakultas Pertanian, Universitas Sam Ratulangi, Manado, Indonesia
[2] Fakultas Pertanian, Universitas Padjadjaran, Bandung, Indonesia

omega 6 and omega 9 in peanuts has not been done. Research is still oriented cultivation techniques and obtaining high yields (Rashad and Anhar, 2007; Wanget et al., 2002). Because of the need for research on the development of food crops, especially groundnuts very necessary.

In the peanut oil contained compounds which are natural antioxidants tocopherol and effective in inhibiting the oxidation process peanut oil. The tocopherol compounds contained in the unsaturated fatty acids of peanut oil (Ketaren, 1986). The presence of double bonds in the structure of unsaturated fatty acid compounds influence the importance of the resulting compounds. The closer the location of the double bond in the carbon chain structure, such as omega 3 and omega 6, the easier it reacts and the greater nutritional value than other unsaturated fatty acids. Proportionally, saturated fatty acids abundant in animal protein, whereas unsaturated fatty acids found in many plant proteins, including the peanut oil.

Flavonoids and isoflavonoida is one class of secondary metabolites found in many plants, particularly from groups *Leguminaceae*. The content of flavonoids compounds in the plant itself is very low, around 0.25%. These compounds are generally in a state of bound / conjugation with sugar compounds (Snyder and Kwon, 1987). Isoflavone compounds are widely distributed in the plant parts, both in the roots, stems, leaves, and fruit, so that these compounds also unwittingly consumed in the daily diet. In fact, because of its ubiquitous distribution in the plant it is said that when a virtually normal diet without containing flavonoids. It shows that flavonoids are not harmful to the body and even otherwise may provide health benefits.

Studies have shown that isoflavones have antioxidant properties equivalent to the well-known antioxidant vitamin E. The antioxidant power of isoflavones can reduce the long-term risk of cancer by preventing DNA damage. Genistein is an isoflavone among the most powerful antioxidants in soybean, followed by daidzein.

MATERIAL AND METHOD

The research method used was a randomized block design experiment with the treatment of 22 accessions of peanuts in 2 replications, the first planting season and planting season III. The analysis was performed on total fat content, content of Omega-3 Fatty Acids, Fatty Acid Content of Omega-6, Omega Fatty Acid Content-9. Isoflavone content analysis is only done during the growing season I.

The analysis carried out in the Laboratory of Food Crop Post-Harvest in Cimanggu Indonesian Ministry of Agriculture, Bogor in April 2012 and in July 2013.

The material analyzed was a sample of 22 peanut accessions (70 accessions selected from peanuts available based representation of regions in Indonesia peanut spread) with 2 replications, so the number of samples analyzed was 44 samples at each crop growing season (planting season the first and third growing season).

Analysis of laboratory testing performed in the laboratory of the Center for Post-Harvest Food Crops Ministry of Agriculture of Indonesia in Bogor. Tools and materials used laboratory analysis followed the standard operating procedure the international level.

Laboratory and Data Analysis

Laboratory analyzes the content of total fat, polyunsaturated fatty acids (Omega 3, 6, and 9), and isoflavones using samples of peanut seed as much as 100 grams of each accession were observed. Analysis using GC method and Sochlet. Chemicals and reagents used were sulfuric acid 1.25%, 3.25% sodium hydroxide, hexane, sulfuric acid, boric acid 4%, indicator conway, 0.1N hydrochloric acid, sodium hydroxide in methanol, boron triflorida 20 %, saturated sodium chloride and selenium mixture.

The tools used in this analysis is soklet, furnaces, ovens, tube destruction, a set of distillation equipment, electric bath, rotary evaporator, desiccator, filter paper, and other glassware and gas chromatography can separate the components by means of a carrier gas and is recorded as a function of time by the detector (McNair and Bonelli, 1997). Chromatography also provide a short analysis of the sensitivity ppm (Khopkar, 1990) Hitachi brand-263.50 with FID detector. System carrier gas in gas chromatography filter typically contains a molecule of water and other impurities that would be seen in the results recorder (Skoog, 1994).

The observations made by an analysis of the total fat content, the content of unsaturated fatty acids (omega 3, omega 6, omega 9) and isoflavones. Observations were made on samples of peanut seeds that have been dried to a water content of 10-15%.

Data were analyzed statistically by analysis of variance and PCA.

RESULTS AND DISCUSSION

The data results of laboratory analysis of total fat, omega-3, omega-6, omega-9, and isoflavones analyzed variance with randomized block design method. Quadratic mean error value of each character Anova Barlet tested for homogeneity between growing seasons, are in Table 1.

Table 1

Barlet Test Results of 22 Peanut Accession Character Quality

Variance	KTe MT 1	KTe MT 3	Combine	Barlet Test	Criteria
Total Fat	21.30038	35.7779	28.53914	4.555362	inhomogeneous
Omega-3	0.912674	3.977573	2.445124	34.17637	inhomogeneous
Omega-6	19.58171	30.59719	25.08945	3.383465	homogeneous
Omega-9	29.45109	45.58558	37.51834	3.242786	homogeneous

Barlet test results in two cropping seasons on the character quality of the results showed that only two characters are homogeneous. The second character is the homogeneous character of omega-6 and omega-9. Thus the character can be analyzed both combined in the second growing season. The characters are not homogeneous character of omega-3 and total fat content. Both of these characters were analyzed independently of each growing season. In this research, an analysis of the isoflavone content. In the statistical analysis only independent analysis conducted in the first growing season.

Results of laboratory analysis of total fat, unsaturated fatty acids and isoflavones in the two cropping seasons are presented in Table 2.

The results of analysis of diverse quality of the 22 accessions of groundnut is shown in Table 3 Almost all the character quality of the results showed a narrow genetic diversity unless the character content of Omega-3. Character content of Omega-3 has a broad genetic diversity and extensive phenotypic diversity.

Character quality of the results is controlled by many factors, both genetic and environmental interactions, or both. Phenotypic diversity of all the character qualities calculated results is to have a wide variety of categories. This suggests environmental factors strongly influence the quality of the appearance of the character 22 peanut accessions studied.

Table 2

Average content of total fat, unsaturated fatty acids, and isoflavones

Accession	Total Fat	Omega 3	Omega 6	Omega 9	Isoflavons
Atambua	38,3975	2,163	35,03225	35,58225	166,31
Bm 3	36,635	1,5095	28,861	22,0685	288,05
Bm 4	33,73	1,6075	28,283	29,18125	514,01
Gajah	38,04	2,87475	32,6865	33,17375	256,76
Gorontalo A	38,87	2,3735	33,9455	38,21225	420,29
Gorontalo B	38,53	2,206	32,1565	32,734	143,31
Gorontalo C	42,5025	1,923	28,7045	35,812	379,14
Jerapah	41,3325	1,829	33,357	37,18725	247,25
Kanonang Merah	39,03	2,016	27,53075	31,21475	441,53
Kanonang Putih	43,5	2,82425	37,694	38,08025	347,29
Kefa Timor	39,6175	2,0635	33,50725	30,0805	199,89
Kinali Merah	38,465	2,44825	31,13075	32,8115	395,5
Kinali Putih	38,45	1,899	27,06675	36,29775	208,86
Larantuka	35,68	3,320667	40,46933	35,55933	438,62
Madura 1	36,4975	4,064	37,67925	37,15025	152
Siborongborong	39,065	1,3365	30,668	33,9705	152,02
Sima	34,09	2,0845	35,67	36,755	277,81
Soe Timor	35,6325	1,554	30,23525	29,5555	122,98
Sumba Timor	41,2075	4,16175	36,366	32,96675	210,39
Tondegesan Merah	40,265	3,10025	37,243	35,406	272,22
Tondegesan Putih	43,65	1,69	32,4545	35,28175	270,92
Tuban	40,7025	2,35525	31,472	33,17725	342,69

A superior crop varieties should be supported by the potential quality of the results. The quality of peanut yield determined by including the fat content of unsaturated fatty acids and isoflavones content. The results of a study of 22 peanut accessions in two growing seasons, in Table 3, showed a narrow genetic diversity and extensive phenotypic diversity in the character of the total fat content, isoflavones, omega-6, omega-9 and omega-3 (season two). While the character content of Omega-3 in the growing season of the genetic and phenotypic diversity extensive. The results of this study are supported by research over several years of testing at several locations, species strain Valencia peanuts produce oleic acid slightly higher or almost equal to the cultivar New Mexico Valencia C (Burow and Ayers, 2012). Research on the growth of three types of groundnut showed oil content and fatty acids were not significantly different (Raheja et al., 1987), as well as research on four peanut varieties originating from different regions in Pakistan (Akhtar et al., 2005) on the fatty acid composition showed a narrow genetic diversity. Variation of the ratio of high and low grade oleic peanut type in Spanish indicates that factors other than genetics may be involved in determining the ratio of oleic / linoleic (ratio O / L) with appropriate (Lopez et al., 2001). It is claimed that the character quality of peanut plants is strongly influenced by the environment.

Table 3

Diversity of Quality Character of 22 Peanut Accessions

Growing season	Two growing season		One growing season			
			MT 1		MT 2	
Character	σ_g	σ_f	σ_g	σ_f	σ_g	σ_f
Omega-6	-1,79	43,83				
SD	5,27	4,52				
	Narrow	Wide				
Omega-9	-1,91	37,71				
SD	5,24	4,68				
	Narrow	Wide				
Isoflavon			6262,99	18362,06		
SD			4421,22	3630,77		
			Narrow	Wide		
Total Fat			4,62	25,93	-8,47	27.31
SD			6,33	4,50	7,96	2,78
			Narrow	Wide	Narrow	Wide
Omega-3			0,89	1,80	-0,86	3,12
SD			0,44	0,40	0,89	0,33
			Wide	Wide	Narrow	Wide

Analysis of isoflavone content narrow genetic diversity while extensive phenotypic diversity. A similar study by Kirakosyan et al. (2007) on the concentration of isoflavone content in seeds and seedlings on 20 peanut genotypes originating from different geographical sources show a wide diversity. In their

study they also found isoflavone content in groundnut seeds 0.8-fold higher concentration than in the isoflavone content of peanut seeds.

The results of PCA to 5 characters on the quality of the two main components are mapped on a graph as in Figure 1 Biplot Biplot graph shows the distribution of the data character that describes the four main groups of characters that affect the population of 22 peanut accessions were observed. In four distribution groups are groups consisting of two characters, and there are also only consists of a single character. Some characters who are members of the group showed that these characters are-jointly affect changes in a population.

Table 4

Eigenvalue, percentage of variation and percent kumulatif 5 character quality results

PC	Total Eigenvalue	% of Variance	Cumulative %
1	1,897	37,935	37,935
2	1,33	26,6	64,535

Table 4 shows the cumulative percent of the highest value is to PC2. The highest percentage of variance indicated that PC1 character states with high values on PC1 is the most influential character in the diversity of the population of 22 peanut accessions.

Table 5

Component Value Matrix 5 characters at 22 peanut accessions

Characteristics	PC	
	1	2
Omega-6	**0,917**	0,159
Omega-3	**0,888**	
Total Fat	-0,127	**0,923**
Omega-9	0,495	**0,649**
Isoflavon	-0,156	

Table 5 shows the most influential character is the character of Omega-6 and Omega-3 on PC 1 column variation caused by the character of Omega-3 and Omega-6 are jointly affect the diversity of the population of 22 peanut accessions. Biplot diagram (Figure 1) clarify the role of the character of Omega-3 and Omega-6 which together are in quadrant I.

Temperature is the main controller which increase the solubility of oxygen in the water along with the drop in temperature that provides O2 as the recipient of a hydrogen atom essential for the process of unsaturation in the ER so that yield more unsaturated fatty acids. The formation of fatty acids is much faster on the state of the light than in the dark. Plants or parts of plants that received the higher light intensity will result in a higher fatty acid. Ability accession accept a variety of light and temperature cause the expression of diverse fatty acid content.

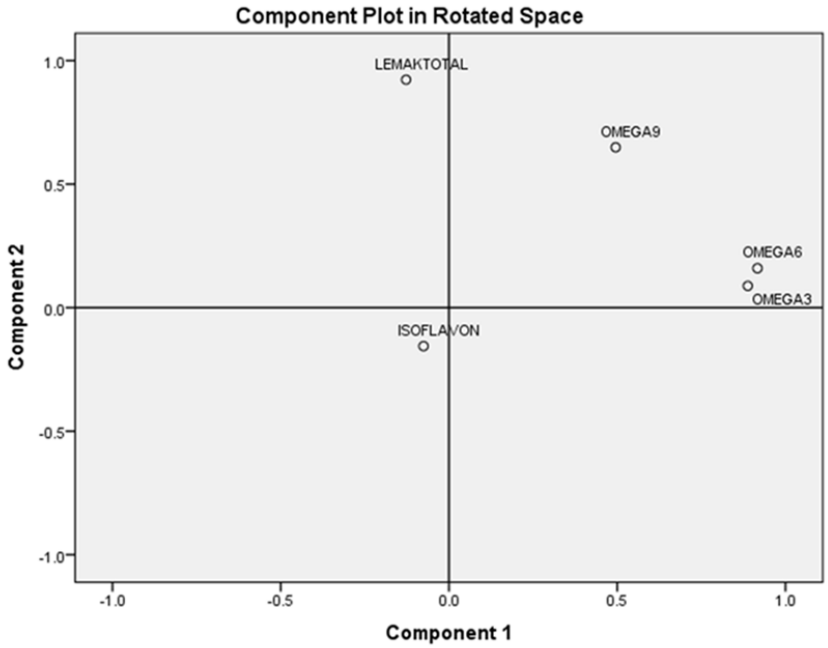


Fig. 1 - Biplot Graphic Pattern Spread the character quality of the 22 accessions of groundnut

The results of PCA for 22 accessions of peanuts on two main components Biplot mapped on a graph as shown in Table 5 and Table 6 and Figure 2 Biplot graph illustrates the distribution of accessions that clustered and distributed almost linear. The results showed that there were 13 accessions most defining quality of the diversity in the population 22 peanut accessions in the study.

Table 6

Eigenvalue, percentage of variation and the percentage of 22 accessions of cumulative groundnut

PC	Total Eigenvalue	% of Variance	Cumulative %
1	11,467	52,124	52,124
2	10,525	47,839	99,963

The data in Table 6 shows that the PC 2 has a higher cumulative percentage values of PC 1, but the higher percetage of variance shows by PC1, 52,124% indicating that accessions having the highest score on PC1 are most influential on the population diversity of 22 accessions of groundnut based character quality results.

Table 7 shows all accessions had values ≥ 0.5 on PC1 and PC2 which states that the accession-accession together create diversity in the population. The

diversity of each accession are displayed on the PC showed extensive phenotypic diversity.

Table 7

Component Value Matrix 22 peanut accessions based on the character quality of the results

Accession	PC		Accession	PC	
	1	2		1	2
Bm 4	0,784	0,621	Tondegesan merah	0,731	0,682
Kanonang merah	0,774	0,633	Tondegesan putih	0,727	0,687
Larantuka	0,77	0,638	Jerapah	0,717	0,697
Kinali merah	0,767	0,642	Kinali putih	0,708	**0,706**
Gorontalo A	0,766	0,643	Soe timor	0,617	**0,786**
Gorontalo C	0,759	0,65	Gorontalo B	0,639	**0,769**
Tuban	0,755	0,656	Siborongborong	0,647	**0,762**
Bm 3	0,753	0,657	Madura	0,648	**0,76**
Kanonang putih	0,747	0,664	Atambua	0,661	**0,75**
Sima	0,738	0,674	Kefa	0,7	**0,714**
Gajah	0,734	0,68	Sumba timor	0,705	**0,709**

The results of the PCA analysis of the population of 22 accessions of groundnut in the form of scores on PC1 and PC2 biplot mapped on a graph as shown in Figure 2.

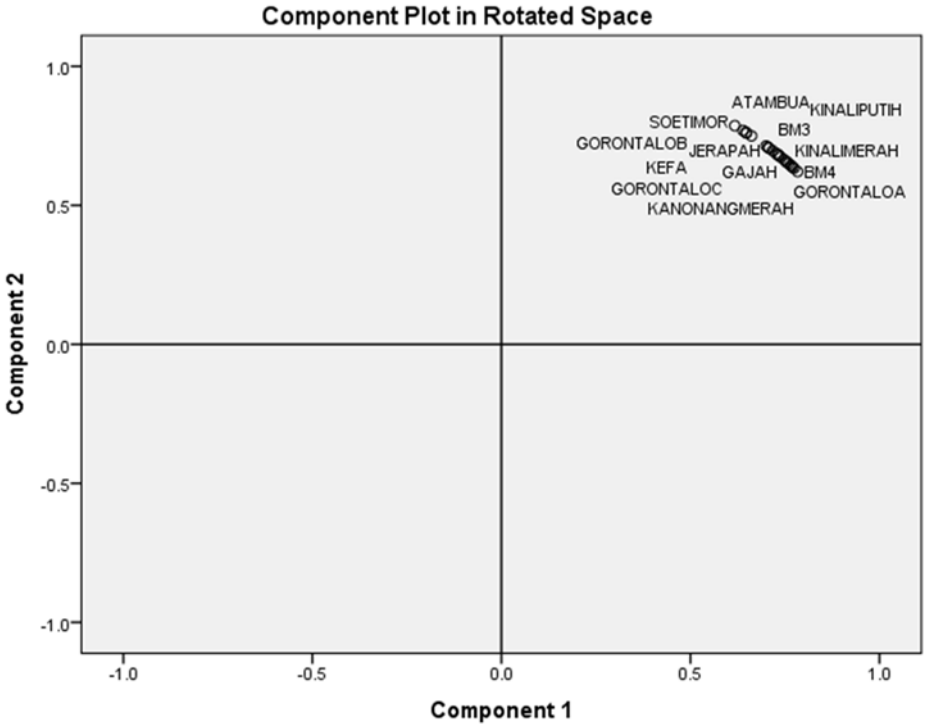


Fig. 2 - Biplot Pattern Chart 22 Spread peanut accessions based on the character quality

Distribution of 22 peanut accessions based on PC1 and PC2 show all accessions form a group on quandran I which showed a narrow genetic diversity of all accessions were observed.

CONCLUSIONS

Phenotypic diversity of all characters analyzed the quality of the results is large, while genetic diversity is narrow, because the metabolism of fats and unsaturated fatty acids and isoflavones are highly influenced by environmental factors so that the selection is based on the diversity of character qualities ineffective results.

Acknowledgements: *Thanks to the Center for Food Crops Republic of Indonesia, in Cimanggu Bogor-Indonesia, which has helped in the process of analysis of the total fat content, the content of unsaturated fatty acids (omega-3. omega-6 and omega-9) and the isoflavone content .*

REFERENCES

1. **Akhtar H., Hamid S., Khan J.I., 2005** - *Comparative Study of Fatty Acid Composition and Characterization of Fixed Oil of Four Peanut Varieties Available in Pakistan*. Natural Product Sciences 11(4), pp. 225-228.
2. **Baker G. L., 2002** - *Flavor Formation and Sensory Perception of Selected Peanut Genotypes (Arachis hypogaea L.) as Affected by Storage Water Activity, Roasting, and Planting Date.* Florida: The University of Florida.
3. **Burow M. A., 2012** - *Development of High-Yielding, High-Oleic acid Valencia Peanuts. American Peanut* Volume 44 Proceedings.14. Raleigh, North Carolina: The American Peanut Research and Education Society, Inc.
4. **Ketaren S., 1986** - *Pengantar Teknologi Minyak dan Lemak Pangan*. Jakarta: UI-Press.
5. **Kirakosyan A., Kaufman P.B., Duke J. A., Seymour E.M., Warber S., Bolling S.F., 2007** - *Production of Isoflavones in Seeds and Seedlings of Different Peanut Genotypes*. Crop Science. 47 (2), pp. 717-719.
6. **Lopez Y, Smith O.D., Senseman S.A., Rooney W.L., 2001** - *Genetic Factors Influencing High Oleic Acid Content inSpanish Market-Type Peanut Cultivars*. Crop Science. 41, pp. 51-56.
7. **McNair H. M., Bonelli E.J., 1988** - *Dasar Kromatografi Gas.* Malang: Bandung: ITB Bandung.
8. **Raheja R.R., Batta S.K., Ahuja K.L., Labana K.S., Singh M., 1987** - *Comparison of Oil Content and Fatty acid composition of Peanut genotype differing in growth habit.* Plant Foods for Human Nutrition 37, 103-108.
9. **Rasyad A., Anhar A., 2007** - *Interaksi Genetik x Lingkungan untuk Berbagai Komponen Hasil dan Stabilitas Hasil pada Beberapa Varietas Padi Lokal Sumatera Barat.* Zuriat, vol.18, no. 2, p. 100.
10. **Salisbury F.B., Ross C.W., 1991** - *Plant Physiology.* Belmont, California: Wadsworth Publishing Company.
11. **Skoog D.A., West D.M., Holler F.J., 1994** - *International Edition Analytical Chemistry an Intrduction.* 6th Edition. Sounders College Publishing, Appendix 4.
12. **Snyder H.E., Kwon T.W. 1987** - *Soybean Utilization.* AVI Book, New York, pp. 104-111.

13. **Sudjadi M., Supriati Y., 2001 -** *Perbaikan Teknologi Produksi Kacang Tanah di Indonesia.* Buletin Agrobio. 4(2), pp. 62-68.
14. **Wanget S.A., Setiamihardja R., Hermiati N., Yuwariah Y., 2002** - *Pola Pewarisan Karakter Warna Kulit Biji dan Jumlah Biji per polong Tanaman Kacang Tanah.* Bandung, Indonesia: Program Pascasarjana Universitas Padjadjaran. Tesis.

THE NEED TO CONTINUE VEGETABLES BREEDING IN ROMANIA IN THE YEARS 2015-2025

NECESITATEA CONTINUARII AMELIORĂRII LEGUMELOR ÎN ROMÂNIA ÎN PERIOADA 2015-2025

SCURTU I.[1]
e-mail: ucb_scurtu@yahoo.com

Abstract*: Improved varieties have had a main role in the increases in yield and quality of vegetable crops. In this respect, the vegetable varieties are quickly replaced by new ones. During the last years, an immense quantity of new knowledge on the genetic diversity of vegetables and the utilization of genetic resources, breeding methods and techniques, and utilization of modern biotechnologies in vegetables crop breeding has accumulated.*
Unfortunatelly, în our country, we have lost a great part of genes stock; many breeding laboratories have not enough modern devices and international cooperation is not too strong. But we beleive that the breeding vegetable in our country is very necessary and Ministry of Agriculture must to grant more funds for this activity. We discuss what is possible to do în the next years in order to solve the main problems of this field: researchers training; devices for laboratory and field; breeding objectives în main cultivated vegetable species.
Keywords: *breeding vegetable, new varieties, objectives, breeding team, pathogens resistance.*

Rezumat: *Soiurile ameliorate au avut un rol principal în creşterea producţiei şi calităţii legumelor. Structura soiurilor şi hibrizilor de schimbă foarte repede, cultivarurile vechi fiind înlocuite de cele noi. În ultima perioadă s-au acumulat multe cunoştinţe în domeniul diversităţii genetice a legumelor, a utilizării resurselor genetice, a metodelor şi tehnicilor de ameliorare, inclusiv prin utilizarea biotehnologiilor moderne. Din păcate, în ţara noastră am pierdut o parte din resursele genetice la legume, laboratoarele rămase nu au dotarea necesară şi cooperarea internaţională în acest domeniu a slăbit. Dar credem că ameliorarea legumelor în România este foarte necesară şi are nevoie de fonduri pentru relansare. În articol abordăm ce este necesar de întreprins în anii viitori pentru rezolvarea problemelor din acest domeniu: pregătirea cercetătorilor, procurarea de echipamente pentru câmp şi laborator, principalele obiective care se cer rezolvate.*
Cuvinte cheie: *ameliorarea legumelor, cultivare noi, obiective de ameliorare, echipe de cercetare, rezistenţa la patogeni*

1. THE NEED TO ACHIEVE A NEW BREEDING PROGRAMMES OF VEGETABLE SPECIES

The production and consumption of vegetables have risen sharply in recent decades. Worldwide, in the last 15 years, the production has increased by 15%, a growth rate higher than in other groups of plants. The increasing of the production and

[1] „Constantin Brâncovenu" University of Piteşti, Romania

areas in the last decade is a continuation of the trend occurred after the Second World War. In the period 1961-2000 the areas planted with vegetables and melons have increased by 153% and the total production by 253% (Voican et al., 2006).

The vegetables are an important part of a healthy and varied nutrition, enriching the body with more substances which may prevent diseases, such as vitamins (A, B1, B2, C, PP), antioxidants and minerals (Dumitrescu et al., 1998). As a result, we expect that in the near future, the need of vegetable products will increase. The improvement activity has had and will continue to have a crucial role in increasing production, in diversifying the product range, in the quality of vegetable products. From this point of view, the market of vegetable seed is very dynamic, there is a strong competition between the producing companies. Varieties and vegetable hybrids generally have a short life, being quickly replaced by new cultivars. Therefore, genetics and breeding methods, but - especially novelties, are of great interest to the breeders of the vegetable species, researchers and students.

Especially after 1980, an enormous amount of information on the genetic diversity of vegetable genetic resources has been accumulated, of the breeding methods and techniques, of the development and use of modern biotechnology. The activity of obtaining new cultivars is currently the attribute of large specialized companies, in which the powerful teams of specialists with top equipment activate who keep the secrecy of the improvement techniques for maintaining competitive advantage. Unfortunately, the Romanian researchers' access to all current knowledge of genetics, biotechnology and breeding techniques vegetables has declined drastically in the last 20 years and just in the period when the most important findings are obtained.

Vegetable breeding activity in Romania decreased in intensity due to the unprecedented decrease of the research funds and, hence, the number of researchers; both the laboratory equipment and mechanical means in the field have become obsolete or have been decommissioned and the area has become unattractive for the young graduates of faculties.

However, the improvement vegetables activity should not be discontinued in pending better times. The researchers who still activate get good results, despite the difficulties. They need to join young people to acquire basic technique and try to obtain varieties and new hybrids with superior characteristics, adapted to the local culture conditions and responding to the Romanian consumer preferences.

2. MAIN RESULTS OBTAINED BY ROMANIAN BREEDERS OF VEGETABLE DURING 1995-2014 PERIOD

The main argument in the possibility of reviving the work of improvement, is that the breeders of the vegetable species from Romania have achieved in the last 25 years numerous successes in the difficult conditions of transition to market economy, followed by a prolonged economic crisis. Very valuable varieties for consumption tomatoes of summer-autumn and for the tomatoes for processing have continued to appear. After a long period in which early tomato hybrids for field and greenhouses missed, at SCDL Buzau, the Siriana F1 hybrid was created, which extends in production due to high yielding, to the earliness and outstanding organoleptic qualities (Scurtu and Lăcătusu, 2013).Breeders from SCDL Buzau are proud of not only the Siriana hybrid but also of the cherry tomato creations also a novelty in the domestic assortment. Nationally, we notice new varieties of chili pepper, bell pepper, long pepper and eggplant approved in the last 15 years that make up a variety conveyor that can cover long periods of consumption with fresh fruit from the early to the late varieties. In this group of vegetables the first F1 hybrids appeared, productive, uniform

as vigor of plants, with the fruit shape and size that is very attractive (Andra and Felicia F1 hybrids, with eggplants). At pumpkin vegetables appeared the first F1 hybrid zucchini and "cornichon" cucumbers, and at peas and beans the native assortment now includes varieties with different growing seasons for different destinations (fresh consumption or industrialization) and for different methods of culture. Another remarkable result of the last years is the getting of the first Romanian hybrid carrot that joins several other new varieties obtained from the breeders from ICDLF Vidra and SCDL Buzau.

A review of the most significant results of the improvement of vegetables is shown in Table 1, which shows the percentage of Romanian varieties and hybrids in production. It can be observed that scientific research in Romania has provided growers valuable varieties and hybrids, which in many cases have more weight in production than the imported cultivars. At the same time, it should not be overlooked that with the assortment destined to the crops for greenhouses, a field that will be expanded more and more in the future, the achievements are very modest. With the early cabbage, cucumbers, early tomatoes, lettuce and other crops for greenhouses, the domestic varieties weight is between 5 and 10%.

Table 1

The domestic vegetable varieties weight (%, 2012)

Species	Romanian Cultivars Weight, (%)	Species	Romanian Cultivars Weight, (%)
Tomatoes (for open field)	90	Onion	50
Pepper (tomato-pepper, long pepper, cayenne pepper)	90	Garden Peas	30
Eggplants	95	Garden Beans	15
Early cabbage	0	Other vegetables (for open field)	92
Summer and autumn cabbage	85	A total of field vegetables	60
Cucumbers and squashes (for open field)	20	Early tomatoes (protected 2 cycles)	5
Root vegetable (carrot, parsnip, parsley, celery, beetroot, radishes)	50	Other protected crops (cucumbers, peppers, eggplant, lettuce, zucchini, beans)	5

It is only in the period 1990-2012, with all the difficulties related to the decreasing numbers of researchers, the precarious material basis and the insufficient funding, the research in the field has managed to create and introduce into production a large number of varieties and hybrids, as shown in Table 2.

The improvement activity in the surveyed period got 227 new cultivars to 38 vegetable species, but the number of F1 hybrids is still modest (only 18 hybrids to 209 varieties).

There are numerous examples of varieties and vegetable hybrids created in Romania and approved both by growers and consumers. The best known are (the cultivars marked with an asterisk are recommended for the culture in plastic tunnels): *Siriana F1*, Pontica 102, Viorica, Darsirius,Buzău 47, Kristinica, Carisma*, Coralina*,* for tomatoes*; Cornel 209, Asteroid 204* for round pepper; *Bârsan, Galben Superior, Ceres, Arum, Buzău 10*, Vidra 9** for bell pepper; *Siret* for long pepper; *Andra F1*, Luiza, Contesa, Daniela, Belona**

Drăgaica , Buzău H1* and Eleonora* for eggplants*; Triumf F1 and Bucovina F1* for carrot*; Bistrița* for celery; *De Buzău* for onion*; Buzoiana, Mocira, Poiana* for cabbage; *Auria Bacăului*, Menuet, Ioana** for garden beans*; Vidra 187, Armonia, Işalnița 60, Diana* for garden peas; *Sirius F1*, Ierprem*; Cornişa* F1 for cucumbers.

The main features and characteristics of the new cultivars are most often higher than those made previously.

There are many pathogens attacking some vegetable species and only at few cultivars we can find the authors' mention on resistance or tolerance to attack. It is noted that most claims of tolerance or resistance to diseases are found in peppers and eggplants, while in other species, no such claims, so growers do not know whether or not such cultivars possess resistance. The lack of claims relating to certain pathogens expresses most often, the authors' uncertainty regarding the degree of tolerance or resistance due to the inability of testing on controlled infections.

Table 2

Vegetable Varieties and Hybrids Registered in the Catalogue during 1990-2012

Species	Number	Observations	Species	Number	Observations
Witloof cicory	2	-	Carrot	5	Hybrids – 2
Pepper	37	Chili pepper –13 Mild pepper – 12 Long pepper –11 Cayenne pepper1	Fennelflower	1	-
Okra	2	-	Parsley leaves	1	-
Basil	1	-	Parsley Root	1	-
Cuccumbers	20	Hybrids – 7	Watermelon	2	-
Onion	17	Hybrids – 2 Red Onion – 3 Chives – 1	Melon	3	-
Chicory	2	-	Parsnip	1	-
Savory (thime)	3	Wild thyme – 1	Eggplants	11	Hybrids – 2
Cauliflower	2	Autumn	Sweetcorn	1	-
Zucchini	6	Hybrids 2	Rhubarb	1	-
Tomatoes	28	Hybrids – 3 Cherry Type – 2 For processing –8 Fresh consumption-15	Redishes	6	Early – 1 Summer – 4 Summer-autumn – 1
Cabbage	12	Hybrids – 2 Autumn – 9 Summer – 1	Lattuce	8	-
Dwarf Beans	17		Sage	1	-

Species	Number	Observations	Species	Number	Observations
Climbing Beans	5		Beetroot	2	-
Fennel	1		Spinach	3	-
Kohlrabi	2		Origanum	1	-
Lovage	1		Dill	2	
Orache	3		Celeriac	2	-
Peas	10		Garlic	4	-

3.THE MAIN OBJECTIVES OF IMPROVING VEGETABLES IN THE NEXT PERIOD

The success of a long-term breeding programme is related to the adoption and implementation of a coherent strategy. Random and uncertain funding with values that may decrease from one year to another, cannot lead to competitive results with what is already obtained in the traditional countries and companies. As in any strategy we must start from: a) what it is (where we are); b) to establish our goals; c) how we shall achieve the proposed objectives.

a) One must first start from the existing situation, showing the strengths, weaknesses, opportunities and threats in the system that allow us to determine exactly where we are. We can easily identify, among the *strengths*, the existence of the organized research units and of a number of specialists with knowledge and passion for improvement. Other strengths relate to:

- the experience gained during almost 50 years of functioning of the vegetable research network;
- the accumulation within the institute's portfolio and the research stations of some patents on some valuable varieties (exclusivity);
- the growers' knowledge of some varieties developed in the country and the existence of a constant demand for these due to the credibility of the research stations, gained over time;
- the existence of the stations' sites that contain important information that growers need.

Among *weaknesses* (much more numerous than strengths) one may identify the lack of funding which means the small number of staff and the impossibility of creating multidisciplinary teams, low wages, the lack of equipment and means of production in the field, difficulties in accumulating strictly specialized knowledge, the small number of international partners, the sharp decrease of the germ-plasma sources, etc.

The main *opportunities* are the relatively large areas cultivated with vegetables (around 150,000 hectares), the relatively high cost of vegetable seed from abroad, the consumers' attachment to the Romanian vegetables, characterized by high quality nutrition and taste - and the emergence of some European research funds for 2014-2020;

- the ability to regain the land taken illegally in court, the land required for the isolation in space and the producing of the basic seed.

The program has many *threats* related to the market conquest by large international companies and the difficulty of competing with them without a good marketing programme in place.

- the insufficient concern or lack of concern of the state authorities to support this activity sector;
- the land and the stations' buildings are located in the space within or in close proximity to cities, have a very high value, and therefore of particular interest for the "hunters in the real estate", with permanent risk of losing their ownership;

- the danger of losing indigenous genetic heritage, extremely valuable, collected and improved over 50 years of activity.

b) The second factor to be considered is related to the mission of breeding (where we want to go), i.e. assuming clear objectives: what species will be improved; to what directions of culture and whose purpose will be to the new creations addressed (open field crops, greenhouse or solar, for fresh consumption or industrialization), what place their own creations in the cultivated area will occupy, etc. It is very important for what kind of creations we turn our efforts, respectively to what features and qualities will print to the new creations so that they can be requested by vegetable producers and consumers.

c) The third factor to be noted in the strategy is how we achieve the proposed objectives (providing financial resources, researchers training, sources of germ-plasma, the field and the laboratory equipment, marketing programmes, multiplication, the approval and certification of creations).

The main issue on which we will focus more is about setting goals for improvement, which eventually will make the size and expenditure of each species breeding programme.

Often, there are, in every species, many current and future goals that a breeding programme must solve. The breeder should identify important issues affecting the production and quality of each species in the area that new cultivars will be cultivated. The goals should be prioritized and worked on many projects the budget allows us, the personnel and material basis. Almost all the breeding programmes should aim at three objectives: the resistance to pathogens and insects, the increase of production and the improving of quality (Prohens and Nuez, 2008). Each of these has, in each species, many research directions.

The large number of species does not allow us to detail the myriad of issues concerning the improvement objectives. Significant progress on long-term in breeding programmes cannot be achieved without the development of “in vitro” culture techniques, which allow overcoming barriers of incompatibility to hybridization between cultivated species and wild relatives from which specific resistance genes should be introduced. Other targets for improvement should relate to improving resistance to soil salinity which is or may become a problem of land cultivated with vegetables, and increasing resistance to low or too high temperatures. Throughout the world is already working to introduce resistance to insects’ attack or other pests (mites, nematodes) and it is possible that such creations to get in our country. Therefore, the future improvement teams should be able to address these new issues.

REFERENCES

1. **Dumitrescu M., Scurtu I., Stoian L., Glăman Gh., Costache M., Lăcătuş V., 1998** - *Producerea legumelor* Ed. Artprint,.
2. **Gallais A., Bannerot H., 1992** - *Amelioration des especes vegetales cultivee* -INRA France.
3. **Prohens J., Nuez F**., **2008** - *Handbook of Plant Breeding-Vegetables-* Springer.
4. **Scurtu I., Lăcătuş V., 2013** - *Romanian Vegetable Growing– Present and Prospective for 2020-2025* Management strategies, year VI, special issue.
5. **Voican V., Scurtu I., Costache M., Lăcătuş V., Stoian,L., Roman T., Dumitrescu M. 2006** - *Cultura legumelor în câmp,* Ed. Phoenix, Bucuresti.

DYNAMICS OF THE FLAVONOIDS CONTENT IN SOME TOMATO CULTIVARS FROM NORD - EAST ROMANIA

EFECTUL STRESULUI SALIN ASUPRA DINAMICII CONȚINUTULUI DE FLAVONOIZI A UNOR POPULAȚII LOCALE DE TOMATE DIN NORD-ESTUL ROMÂNIEI

***JITĂREANU Carmenica Doina*[1], *SLABU Cristina* [1], *MARTA Alina Elena*[1]**
***BOLOGA (COVAȘĂ) Mihaela* [1]**
e-mail: doinaj@uaiasi.ro

Abstract. *The aims of the present work were to determine effects of salt stress on the flavonoids content in some tomato cultivars, to find the differeces of salt response in these cultivars. The impact of excess soil salinity on crop productivity is sometimes disastrous which results, in the identification and design of new genotypes of plants tolerant to osmotic stress conditions. Flavonoids, the most common group of polyphenolic compounds that are found ubiquitously in plants. The biological material was represented by local tomatoes populations collected from areas with saline soils from Moldavia region and compared with commercial type salt-tolerant tomato. The bifactorial experience was conducted in a pots experiment in randomized blocks with four repetitions. Ten tomato genotypes studied were subjected to salt stress for a period of 30 days is constantly wetted with saline solution to a concentration of 100 mM and 200 mM. Determination of flavonoids content of leaves was done by the spectrophotometric method. The results show that seven genotypes maintain a high level of flavonoids in the exposure of the two different solutions concentration compared to untreated.*
Key words: *Lycopersicon esculentum, salinity stress, flavonoids.*

Rezumat. *În cadrul acestui studiu a fost analalizat efectul stresului salin asupra conținutului de flavonoizi din frunzele unor genotipuri de tomate. Impactul salinității solului asupra productivității este de cele mai multe ori devastator, ceea ce duce la identificarea unor noi genotipuri de tomate rezistente la salinitate. Flavonoizii reprezintă grupul cel mai comun de compuşi polifenolici care se găsesc în plante. Materialul biologic a fost reprezentat de populații locale de tomate colectate din diferite areale cu soluri saline din regiunea Moldovei şi un soi martor rezistent la salinitate. Experiența de tip bifactorial cu 4 repetiții a fost înființată în vase de vegetație. Pentru o perioadă de 30 de zile, 10 genotipuri de tomate şi un soi rezistent la salinitate au fost supuse stresului salin. Plantele au fost udate cu soluție salină de 100 mM şi 200 mM. Metoda utilizată pentru determinarea conținutului de flavonoizi din frunze s-a realizat prin metoda spectrofotometrică. Rezultatele arată că la 7 genotipuri se menține un nivel ridicat de flavonoizi în cazul expunerii la cele două concentrații diferite de soluții saline față de varianta netratată.*
Cuvinte cheie: *Lycopersicon esculentum, stres salin, flavonoizi.*

[1] University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

INTRODUCTION

Flavonoids, the most common group of polyphenolic compounds that are found ubiquitously in plants. These are widely distributed in plant fulfilling many functions. Flavonoids and other plant phenolics are especially common in leaves, flowering tissues and woody parts such as stems and bark. They are important in plant for normal growth development and defense against infection and injury. Flavonoids are the most important pigments for flower coloration producing yellow or red/blue pigmentation in petals (Khatiwora et. al., 2010).

Flavonoids represent one of the largest and most studied classes of phenylpropanoid-derived plant specialized metabolites, with an estimated 10.000 different members. Structurally, they consist of two main groups, the 2-phenylchromans (the flavonoids, including flavanones, flavones, flavonols, flavan-3-ols, and anthocyanidins) and the 3-phenylchromans (the iso- flavonoids, including isoflavones, isoflavans, and pterocarpans). Flavonoids act as attractants to pollinators and symbionts, as sunscreens to protect against UV irradiation, as allelochemicals, and as antimicrobial and antiherbivory factors. Their importance in plant biology goes beyond their specific functions within the plant (Dixon and Pasinetti, 2010).

In plants, polyphenol synthesis and accumulation is generally stimulated in response to biotic/abiotic stresses, such as salinity. Indeed, polyphenolic compounds participate in the defence against reactive oxygen species (ROS), which are inevitably produced when aerobic or photosynthetic metabolism is impaired by environmental stresses. Halophytes are naturally salt-tolerant plants that may be potentially useful for economical (oilseed, forage, production of metabolites) applications (Ksouri et. al., 2007).

To prevent the potential cytotoxic effects of ROS, the stimulation of antioxidant systems can assist in plant protection from oxidative stress. Plants have developed antioxidant enzymes such as superoxide dismutase, ascorbate peroxidase, glutathione reductase, catalase, peroxidase and non-enzymatic scavengers like glutathione, ascorbic acid, carotenoids and flavonoids which regularly maintain ROS balances within the cell. Flavonoids are reported as antioxidant agents by scavenging ROS, which are functioned by virtue of the number and arrangement of their hydroxyl groups attaches to ring structures. Their ability to act as antioxidants depends on the reduction potentials of their radicals and accessibility of the radicals (Chutipaijit et. al., 2009).

Some physiological responses to salt stress have been used in determining salt tolerance of plants. Plant hormone levels, antioxidant enzyme activities, pigment contents, osmotic potential reduction, gas exchange characteristics, total soluble protein contents and proline, amounts were determined in different tolerant and sensitive plant varieties at wide range of salt concentrations (Doganlar et. al., 2010). The reported results suggest that an increase of the total phenolic content in rice genotypes as a result of salt stress protects plants from oxidative damage. It was swon that the total flavonoid content increased in salt-stressed

seedlings of salt tolerant rice varieties by 6.34-7.31% and 1.72-3.48% in alt sensitive plants, which indicates that probably flavonoides similarly to proline compounds serve a protective role under stress conditions (Parvaiz, 2013).

MATERIAL AND METHOD

The research was conducted under greenhouse condition and the analysis in the Laboratory of Plant Physiology, Faculty of Agriculture, from USAMV Iaşi.

The biological material was represented by local tomatoes populations collected from areas with saline soils from Moldavia region and compared with commercial type salt-tolerant tomato (*Ursula* $_{F1}$) from Israel.

The bifactorial experience was conducted in a pots experiment in randomized blocks with four repetitions. Ten tomato genotypes (*Moşna* $_{2}$*, Şcheia, Dorohoi* $_{4}$*, Dorohoi* $_{6}$*, Dorohoi* $_{8}$*, Copalău* $_{2,}$ *Copalău*$_{3}$*, Copalău* $_{4}$*, Copalău* $_{5}$*, Moşna* $_{3}$) studied were subjected to salt stress for a period of 30 days is constantly wetted with saline solution to a concentration of 100 mM and 200 mM (fig. 1).

For determination of photosynthetic pigments of leaves we used a UV spectrophotometer type – 1800. The content of chlorophyll pigments was assessed by light absorption ability of the acetone extract of pigments (1%) in the visible spectrum (320-325 nm).

Fig. 1 - The biological material for research (local tomatoes populations)

RESULTS AND DISCUSSIONS

After 15 days the application of saline treatments, the experimental results showed for the plants watered with water only, values of flavonoid

pigment content appreciated on the ability to absorb light in the 320 nm wavelengthn, between 0.66 and 3.23 a.u. In seven of the genotypes studied values between 1.93 and 3.23 a.u. are superior to the variety *Ursula* $_{F1}$, resistant to salinity. The exposure to 100 mM *NaCl* caused an increase of absorbance in 6 genotypes (*Ursula* $_{F1}$, *Copalău* $_{2}$, *Copalău* $_{3}$, *Copalău* $_{4}$, *Copalău* $_{5}$, *Moşna* $_{3}$), subjected to salt stress compared to the control which is between 2.20 and 3.26 a.u. and compared to salt-tolerant type *Ursula* $_{F1}$ (1.70 a.u.) 100 mM a single cultivar (*Moşna* $_{3}$) showed higher values, namely 3.26 a.u. The others 9 genotypes had lower values between 1.70 and 3.13 a.u. The exposure to 200 mM *NaCl* caused an increase of absorbance values at 7 genotypes (*Ursula* $_{F1}$, *Dorohoi* $_{8}$, *Copalău* $_{2}$, *Copalău* $_{3}$, *Copalău* $_{4}$, *Copalău* $_{5}$, *Moşna* $_{3}$) subject to salt stress compared to the control which is between 2.10 and 3.23 u.a, and compared to salt-tolerant type Ursula F1 (2.63 a.u.) 200 mM, 4 genotypes (*Şcheia, Dorohoi* $_{4}$, *Dorohoi* $_{6}$, *Dorohoi* $_{8}$) showed higher values between 2.86 and 3.23 a.u. It is noted these genotype: *Ursula* $_{F1}$, *Copalău* $_{2}$, *Copalău* $_{3}$, *Copalău* $_{4}$, *Copalău* $_{5}$, *Moşna* $_{3}$ maintain a high level of flavonoids in the exposure of two different saline concentrations compared untreated variant (fig. 2).

After 30 days the application of saline treatments, the experimental results showed for the plants watered with water only, values of flavonoid pigment content appreciated on the ability to absorb light in the 320 nm wavelengthn, between 0.00 and 3.30 a.u. In 3 of the genotypes studied values between 2.90 and 3.30 a.u. are superior to the variety *Ursula* $_{F1}$ (2.86 u.a).

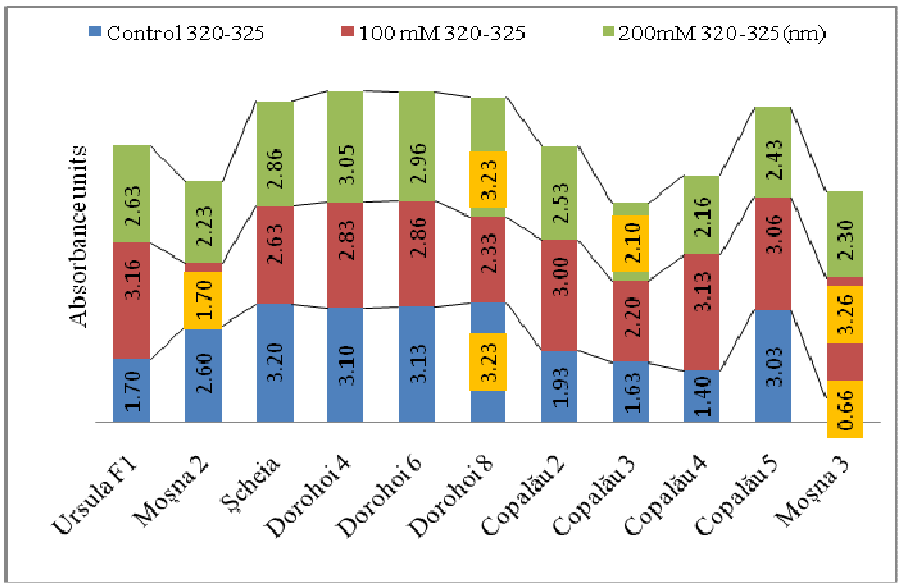


Fig. 2 - The content of flavonoid, after 15 days of saline treatment

The exposure to 100 mM *NaCl* caused an increase of absorbance in all genotypes subjected to salt stress compared to control and to *Ursula* $_{F1}$ values is the same for all genotypes, namely 4.00 a.u.

The exposure to 200 mM *NaCl* caused an increase of absorbance in 9 genotypes compared to control, with values between 2.73 and 3.56 a.u. A single genotype (*Copalău* $_5$) with a value less than the correspondent untreated variant. Compared with *Ursula* $_{F1}$ (3.30 a.u.) 200 mM, 7 genotypes (*Şcheia, Dorohoi* $_4$, *Dorohoi* $_6$, *Dorohoi* $_8$, *Copalău* $_3$, *Copalău* $_4$, *Moşna* $_3$) showed the hight values between 3.33 and 3.56 a.u. (fig. 3).

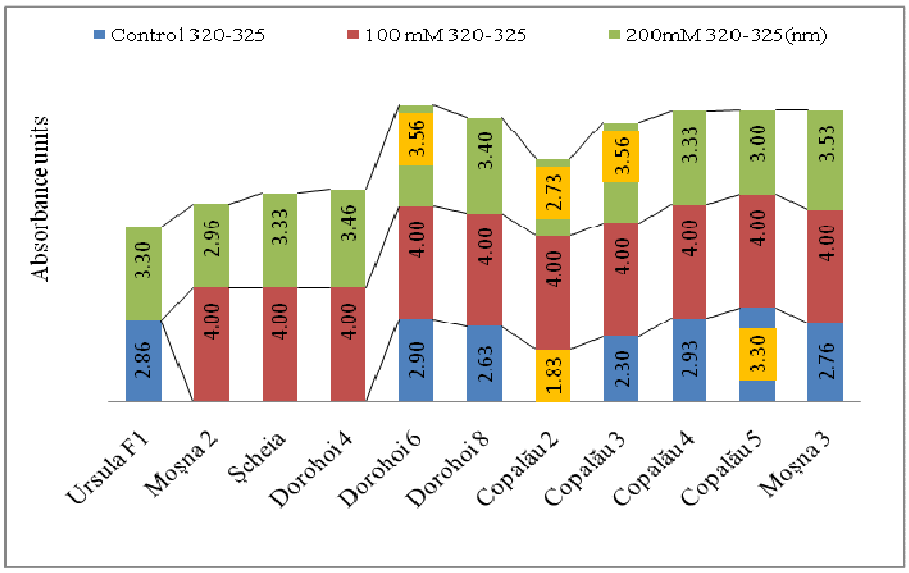


Fig. 3 - The content of flavonoid, after 30 days of saline treatment

CONCLUSIONS

After 15 days of saline treatment it is noted that's genotype: *Ursula* $_{F1}$, *Copalău* $_2$, *Copalău* $_3$, *Copalău* $_4$, *Copalău* $_5$, *Moşna* $_3$ maintain a high level of flavonoids in the exposure of two different saline concentrations compared untreated variant.

After 30 days of saline treatment the exposure to 100 mM *NaCl* caused an increase of absorbance in all genotypes subjected to salt stress compared to control and the exposure to 200 mM *NaCl* caused an increase of absorbance in 9 genotypes compared to control.

Compared with commercial type salt-tolerant tomato (*Ursula* $_{F1}$) after 15 days of saline treatment , 5 genotypes (*Moşna* $_3$, *Şcheia, Dorohoi* $_4$, *Dorohoi* $_6$, *Dorohoi* $_8$) showed a higher level of the flavonoid contents and after 30 days it is noteds that the exposure to100 mM *NaCl* showed a higher level for the all ten

genotypes. The exposure to 200 mM, showed a higher values for 7 genotypes (*Şcheia, Dorohoi $_4$, Dorohoi $_6$, Dorohoi $_8$, Copalău $_3$, Copalău $_4$, Moşna$_3$)* .

Acknowledgement. *This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number PN-II-PT-PCCA-2011-3.1-0965.*

REFERENCES

1. **Chutipaijit. S, Cha-Um S.**, **Sompornpailin K., 2009 -** *Differential accumulations of proline and flavonoids in Indica rice varieties against salinity***.** Pakistan Journal of Botany, 41(5), pp. 2497-2506.
2. **Dixon Richard A., Pasinetti Giulio M., 2007** - *Flavonoids and Isoflavonoids*. Plant Biology to Agriculture and Neuroscience, 154(2), pp. 453-457.
3. **Doganlar Z. B., Demir K., Basak H, Gul I., 2010** - *Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars*. African Journal of Agricultural Research 5(15), pp. 2056-2065.
4. **Khatiwora E., Vaishali B. Adsul, Manik M. Kulkarni, Kashalkar R.V., Deshpande N.R., 2010** - *Spectroscopic determination of total phenol and flavonoid contents of Ipomoea carnea* International Journal of ChemTech Research, 3 (2), pp 1698-1701.
5. **Ksouri R., Megdiche W., Debez A., Falleh H., Grignon C., Abdelly C., 2007** - *Salinity effects on polyphenol content and antioxidant activities in leaves of the halophyte Cakile maritime.* Plant Physiology and Biochemistry 45, pp. 244-249.
6. **Parvaiz A., Azooz M.M, Prasad M.N.V., 2013** - *Ecophysiology and responses of plants under salt stress.* Springer 15, p. 298-299.

CHLOROPHYLL CONTENT INDEX OF SOME NE-ROMANIA *PHASEOLUS VULGARIS* L LOCAL CULTIVARS, UNDER SALT STRESS

INDICELE CONȚINUTULUI DE CLOROFILĂ A UNOR POPULAȚII LOCALE DE FASOLE DIN NE-ROMÂNIEI, EXPUSE STRESULUI SALIN

***MARTA Alina Elena*[1], *JITĂREANU Carmenica Doina*[1], *SLABU Cristina*[1]**
e-mail: cristinaslabu@yahoo.com

Abstract. *Soil salinity is a significant limiting factor affecting crop productivity in many areas of the world. The development of salt-resistant crops has made very limited progress despite tremendous efforts. From this reason, the capitalization of salt tolerance variability in local landraces should be a first step in plant breeding for the improvement of salt tolerance of cultivated species. Phaseolus vulgaris L is a species particularly sensitive to salt stress A decrease in chlorophyll content under salt stress is reported as a commonly phenomenon in various studies. For this reason, the aim of this work was to determinate the effect of NaCl excess on chlorophyll content index at 19 common bean local landraces, at different growth stages, as an indicator of salt stress tolerance. After NaCl treatments, the most salt sensitive local landraces were Tudora 1, Balş 1, Copalău 2 and 3, Iezer 2, Moşna 6, and the most tolerant Coropceni 1 and 2, Iezer 4 and 5, Moşna 3 and 5, Săveni 1 and 7 and Truşeşti 5.*
Key words: *salt stress, Phaseolus vulgaris, chlorophyll content index*

Rezumat. *Salinitatea solului reprezintă un factor limitativ important pentru productivitatea culturilor agricole, în multe regiuni ale globului. Cu toate eforturile depuse, crearea de plante agricole rezistente la salinitate a înregistrat mici progrese. Din acest motiv, capitalizarea variabilității genetice, referitor la rezistența la salinitate a populațiilor locale, poate constitui un prim pas în lucrările de ameliorare, în vederea îmbunătățirii toleranței la salinitate a speciilor cultivate. Phaseolus vulgaris L este o specie deosebit de sensibilă la stres salin. Reducerea conținutului de clorofilă, sub efectul stresului salin, este descrisă ca un fenomen comun, în numeroase studii. Din acest considerent, scopul lucrării de față a fost determinarea efectului excesului de NaCl asupra dinamicii indicelui conținutului de clorofilă în cazul a 19 populații locale de fasole, ca un indicator al toleranței la stres salin. După tratamentul cu NaCl, cele mai sensibile cultivare s-au dovedit a fi: Tudora 1, Balş1, Copalău2 şi 3, Iezer 2, Moşna 6, iar cele mai tolerante: Coropceni 1 şi 2, Iezer 4 şi 5, Moşna 3 şi 5, Săveni 1 şi 7 şi Truşeşti 5.*
Cuvinte cheie: *stres salin, Phaseolus vulgaris, indicele conținutului de clorofilă*

INTRODUCTION

Soil salinity is a significant factor affecting crop productivity in many areas of the world. The decreases of plant growth and yield, depend on the plant

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

species, salinity levels, and ionic composition of the salts (Husain et al., 2010, Slabu et al., 2009).

Phaseolus vulgaris L. is one of the most important food plants, cultivated in several cultivars (Burescu and Toma, 2005), for its seeds rich in protein (15%), fat (2%) and starch (80%). Is a species particularly sensitive to the presence of NaC1 in the root zone (Greenway and Munns, 1980). During the last decades, the development of salt-resistant crops has made very limited progress despite tremendous efforts (Schubert et al., 2009). The investigation of stress exposed plants and subsequent identification of certain stress-specific biomarkers revealed important information about the limits of stress tolerance. (Gostin, 2007). The genetic variation in cultivated beans germplasm for salinity tolerance is limited (Gama et al., 2007). Although, it is important to test several cultivar regarding this quality. From this reason, the capitalization of salt tolerance variability in local landraces should by a first step in plant breeding for the improvement of salt tolerance of cultivated species.

Under salt stress, the decrease in chlorophyll content is a commonly reported phenomenon in various studies (Taffouo et al., 2009, Nazarbeygi et al., 2011, Mehr , 2013). Salinity and salt stress duration significantly affected photosynthesis (Bayuelo-Jimenez, 2012). For pinto bean found Ghassemi-Golezani et al. (2012) that under salt stress, the chlorophyll content index (CCI) of leaves diminished with progressing plant development at reproductive stages and during reproductive stages CCI of all cultivars decreased with increasing plant senescence. For faba bean found Slabu et al. (2009) that the reduction of chlorophyll in leaves after NaCl exposure may be explained as a result of high Cl^- concentrations in the chloroplasts, which is amplified by a simultaneously high Na^+ concentration. On the other hand, it is known that under salt stress, the plants have a reduced growth and an intense green color (Mengel, 1991), respectively a higher chlorophyll concentration in the leaves. These aspects can be explained by the biphasic response of plants to salinity, proposed by Munns (2002): a rapid, osmotic phase that inhibits growth of young leaves, and a slower, ionic phase that accelerates senescence of mature leaves. In the second phase of salt stress, NaCl decreased total chlorophyll concentration in the leaves by destruction of chloroplast structure and the instability of pigment protein complexes (Koyro, 2002). Plant adaptations to salinity are of three distinct types: osmotic stress tolerance, Na^+ or Cl^- exclusion, and the tolerance of tissue to accumulated Na+ or Cl^- (Munns and Tester, 2008). Therefore maintaining high levels of chlorophyll content in leaves can be an indication of greater salinity tolerance of plants.

The aim of this work was to determinate the effect of salt stress on chlorophyll content index at 19 common bean local landraces, at different growth stages, as an indicator for salt stress tolerance.

MATERIAL AND METHOD

A pot experiment with factorial arrangements on the bases of randomized complete block with three replications was conducted in 2014 to investigate the

salinity tolerance at 19 common bean local landraces. The cultivars were collected from NE Romania Iasi and Botosani districts from areas with saline excess. Six seeds were sown 3 cm deep in each pot, filled with 10 kg garden soil. The pots were placed in the greenhouse. The temperature variation in the greenhouse was 17- 30ºC. After the emergence were kept only three plants per pot. After the occurrence of the second true leaves, the plants were exposed to salt stress experimental variants: V1 – control, watered with water only; V2 - 4 watering with 1 liter 100 mM NaCl solution for each watering; V3 - 4 watering with 1 liter 200 mM NaCl solution for each watering. Plants were grown to full maturity.

Chlorophyll content index was measured in each plot by using a portable chlorophyll content meter (CCM-200, Opti-Sciences Inc., NH, USA). Ten measurements were taken for each pot. 4 determinations were made: d1 - after a week of exposure of plants to salt stress; d2 - alt flowering stage; d3 - beginning of pot development; d4 - ripening of fruit and seed.

RESULTS AND DISCUSSIONS

At the first determination (d1) control plants showed variations of CCI values between 11.7 (Truşeşti 5) and 21.0 (Copalău1). The treatment with 100 mM NaCl (V1) for one week led to an increase of the CCI values, 14.5 (Truşeşti) and 24.7 (Coropceni 1). The same behavior has been observed after the treatment with 200 mM NaCl, CCI values ranging from 12.0 (Coropceni 2) and 23.0 in Copalău 1 (table 1).

Table 1

Chlorophyll content index at vegtative growth stages (d1) and by flowering (d2)

	d1			d2		
Cultivars	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**
Balş 1	17,9	19,2	19,5	21,1	24,1	24,0
Copalău 1	21,0	23,3	23,0	27,9	21,2	22,4
Copalău 2	15,2	17,1	17,6	15,3	17,2	16,1
Copalău 3	13,8	17,1	17,6	15,7	17,7	18,7
Codreni 1	16,3	17,3	13,0	14,4	14,1	13,1
Coropceni 1	17,5	24,7	17,2	24,3	19,2	16,7
Coropceni 2	17,7	17,7	12,0	21,0	16,6	17,7
Iezer 2	14,7	14,7	15,1	19,6	22,5	23,0
Iezer 4	14,3	18,7	18,1	20,0	24,3	17,4
Iezer 5	12,2	16,7	16,2	19,8	28,8	25,9
Moşna 1	18,6	23,9	17,8	17,9	25,6	17,0
Moşna 3	12,3	16,3	17,8	14,9	16,2	17,6
Moşna 4	13,4	17,7	14,7	14,7	23,2	20,7
Moşna 5	21,0	21,7	20,4	21,5	22,8	23,8
Moşna 6	12,3	17,0	13,7	16,7	19,4	18,0
Săveni 1	14,8	18,6	17,9	16,0	19,9	25,2

Săveni 7	12,2	18,4	19,5	16,3	16,4	25,5
Truşeşti 5	11,7	14,5	13,8	18,2	16,8	21,2
Tudora 1	16,6	17,8	18,8	19,6	22,6	17,8
MIN	**11,7**	**14,5**	**12,0**	**14,4**	**14,1**	**13,1**
MAX	**21,0**	**24,7**	**23,0**	**27,9**	**28,8**	**25,9**

The studies done by Beinsan C. et al, 2009 showed for the common beans an increase in chlorophyll content at lower level of stress and a decrease in more stressed variant. Dadkhah and Moghtader (2008) found for the sugar beet that under salt stress leaves appeared healthy and leaf chlorophyll content increased with increasing salinity. The increasing in chlorophyll can be explained due to salinity effect of leaf area. Leaves of stressed plants became thicker than unstressed plants. Thicker leaves contain more cells in a certain leaf area (Dadkhah and Moghtader, 2008). Also in the case of common bean salinity concentration caused considerable reduction of leaf area with differences between bean local landraces on different variant of osmotic stress (Beinsan et al., 2009).

The increase of CCI at vegetative growth phenophase could by explain by a leaf reduction under salt stress without a destruction of chloroplast.

At the flowering stage (d1) a growth of CCI was recorded in 11 of the analysed cultivars, in the rest of the cultivars was observed a reduction of CCI, after the treatment with 100 mM NaCl or with 200 mM NaCl (table 1).

At the beginning of pot development (d3), CCI values remain elevated in most cultivars, ranging between 14.3 and 28.4 (table 2) except cultivars Balş 1 and Tudora 1, wherein the plants are completely chlorotic.

Table 2

Chlorophyll content index at beginning of pot development (d3) and by ripening of fruit and seed (d4)

	d3			d4		
cultivars	**V1**	**V2**	**V3**	**V1**	**V2**	**V3**
Balş 1	22,0	3,3	2,5	12,4	0,0	0,0
Copalău 1	26,9	22,8	21,9	17,4	6,7	0,0
Copalău 2	21,6	17,8	16,7	6,3	0,0	0,0
Copalău 3	16,5	20,2	19,8	7,2	0,0	0,0
Codreni 1	14,3	12,2	11,1	6,5	8,9	0,0
Coropceni 1	28,4	25,5	24,5	14,4	10,0	4,6
Coropceni 2	23,8	18,1	21,1	9,3	7,4	6,0
Iezer 2	24,8	26,5	21,5	10,9	0,0	0,0
Iezer 4	16,3	20,1	15,1	12,2	6,1	6,5
Iezer 5	21,5	19,8	20,5	13,3	10,5	9,5
Moşna 1	22,9	18,1	17,8	0,0	0,0	0,0
Moşna 3	14,7	16,6	17,1	9,8	12,1	8,1

Moşna 4	17,0	20,1	14,8	9,9	3,9	0,0
Moşna 5	24,1	24,6	18,3	9,3	4,0	3,9
Moşna 6	17,3	20,3	14,1	9,2	0,0	0,0
Săveni 1	17,2	26,3	19,6	12,3	11,0	8,3
Săveni 7	16,6	22,1	23,4	10,3	5,0	5,9
Truşeşti 5	18,6	18,5	23,7	11,6	4,7	7,8
Tudora 1	20,4	0,0	0,0	17,6	0,0	0,0
MIN	**14,3**	**0,0**	**2,5**	**6,3**	**0,0**	**3,9**
MAX	**28,4**	**26,5**	**24,5**	**17,6**	**12,1**	**9,5**

At ripening of fruit and seed (table 2), normally occurs a decrease of CCI values by control variants, which are between 6.3 (Copalău 2) and 17.6 (Tudora 1). The treatments with 100 mM NaCl caused the drying of cultivars: Balş 1, Copalău 2 and 3, Iezer 2, Moşna 6. The treatments with 200 mM caused plants death from cultivars: Copalău 1, Codreni 1, Moşna 4. The decrease of CCI values after saline treatment may be explained by the toxic effect of Na^+ and Cl^- ions that disorganize the cells, especially chloroplasts, resulting a reduction of chlorophyll content.

After this salt treatments, the most tolerant cultivars, by which the chlorosis occurred less, were Coropceni 1 and 2, Iezer 4 and 5, Moşna 3 and 5, Săveni 1 and 7, and Truşeşti 5.

CONCLUSIONS

1. At the analysed cultivars, the control variants, watered with water only, the chlorophyll content index shows high variability.

2. NaCl treatment causes an increase in CCI, excepting of three local populations at 200 mM NaCl: Codreni 1, Coropceni 1 and Coropceni 2.

3. By the sensitive variants, elevated CCI is maintained until flowering and by the salinity tolerant variants until the pods formation.

4. After NaCl treatments, the salt sensitive local landraces were Tudora 1, Balş 1, Copalău 2 şi 3, Iezer 2, Moşna 6, and the most tolerant Coropceni 1 and 2, Iezer 4 and 5, Moşna 3 and 5, Săveni 1 and 7 and Truşeşti 5.

***Acknowledgments**: This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number PN-II-PT-PCCA-2011-3.1-0965.*

REFERENCES

1. Bayuelo-Jimenez J. S., N. Jasso-Plata, and I. Ochoa, 2012 - *Growth and Physiological Responses of Phaseolus Species to Salinity Stress*. International Journal of Agronomy doi:10.1155/2012/527673, pp. 1-14.

2. Beinsan C., Camen D., Sumalan R., Babau M., 2009 - *Study concerning salt stress effect on leaf area dynamics and chlorophyll content in four bean local landraces*

from Banat area. 44th Croatian and 4th International Symposium on Agriculture, Opatija, Croatia, 16-20 febr., pp. 416-419.
3. **Burescu P., Toma I., 2005** – *Manual de lucrări practice de botanică*. Ed. Universităţii din Oradea, pp. 346-347.
4. **Dadkhah A., Moghtader S. H., 2008** - *Growth and gas exchange response of sugar beet (*Beta vulgaris L.*) cultivars grown under salt stress.*,in :*Photosynthesis-energy from the sun,* Eds: J F Allen; et al. Ed. *Springer Netherlands,* p. 1432 -1436.
5. **Gama P. B. S., Inanaga S., Tanaka K., Nakazawa R., 2007** - *Physiological response of common bean (*Phaseolus vulgaris L.*) seedlings to salinity stress*. African Journal of Biotechnology, vol. 6 (2), pp. 79–88.
6. **Greenway H., Munns R., 1980** - *Mechanisms of salt tolerance in nonhalophytes*. Annu. Rev. Plant Physiol. 31, pp. 149-190.
7. **Ghassemi-Golezani K., Neda Nikpour-Rashidabad, Saeid Zehtab-Salmasi, 2012** - *Physiological performance of pinto bean cultivars under salinity*. IJPAES, vol 2(2), pp. 223 -228.
8. **Gostin Irina, 2007** – *Biomarkeri structurali la plante*. Ed. Univ. „Al I Cuza" Iasi, pp.17.
9. **Hussain N., Sarwar G., Schmeisky H., Al-Rawahy S., Mushtaque A., 2010** - *Climate change and management of cool season grain legume crops*. Eds: Yadav SS, McNeil DL, Redden R, Pati. Ed. Springer, Heidelberg, pp.178.
10. **Koyro H. W., 2002** - *Ultrastructural effects of salinity in higher plants*. In: *Salinity: Environment – Plants – Molecules*. Eds: A. Läuchli and U. Lüttge, Ed. Kluwer Academic Publ. pp.139-158.
11. **Mehr Z. S., 2013** - *Salt-induced changes in germination and vegetative stages of* Anethum graveolens L. Journal of Stress Physiology & Biochemistry Vol. 9(2), pp. 190 -198.
12. **Mengel K., 1991** - *Ernährung und Stoffwechsel der Pflanze*. Ed. G Fischer, Jena, pp. 267.
13. **Munns R., 2002** - *Comparative physiology of salt and water stress*. Plant, Cell and Environment 25, pp. 239–250.
14. **Munns R., Tester M., 2008** - *Mechanisms of Salinity Tolerance* . Annual Review of Plant Biology. Vol. 59: pp. 651-681.
15. **Nazarbeygi E., Yazdi H.L., Naseri R., Soleimani R., 2011** - *The effects of different levels of salinity on proline and a-, b- chlorophylls in canola*. Amer-Eurasian J. Agric. Environ. Sci., Vol.10, pp. 70-74.
16. **Slabu C.,. Zörb Ch, Steffens D., Schubert S., 2009** - *Is salt stress of faba bean* (Vicia faba) *caused by* Na^+ *or* Cl^- *toxicity*? J. Plant Nutr. Soil Sci.172, pp. 644–650.
17. **Schubert S., Anja Neubert, Antje Schierholt, Ali Sümer and Christian Zörb, 2009** - *Development of salt-resistant maize hybrids: The combination of physiological strategies using conventional breeding methods*. Plant Science 177, pp.196–202.
18. **Taffouo V. D., J. Kemdem Kouamou, L. M. Tchiengue Ngalangue, B. A. Nandjou Ndjeudji, A. Akoa, 2009** - *Effects of salinity stress on growth, ions partitioning and yield of some cowpea* (Vigna unguiculata L. *Walp*.) *cultivars.* International Journal of Botany, 5, pp.135-143.

CHLOROPHYLL CONTENT INDEX AND LEAF AREA OF SOME TOMATO LOCAL CULTIVARS FROM N-E ROMANIA, UNDER SALT STRESS

INDICELE CONȚINUTULUI DE CLOROFILĂ ȘI SUPRAFAȚA FOLIARĂ A UNOR POPULAȚII LOCALE DE TOMATE DIN NORD-ESTUL ROMÂNIEI, EXPUSE STRESULUI SALIN

BOLOGA (COVAȘĂ) Mihaela[1], JITĂREANU Carmenica Doina[1], MARTA Alina Elena[1], SLABU Cristina[1]
e-mail: miha_bologa@yahoo.com

Abstract: *Soil salinity is an important abiotic stress factor seriously affecting plant productivity and survival. Photosynthesis and growt of many plants are inhibited under NaCl salinity. The research was conducted under greenhouse condition. The biological material was represented by four local tomatoes populations collected from areas with saline soils from Moldavia region and compared with commercial type salt-tolerant tomato. Tomato genotypes in the study were subjected to salt stress for a period of 30 days is constantly wetted with saline solution to a concentration of 100 mM and 200 mM. The chlorophyll content was determinated whit chlorophyll meter and the leaf area with portable scanner AreaMeter AM 300 – 0002. Analyzed the results based on biphasic model of growth response under salt stress proposed by Munns (1993), and found that tomatoes reacted similarly. The transition from phase I to phase II, is done by salt stress intensity, but mostly by cultivated genotype.*
Key words: *Soil salinity, tomato, leaf area, chlorophyll content.*

Rezumat: *Dintre factorii de stres din mediu, salinitatea rămâne principalul factor care pune sub semnul întrebării viitorul agriculturii. Procesul de fotosinteză pentru multe specii de plante este inhibat de concentrația NaCl. Experiența a fost înființă în vase de vegetație în condiții de seră. Au fost luate în studiu 4 genotipuri de tomate colectate din solurile saline ale Moldovei și un soi comercial rezistent la salinitate. Acestea au fost expuse stresului salin pe o perioadă de 30 de zile, fiind udate constant cu soluții saline de concentrație 100 mM și 200 mM. Conținutul de clorofilă al frunzelor a fost determinat cu ajutorul clorofilometrului iar, pentru suprafața foliară s-a utilizat aparatul portabil AreaMeter AM 300 – 0002. Analizând rezultatele pe baza modelului bifazic de reacție al plantelor la salinitate propus de către Munns (1993), s-a constatat că și tomatele se înscriu în același model. Trecerea de la faza I la faza a II-a, făcându-se în funcție de intensitatea stresului salin și de genotipul cultivat.*
Cuvinte cheie: *salinitatea solului, tomate, suprafață foliară, conținut de clorofila*

INTRODUCTION

Soil salinity is an important abiotic stress factor seriously affecting plant productivity and survival. Growth and development of glycophytes are negatively

[1] University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

affected but halophytes tolerate high salt concentrations (Doganlar et. al., 2010). Salinity resistance of crops is determined by the physical properties of the soil, physiological particularities of plant, growth and development phase (Jităreanu, 2007).

The tolerance to Na^+ of plants under salt conditions (*NaCl*), the prevention of the replacement of Mg^+ with Na^+ and the continuous increase of chlorophyll amount is accepted as an important indicator of salt tolerance. It is stated that plants with high chlorophyll content under salinity stress are more tolerant to salt (Yaşar and Esra, 2012). The decrease in chlorophyll content of the leaves of the plants treated with *NaCl*, may be caused by the increase in the concentration of Cl^- in the chloroplast, which may be amplified simultaneously by increasing the concentration of Na^+, as a result of synergistic effect. An increase in the concentration of Mg^+ in the nutrient solution prevents effectively lowering the concentration of chlorophyll (Slabu, 2005).

Photosynthesis is one of the mostly affected factors due to salt stress (Babu et. al., 2011). The decline in photosynthesis due to salinity stress could be due to lower stomata conductance, depression in carbon uptake and metabolism, inhibition of photochemical capacity or a combination of all these factors (Zhani et. al., 2012).

Leaf area represent an important physiologic index in characterization of intensity to some metabolic process (groweing, transpiration, photosynthesis, respiration, etc) (Șumălan and Dobrei, 2002). Ciobanu (Popescu) and Șumălan (2009), shwoed that at the plant exposed to saline environment generally has the leaf area reduced.

MATERIAL AND METHOD

The research was conducted under greenhouse condition from USAMV Iași.

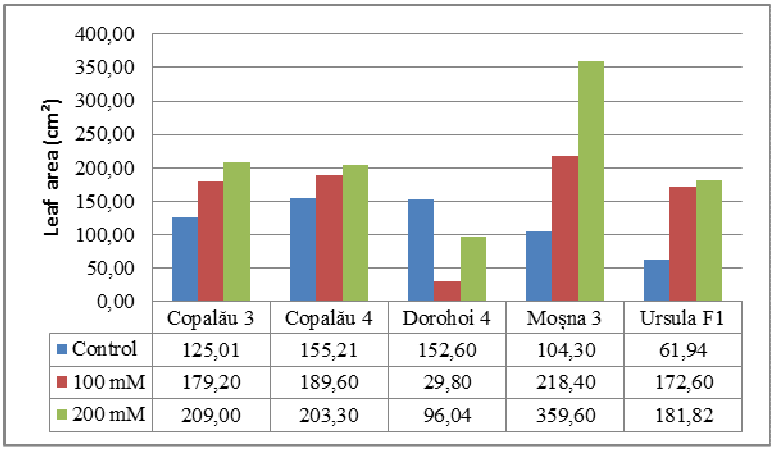
The biological material was represented by local tomatoes populations collected from areas with saline soils from Moldavia region and compared with commercial type salt-tolerant tomato (*Ursula* $_{F1}$) from Israel.

The bifactorial experience was conducted in a pots experiment in randomized blocks with four repetitions. Four tomato genotypes (*Copalău*$_3$, *Copalău* $_4$, *Dorohoi* $_4$, *Moșna* $_3$) studied were subjected to salt stress for a period of 30 days is constantly wetted with saline solution to a concentration of 100 mM and 200 mM.

The chlorophyll content was determinate whit chlorophyll meter and the leaf area with portable scanner Area Meter AM 300 – 0002.

RESULTS AND DISCUSSIONS

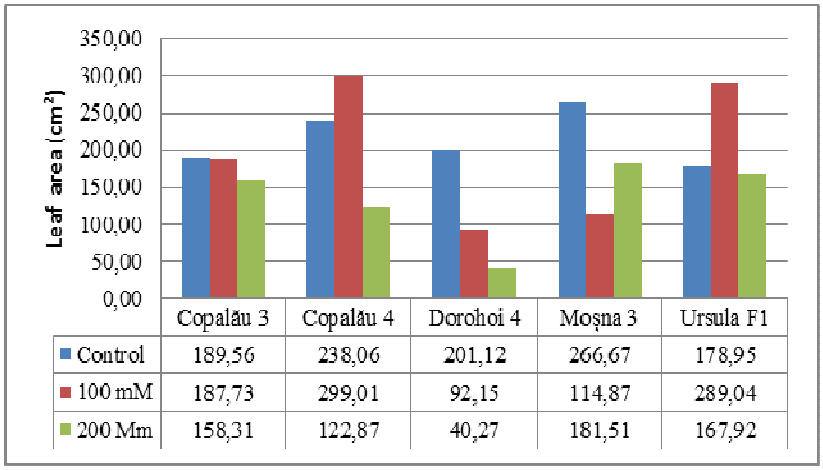
After 30 days, treatment with *NaCl* did not interfere with the foliar growth in the base of the stem. Found an increase in leaf area in the two concentrations of *NaCl* compared to control, in tomato genotypes under study, which shows that the leaves appeared before applying treatments with *NaCl*, grown in the absence of stress are not affected. An exception is genotype *Dorohoi*$_4$ exposed to a concentration of 100 mM (fig. 1).



	Copalău 3	Copalău 4	Dorohoi 4	Moșna 3	Ursula F1
Control	125,01	155,21	152,60	104,30	61,94
100 mM	179,20	189,60	29,80	218,40	172,60
200 mM	209,00	203,30	96,04	359,60	181,82

Fig. 1 - Effect of salt stress on leaf growth in the basal third of the stem

The middle third of the stem, salinity affects leaf growth compared to control to 2 genotypes (*Dorohoi*$_4$, *Moşna*$_3$,) exposed to 100 mM *NaCl* concentration. In the exposure 200 mM all genotypes showed lower values of leaf area. Compared to *Ursula* $_{F1}$, salinity resistant varieties all untreated genotypes showed higher values. When exposed to excess salt *Copalău*$_4$ one genotype showed higher values (fig. 2).



	Copalău 3	Copalău 4	Dorohoi 4	Moșna 3	Ursula F1
Control	189,56	238,06	201,12	266,67	178,95
100 mM	187,73	299,01	92,15	114,87	289,04
200 Mm	158,31	122,87	40,27	181,51	167,92

Fig. 2 - Effect of salt stress on leaf growth in the middle third of the stem

The manifestation of the negative effects of excess salt, to the leaves of the middle, can be explained by the fact that the leaves have started to grow under salt stress effect, and by the fact that the ions are transported with the mineral water to areas where they accumulate increased sweating.

Increased leaf from the top stem was strongly affected by excess salt to the all cultivars studied (fig. 3).

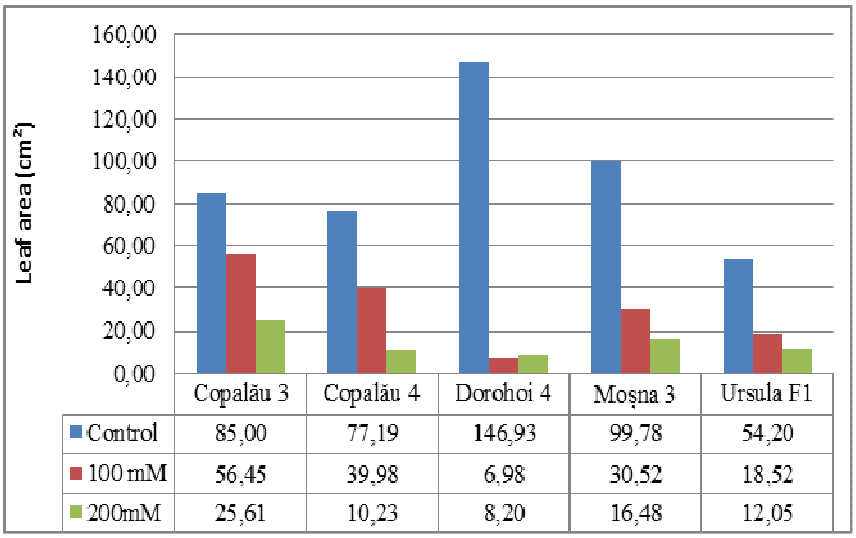


Fig. 3 - Effect of salt stress on leaf growth in the top of the stem

The chlorophyll content of leaves was determined as chlorophyll content index (CCI).

After 15 days salt stress has been found differences between the genotypes in the concentration of chlorophyll in the leaf level. In the case of tomatoes exposure to 100 mM concentration, in comparison with the plants watered only with water there is a lower value genotypes *Moşna*$_3$, *Copalău*$_3$ and an increase in chlorophyll index for the other genotypes.

When exposed to 200 mM the chlorophyll content is higher for all genotypes (fig. 4). This shows that plants to chlorophyll content index registers values higher compared with control variant, are in the phase osmotic stress.

After 30 days exposure to 100 mM, compared to control variant observed higher values of chlorophyll content of leaves for all genotypes, except genotype *Dorohoi*$_4$ which shows their maintenance during osmotic stress. In the exposure of 200 mM genotypes *Dorohoi*$_4$, *Moşna*$_3$ and *Ursula* $_{F1}$ have higher values compared to plants watered with water only, this means that they are still in the process of osmotic stress.

Genotypes *Copalău*$_4$ *Copalău*$_3$ has lower values compared with to control variant (fig. 5). In this case switch-on the second phase of stress, the ion toxicity, disturbances in the chloroplast. The transition from phase I (osmotic stress) to phase II (ions toxicity), is done by salt stress intensity, but mostly by cultivated genotype (Muuns, 1993).

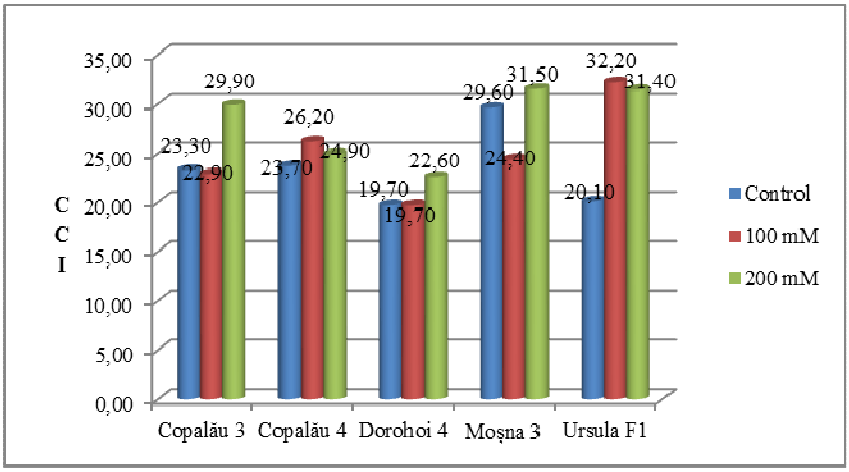


Fig. 4 - The index chlorophyll content after 15 days of exposure to salt stress

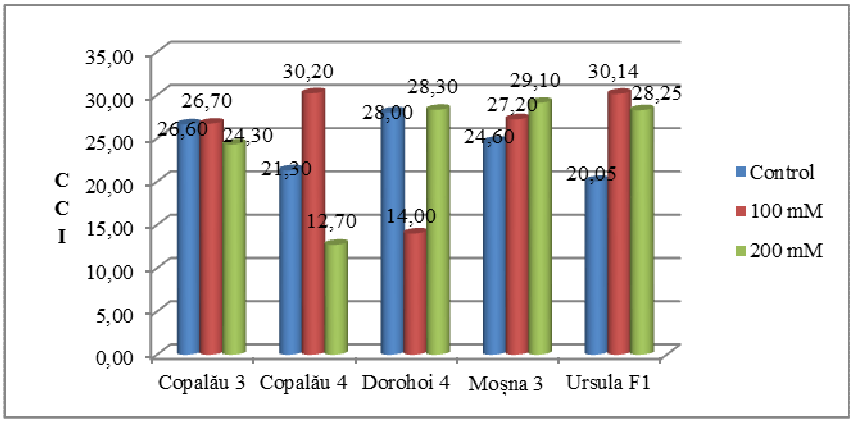


Fig. 5 - The index chlorophyll content after 30 days of exposure to salt stress

CONCLUSIONS

After 30 days, treatment with *NaCl* did not interfere with the foliar growth in the base of the stem but the middle third of the stem, salinity affects leaf growth compared to control to 2 genotypes. In the exposure 200 mM all genotypes showed lower values of leaf area.

Increased leaf from the top stem was strongly affected by excess salt to the all cultivars studied.

The plants to chlorophyll content index showing the higher values compared with control variant, are in the phase osmotic stress.

The transition from phase I (osmotic stress) to phase II (ions toxicity), is done by salt stress intensity, but mostly by cultivated genotypes.

Acknowledgement. *This work was supported by a grant of the Romanian National Authority for Scientific Research, CNDI-UEFISCDI, project number PN-II-PT-PCCA-2011-3.1-0965.*

REFERENCES

1. **Babu M.A., Singh D., Gothandam K.M., 2011** - *Effect of salt stress on expression of carotenoid pathway genes in tomato*. Journal of Stress Physiology & Biochemistry, 7(3), pp. 87-94.
2. **Doganlar Z.B., Demir K., Gul I., 2010** - *Effects of salt stress on pigment and total soluble protein contents of three different tomato cultivars*. African Journal of Agricultural Research, 5(15), pp. 2056-2065.
3. **Jităreanu C. D., 2007** - *Fiziologia plantelor*. Ed. Ion Ionescu de la Brad, Iasi.
4. **Munns R.,** 1993 - *Physiological processes limiting plant growth in saline soils: some dogmas and hypotheses.* Plant Cell and Environment 16, pp. 15-24.
5. **Popescu (Ciobanu) I., Şumălan R., 2009** - *The effects of the salinity stress on the growing rates and physiological characteristics to the Lycopersicum esculentum specie*. Buletin UASVM Horticulture, 66 (2), pp. 616-620.
6. **Slabu C.**, 2005 – *Physiologische Reaktion der Ackerbohne (Vicia Faba L.) auf Natrium und Chlorid*. Ed. Ion Ionescu de la Brad, Iasi.
7. **Şumălan R., Dobrei C., 2002** - *Fiziologie vegetală lucrări practice*. Ed. Marineasa Timișoara.
8. **Yaşar A., Esra S., 2012** -*The effect of salt stress on growth, chlorophyll content, proline and nutrient accumulation, and K/Na ratio in walnut*. Pakistan Journal of Botany, 44(5), pp. 1513-1520.
9. **Zhani K., Mariem B.F., Fardaous M., and Cherif H., 2012** - *Impact of salt stress (NaCl) on growth, chlorophyll content and fluorescence of Tunisian cultivars of chili pepper (Capsicum frutescens L.)*. Journal of Stress Physiology & Biochemistry, 8(4), pp. 236-252.

EXPERIMENTAL RESULTS ON RUNNER BEAN CULTIVATION (*PHASEOLUS COCCINEUS* L.) IN INTERCROPPING SYSTEM

REZULTATE EXPERIMENTALE PRIVIND CULTIVAREA FASOLEI MARI (*PHASEOLUS COCCINEUS* L.) ÎN SISTEM INTERCROPPING

HAMBURDĂ Silvia Brîndușa[1], MUNTEANU N.[1], STOLERU V.[1], BUTNARIU Gianina[1], TELIBAN G. C.[1], POPA Lorena Diana[1]
e-mail: silvia_hamburda@yahoo.com

Abstract. *This paper presents the behavior of runner bean (Phaseolus coccineus L.) in three intercropping systems, with maize, sunflower and Jerusalem artichoke, compared to a pure culture system (available in three plant display variants - trellis with individual string on a single row, individual string trellis on two rows and synthetic net trellising). The results were evaluated based on the main morphological and phenological plant characteristics, including agro-productivity. The results revealed significant differences between the studied variants, demonstrating the superiority of pure culture growing on synthetic mesh trellis.*
Key words: *pure crop, associated crop, trellising system, runner bean yield*

Rezumat. *Lucrarea prezintă modul de comportare a fasolei mari (Phaseolus coccineus L.) în trei sisteme de intercropping cu porumb, cu floarea soarelui și cu topinambur, în comparație cu sistemul de cultură simplă (în trei variante de dispoziție a plantelor – palisat pe spalier cu sfoară individual într-un singur rând, palisat pe spalier pe sfoară individual pe două rânduri și palisat pe spalier cu plasă sintetică). Rezultatele au fost evaluate pe baza principalelor caracteristici morfologice și fenologice ale plantelor, inclusiv cele de agroproductivitate. Rezultatele au pus în evidență diferențe semnificative între variantele studiate, demonstrând superioritatea variantei de cultivare în cultură pură palisată pe spalier cu plasă sintetică.*
Cuvinte cheie: *cultură pură, cultură intercalată, sistem palisare, producție de fasole mare*

INTRODUCTION

Runner bean (*Phaseolus coccineus* L.) is an herbaceous, annual and out-crossing species, propagated, usually, by seeds, adapted to a relativ cool and humid climate. Such a climate it is not proper for the North – East region of Romania (Popa, 2010). To achieve cost-effective production, it is necessary to diversify the cultivation of this species, through optimization of design and period setting and the use of various support systems, such as runner beans' interleaving with other cultivated species (maize, sunflower, Jerusalem artichoke), whose stem is also supporting bean plants (Hamburdă et al., 2013).

[1]University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

Intercropping is based on the management of plant interactions, in order to increase the productivity (Vandermeer, 1989; Wiley, 1979 a, b). The success of intercropping depends on thorough knowledge of the botanical, ecological and technological particularities of associated species, especially habitus, root morphology, requirements for water and nutrients. The purpose of this system is to put the growing crops in the same place throughout the growing season or at least for a period of time (Mousavi and Eskandari, 2011). The plants involved in intercropping may be seeded or planted at the same time or at different times (Ofori and Stern, 1987).

This paper presents the behavior of runner bean in three intercropping systems, with common maize (*Zea mays* L.), sunflower (*Helianthus annuus* L.), and Jerusalem artichoke (*Helianthus tuberosus* L.), compared to a pure culture system (available in three plant display variants - trellis with individual string on a single row, individual string trellis on two rows and synthetic mesh trellising). The need for this research stems from the fact that a standard technology for runner bean crop is not yet developed. Thus, it is an attempt to obtain cost-effective productions, by using different means of trellising and determining the most efficient trellising system.

MATERIAL AND METHOD

In accordance with the purpose and research objectives, we organized an experience, in 2013, in the Vegetable growing department's experimental field, at "V. Adamachi" farm, U.A.S.V.M. Iaşi.

The available biological material was the runner bean seeds (C_3 local population), seeds of maize (Flato hybrid), sunflower seeds (Tristan hybrid) and Jerusalem artichoke tubers (Topstar cultivar).

Organization of the experiment was in a randomized blocks device with three repetitions. The studied experimental factor was the trellising system with six graduations, in field conditions:

V_1 = on individual string trellis with double rows,
V_2 = on individual string trellis with a single row,
V_3 = on synthetic net trellis (17 cm mesh) in a single row,
V_4 = intercropping with common maize,
V_5 = intercropping with sunflower,
V_6 = intercropping with Jerusalem artichoke.

Each recurrence plot had a 6 m^2 surface area, which consists of two spaced rows of 1.0 m, ensuring a distance of 0.4 m between runner bean plants. For the pure culture, the system consisted of a reinforced concrete support trellis formed of pillars and a steel wire of about 2 cm on the top thereof.

The establishment of runner bean crop was conducted between 1.05 - 30.05. Sowing maize and sunflower, respectively planting artichokes were made about two weeks before sowing runner bean. Crop establishment was performed by direct seeding, three runner bean seeds/nest and two maize seeds or sunflower seeds/nest. At the time of emergence, there have been left, in each nest, two runner bean plants and one of maize and sunflower plants. Artichokes were planted by two tubers/nest, and at emergence time, only one stem/plant were allowed. Thus, in the case of trellising runner bean plants on maize/sunflower/Jerusalem artichoke, for each repetition, there were 30 nests of runner bean plants and 14 plants of

maize/sunflower/Jerusalem artichoke; in the runner bean mesh and string trellising systems, there were 30 nests of runner bean plants on each repetition.

The experimental culture was conducted according to technical rules arising from the specialized literature consulted (Munteanu et al., 1989, Stan N. et al., 2003, Ruști, 2007; Popa, 2010; Axinte et al., 2006). The culture was performed on well leveled ground; the soil is a cambic chernozem, well-stocked in nutrients, with an organic matter content of 3.2 - 3.4% and a pH of 6.5-6.8. Weather conditions during the growing season (months IV to IX) were characterized by an average temperature of 17.8^{0}C, an average relative humidity of 67% and a rainfall amount of 495.4 mm.

Works were carried out as recommended for common climbing beans (Munteanu et al., 1982; Ruști and Munteanu, 2008; Popa, 2010). The culture was not irrigated.

Basic research methods were the observation and the experiment, in which, for evaluating the performance of studied variants, biometric observations and measurements were conducted on the main morphological and phenological plant features, including agro-productivity. Production results were statistically interpreted according to the specialized literature (Jităreanu, 1994; Săulescu and Săulescu, 1967).

RESULTS AND DISCUSSIONS

Between the studied trellising systems, there was no significant differences in the morphological and phenological characterization of the runner bean plants.

Results in terms of morphological characterization: runner bean plant port is voluble; size (height) of the plant is over two meters; plant vigor is high; number of branches per plant is three to four; the foliage color is dark green; flower color is white; seed color is white; pods of widely varying size, with length of 8-17 cm and a width of 1.4 to 2 cm; it is important to point out that the pod's length correlates with the number of seeds/pod, longer pods having a higher number of seeds; seed size, measured by the length of the longest axis, varies between 15 and 22 mm; the number of seed per pod ranges from two to four.

Results in the phenological characterization: emergence is hypogeic, meaning it is achieved only by the epicotil growing above ground, the cotyledon leaves remaining in the soil; period from sowing to emergence was about seven to ten days, the period from emergence to appearance of the first real trifoliate leaves was around five to seven days, the period from emergence to the first flowers was about 32 - 35 days, the period from emergence to the first pods was about 67-70 days, the period from emergence to seed maturation was around 115-122 days and the period from emergence to the end of the vegetation period was around 130-140 days (tab. 1).

Table 1

Phenological characters (number of days)

Sowing - emergence	Emergence - first real trifoliate leaf	Emergence – first flowers	Emergence – first pods	Emergence – seed maturation	Emergence – end of vegetation
7-10	5-7	32-35	67-70	115-122	130-140

Results in terms of runner bean production - pure culture

Following the investigations, it appears that seed production ranged from 2733 kg/ha to 3325 kg/ha. Highest production was obtained in the variant with trellising runner beans on synthetic mesh (3325 kg/ha), which showed distinctly significant positive differences as compared to the mean (3080 kg/ha), while the lowest production variant was obtained on runner bean individual trellising on double rows (2733 kg/ha), a very significant negative differences from the control being highlighted. The second variant (individual string trellis with a single row), achieved yields within the average experimental variation limits (tab. 2).

Table 2

Results obtained in pure culture of runner bean (year 2013)

Variant			Yield of runner bean		Differences to mean (kg/ha)	Semnification
no.	specification		kg/ha	% of the mean		
1	pure culture	V_1	2733	88,7	-347	ooo
2		V_2	3182	103,3	+102	^{NS}
3		V_3	3325	107,9	+245	**
Mean			3080	100	-	-

^{w}Significance of differences made by ANOVA (analysis of variance) for experimental factors and interaction of them; $_{NS}$, *,**,*** - indicate nonsignificant and positive significant at p≤0.05, 0.01, 0.001, respectively;
o,oo,ooo - negative significant at p ≤ 0.05, 0.01, 0.001, respectively

LSD 5% = 103,43 (kg/ha)
LSD 1% = 171,15 (kg/ha)
LSD0,1% = 320,34 (kg/ha)

Results in terms of runner bean production - intercropping

Following investigations, it appears that seed production ranged from 789 kg/ha and 3093 kg/ha (tab. 3).

Table 3

Results obtained in intercropping system (year 2013)

Variant			Runner bean yield		Differences to mean (kg/ha)	Semnification
no.	specification		kg/ha	% of the mean		
1	intercropping	V_4	1966	100,8	+16,6	^{NS}
2		V_5	3093	158,6	+1143,6	***
3		V_6	789	40,4	-1160,3	ooo
Mean			1949	100	-	-

^{w}Significance of differences made by ANOVA (analysis of variance) for experimental factors and interaction of them;
$_{NS}$, *,**,*** - indicate nonsignificant and positive significant at p≤0.05, 0.01, 0.001, respectively;
o,oo,ooo - negative significant at p≤0.05, 0.01, 0.001, respectively

LSD 5% = 271,37 kg/ha
LSD 1% = 449,03 kg/ha
LSD 0,1% = 840,47 kg/ha

Highest yield was obtained when interleaving runner bean with sunflower (3093 kg/ha), which recorded very significant positive differences as compared to the mean (1949 kg/ha), while the lowest yield was obtained in the intercalation of runner bean with Jerusalem artichoke (789 kg/ha), very significant negative differences from the control being highlighted. The third option (intercropping with maize) achieved yields within the average experimental variation limits.

Results in the production of runner bean - total experience

Following investigations, it appears that seed yield ranged from 789 kg/ha and 3325 kg/ha. Highest yield was obtained in the variant with trellising runner bean on synthetic mesh (3325 kg/ha), which recorded very significant positive differences as compared to the experimental mean (2515 kg/ha), while the lowest production was obtained when interleaving runner bean with Jerusalem artichoke (789 kg/ha), very significant negative differences from the control being highlighted (Tab. 4).

Table 4

Yield of runner bean per total experience (year 2013)

Variant			Runner bean production		Differences to mean (kg/ha)	Semnification
no.	Specification		kg/ha	% of the mean		
1	pure culture	V_1	2733	108,6	+218	x
2		V_2	3182	126,5	+667	xxx
3		V_3	3325	132,2	+810	xxx
4	intercropping	V_4	1966	78,1	-549	ooo
5		V_5	3093	122,9	+578	xxx
6		V_6	789	31,3	-1726	ooo
Experience mean			2515	100	-	-

[w]Significance of differences made by ANOVA (analysis of variance) for experimental factors and interaction of them;

[NS], *,**,*** - indicate nonsignificant and positive significant at p≤0.05, 0.01, 0.001, respectively;

o,oo,ooo - negative significant at p≤0.05, 0.01, 0.001, respectively.

LSD 5% = 163,26 kg/ha
LSD 1% = 232,08 kg/ha
LSD0,1% = 336,04 kg/ha

CONCLUSIONS

1. There were no significant differences in characterization of runner bean plants, regarding plant height, vigour of the plant, pod size, seed size, seed number in a pod, vegetation period.

2. The highest production results were obtained in the pure culture system (3080 kg/ha), in comparison with the intercropped culture (1949 kg/ha).

3 In terms of overall experience production, it ranged from 789 kg/ha (V_6 - intercropping with Jerusalem artichoke) and 3325 kg/ha (V_3 - trellising system on synthetic net).

***Acknowledgments**: This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. **Axinte M., Roman Gh.,V., Borcean I., Muntean L.S., 2006** - *Fitotehnie.* Editura "Ion Ionescu de la Brad", Iaşi.
2. **Hamburdă Silvia Brîndușa, Dascălu T., Munteanu N.**, **2013** – *Preliminary studies for new cultivation systems of runner bean (Phaseolus coccineus L.).* Lucrări științifice, seria Horticultură, USAMV Iași. 56 (2), pp. 167-172.
3. **Jităreanu G., 1994** – *Tehnică experimentală*. Editura "Ion Ionescu de la Brad", Iași, 167 pag.
4. **Munteanu N., Timofte Valentina, Timofte E., 1989** – *Aspecte tehnologice ale culturii fasolei de grădină urcătoare*. Cercetări Agronomice în Moldova, vol. 4, Iași.
5. **Mousavi S.R., Eskandari H., 2011** - *A general overview on intercropping and its advantages in sustainable agriculture*. J.Appl. Environ. Biol. Sci. 1 (11), pp. 482-486.
6. **Ofori F., Stern W.R., 1987** - *Cereal-legume intercropping system*. Advance in Agronomy. 41, pp. 41-90.
7. **Popa Lorena Diana, 2010** - *Cercetări privind agrobiologia speciei Phaseolus coccineus L. în vederea optimizării cultivării*. Teză de doctorat. USAMV Iaşi. 232 p.
8. **Ruști Gr., 2007**- *Cercetări privind îmbunătățirea tehnologiei de cultură a fasolei de grădină urcătoare (Phaseolus vulgaris L. var. comunnis L).* Teză de doctorat. U.Ș.A.M.V. Iași.
9. **Ruşti G., Munteanu N., 2008** – *Cultura fasolei de grădină urcătoare*. Editura „Ion Ionescu de la Brad", Iaşi.
10. **Stan N., Munteanu N., Stan T., 2003** – *Legumicultură*, vol III. Editura "Ion Ionescu de la Brad", Iaşi.
11. **Săulescu N.A., Săulescu N.N., 1967** - *Câmpul de experiență*- ediția a II a. Edit. Agro-Silvică de stat, Bucureşti.
12. **Vandermeer J.H., 1989** - The ecology of intercropping. Cambridge University Press, Cambridge, U.K.
13. **Willey R.W., 1979a** - *Intercropping–Its importance and research needs. Part 1. Competition and yield advantages.* Field Crop Abstr 32, pp. 1–10.
14. **Willey R.W., 1979b** - *Intercropping–Its importance and research needs. Part 2. Agronomy and research approaches.* Field Crop Abstr 32, pp. 73–85.

RESEARCH ON ENTOMOFAUNA OF THE RUNNER BEAN (*PHASEOLUS COCCINEUS* L.) CROP CULTIVATED IN INTERCROPPING SYSTEM IN FIELD

CERCETĂRI CU PRIVIRE LA ENTOMOFAUNA DIN CULTURA DE FASOLE MARE (*PHASEOLUS COCCINEUS* L.) CULTIVATĂ ÎN SISTEM INTERCROPPING ÎN CÂMP

***BUTNARIU Gianina*[1]*, HAMBURDĂ Silvia Brînduşa*[1]*, TELIBAN G.C.*[1]*, TĂLMACIU M.*[1]*, MUNTEANU N.*[1]
e-mail: gianina.butnariu@yahoo.com

Abstract. *This paper presents a study on entomofauna from runner bean crop (Phaseolus coccineus L.) cultivated in intercropping system with maize, sunflowers and Jerusalem artichokes and runner bean pure culture and pure culture of sugar maize, at "V . Adamachi" farm, Univesity of Agricultural Sciences and Veterinary Medicine Iaşi. The purpose of this study is to determine if the intercropping system is good for runner bean culture, regarding entomofauna, as compared with a pure crop, and entomofauna from a sugar maize crop. The collection was performed using the Barber insect trap. The results revealed a great diversity of fauna, useful and harmful species belonging to the Araneae, Coleoptera, Diptera, Lepidoptera, Hymenoptera, Isopoda, Orthoptera, Pulmonata and Homoptera orders. The frequency of these species varied depending on crop species and variants of intercropping.*
Key words**:** *Barber traps, sugar maize, sunflower*

Rezumat. *Lucrarea prezintă un studiu asupra entomofaunei din cultura de fasole mare (Phaseolus coccineus L.) realizată în sistem intercropping cu porumb, floarea soarelui şi topinambur, şi în cultura pură de fasole mare şi cultura pură de porumb zaharat, în condiţiile de la Ferma "V. Adamachi" a USAMV Iaşi. Scopul acestei lucrări este de a stabili dacă sistemul de culturi intercalate este benefic pentru cultura de fasole mare, în ceea ce priveşte entomofauna, în comparaţie cu o cultură pură, şi entomofauna dintr-o cultură de porumb zaharat. Colectarea insectelor a fost realizată folosind capcane de tip Barber. Rezultatele au pus în evidenţă o mare diversitate de specii de faună dăunătoare şi faună utilă din ordinele Araneae, Coleoptera, Diptera, Lepidoptera, Hymenoptera, Isopoda, Orthoptera, Pulmonata şi Homoptera. Frecvenţa acestor specii a variat în funcţie de specia cultivată şi de variantele de intercropping.*
Cuvinte cheie**:** *capcane Barber, floarea soarelui, porumb zaharat*

INTRODUCTION

Runner bean (*Phaseolus coccineus* L.) is a worthwhile species through a series of resistance genes in some pests such as plant fly (*Delia platura*) (Popa, 2010). However, present are also pests in unfavorable years which may

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

completely compromise culture. Among the most commonly pests, include: bean weevil (*Acanthoscelides obtectus* Say), black bean aphid (*Aphis fabae* Scop.), gray slug (*Deroceras Agreste* L.), red spider mite (*Tetranychus urticae* Koch.) etc. (Popa, 2010; Tălmaciu, 2003). In our country, runner bean was studied in pure culture system, currently pursuing research on this species in intercropping system (Hamburdă et al., 2013; Hamburdă et al., 2014; Munteanu, 1985, Munteanu et al., 2013; Popa, 2010). One of the advantages of intercropping is to reduce insect populations due to the diversity of cultures and attracting a large number of insects especially useful one when included in the culture are already flowering plants (Kass, 1978; Koul and Cuperus, 2007; Southwood and Way, 1970; Vandermeer, 1989). Therefore, this knowledge led us to initiate a study to determine if the intercopping system is good for growing runner bean crop in comparison with a pure culture, regarding the present entomofauna and its structure, and entomofauna from a sugar maize crop**.**

MATERIAL AND METHOD

In accordance with the purpose and objectives of the research, an experiment was organized in 2014 in the experimental field of Vegetable growing department within the "V. Adamachi" farm of U.A.S.V.M. Iasi. The experimental device was made of randomized blocks with three repetitions type. Experimental factor studied was present entomofauna in following crops, which represents also proposed variants to study: V1 = pure culture of runner bean, V2 = pure culture of sugar maize (*Zea mays* L. var. *Saccharata*), V3 = runner bean in intercopping system with common maize (*Zea mays* L.), V4 = runner bean in intercopping system with sunflower (*Helianthus annuus* L.) and V5 =runner bean in intercopping system with Jerusalem artichoke (*Helianthus tuberosus* L.). The study of these factors was carried out under field conditions. Each plot had an area of 6 m^2, consisting of two rows located at a distance of 100 cm, and between runner bean plants, was ensured a distance of 40 cm. For pure culture the support system consisted in a trellis support formed of reinforced concrete pillars and a steel wire about 2 cm on the top thereof. The establishment of runner bean crop was conducted between 1.05 and 30.05. Sowing common maize, sugar maize and sunflowers, planting Jerusalem artichoke were made about two weeks before sowing runner bean.

Establishment of runner bean crop was done by direct seeding, three seeds of runner bean / nest and two seeds of common maize, sugar maize and sunflower/nest. At the time of emergence has been left in each nest, two runner bean plants and one plant of maize, sugar maize and sunflower. In the case of Jerusalem artichokes, two tubers /nest were planted, and, in emergence time, only two stems / plant were allowed.

To collect and determine entomofauna from this experimental field, Barber traps were used and were installed on 07/01/2014. Two such traps for each variant were placed. Approximately every two weeks samples were collected respectively in data of 07/14/2014, 01/08/2014, 27/08/2014. The main research method used was observation. Determination of species and their systematic classification was carried out in the Laboratory of Entomology of UASVM, according to the specialized literature (Gaetan du Chatenet, 1990; Panin, 1951; Panin, 1952; Reitter, 1908; Rogojanu and Perju, 1979).

RESULTS AND DISCUSSIONS

The data were processed and presented comparatively for each variant.

Entomofauna collected from the runner bean pure culture highlights insects belonging to nine orders: *Araneae, Coleoptera, Diptera, Hymenoptera, Homoptera, Isopoda, Lepidoptera, Orthoptera* and *Pulmonata.* The highest number of insects , 118, was recorded in the order *Diptera* (tab.1).

Table 1

Results obtained in pure culture of runner bean (V_1)

Orders	14.07.2014	01.08.2014	27.08.2014	Total
Araneae	5	-	1	6
Coleoptera	2	3	1	6
Diptera	56	51	11	118
Hymenoptera	22	17	23	62
Homoptera	-	3	-	3
Isopoda	2	1	-	3
Lepidoptera	1	3	6	10
Orthoptera	2	-	-	2
Pulmonata	2	1	-	3

Entomofauna collected from sugar maize crop belongs to orders: *Araneae, Coleoptera, Diptera, Hymenoptera, Lepidoptera* and *Orthoptera* and the highest number of insects, 58, recorded at the *Hymenoptera* order (tab. 2).

Table 2

Results obtained in pure culture of sugar maize (V_2)

Orders	14.07.2014	01.08.2014	27.08.2014	Total
Araneae	-	2	-	2
Coleoptera	1	2	-	3
Diptera	-	12	2	14
Hymenoptera	15	25	18	58
Homoptera	-	-	-	-
Isopoda	-	-	-	-
Lepidoptera	1	5	-	6
Orthoptera	-	-	1	1
Pulmonata	-	-	-	-

Entomofauna collected from runner bean in intercropping system with common maize belongs to the following orders: *Araneae, Coleoptera, Diptera, Hymenoptera, Lepidoptera and Orthoptera,* and the highest number of insects, 77, recorded at the *Diptera* order (tab. 3)

Table 3

Results obtained in runner bean in intercropping system with common maize (V_3)

Orders	14.07.2014	01.08.2014	27.08.2014	Total
Araneae	1	-	-	1
Coleoptera	2	-	3	3
Diptera	10	21	46	77
Hymenoptera	7	13	-	20
Homoptera	-	-	-	-
Isopoda	-	-	-	-
Lepidoptera	2	3	4	9
Orthoptera	-	3	9	12
Pulmonata	-	-	-	-

Entomofauna collected from runner bean in intercropping system with sunflower belongs to the following orders*: Araneae, Coleoptera, Diptera, Hymenoptera, Isopoda* and *Lepidoptera*. The highest number of insects, 144, was recorded in the *Diptera* order (tab. 4).

Table 4

Results obtained in runner bean in intercropping system with sunflower (V_4)

Orders	14.07.2014	01.08.2014	27.08.2014	Total
Araneae	5	-	-	5
Coleoptera	3	6	-	9
Diptera	9	48	57	114
Hymenoptera	13	14	9	36
Homoptera	-	-	-	-
Isopoda	-	6	1	7
Lepidoptera	-	2	5	7
Orthoptera	-	-	7	7
Pulmonata	-	-	-	-

Entomofauna collected from runner bean in intercropping system with Jerusalem artichoke belongs to orders: *Araneae, Coleoptera, Diptera, Hymenoptera, Homoptera, Isopoda, Lepidoptera* and *Isopoda*, and the highest number of insects, 41, was recorded in the *Diptera* order (tab. 5).

Table 5

Results obtained in runner bean intercropping system with Jerusalem artichoke (V_5)

Orders	14.07.2014	01.08.2014	27.08.2014	Total
Araneae	1	-	1	2
Coleoptera	-	2	-	2
Diptera	7	25	9	41
Hymenoptera	20	7	7	34
Homoptera	2	-	-	2
Isopoda	13	-	-	13
Lepidoptera	-	-	10	10
Orthoptera	1	-	28	29
Pulmonata	-	-	-	-

We determined the following species belonging to the *Coleoptera* order, mostly harmful species (tab. 6).

Table 6

Species belonging to the *Coleoptera* order found in collected samples

Species scientific name	Variant					Total insects
	V1	V2	V3	V4	V5	
Cantharis fusca	1	1	-	-	-	2
Cicindela germanica	1	-	-	-	-	1
Coccinella septempunctata	1	-	-	-	-	1
Coccinella 14 punctata	1	-	-	-	-	1
Oxythyrea funestra	-	1	-	-	-	1
Pseudophonus griseus	-	1	-	5	-	6
Pseudophonus rufipes	1	-	3	4	1	9
Silpha carinata	1	-	-	-	-	1
Staphylinus caesareus	-	-	-	-	1	1

CONCLUSIONS

1. Following collection performed the highest number of insects, 364, recorded in the *Diptera* order.
2. The most diverse entomofauna recorded in pure culture of runner bean, belonging to a number of nine orders.
3. Stage of crop growth and development is directly proportional to entomofauna collected, with the number of insects belonging to each order.
4. Intercropping system is beneficially for the cultivation of runner bean.

***Acknowledgments**: This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. Chatenet du Gaetan, 1990 – *Guide des Coléoptères d'Europe*. Delacrois et Niestlé, Paris.
2. Hamburdă Silvia Brîndușa, Dascălu T., Munteanu N., 2013 – *Preliminary studies for new cultivation systems of runner bean (Phaseolus coccineus L.)*. Lucrări științifice, seria Horticultură, USAMV Iași. 56 (2), pp. 167-172.
4. Hamburdă Silvia Brîndușa, Munteanu N., Popa Lorena Diana, 2014 - *Evaluarea unui sortiment de fasole mare (Phaseolus coccineus L.) în condițiile din județul Iaşi.* Știința Agricolă. Universitatea Agrară de Stat din Moldova, Chișinău. 1, pp. 38-41.
5. Kass D.C.L., 1978 - *Polyculture cropping systems: A rewiew and analysis.* Cornell Univ., Ithaca, N. Y. Cornell Intl. Agr. Bul. 32.
6. Koul O., Cuperus G. W., 2007 - *Ecologically Based Integrated Pest Management*. CABI, 262 p.
7. Munteanu N., 1985 – *Phaseolus coccineus* L. *– o specie legumicolă care merită mai multă atenție*. Producția Vegetală, Horticultura, nr. 4/1985.

8. **Munteanu N., Hamburdă Silvia Brîndușa, Popa Lorena Diana, 2013 -** *Research on the main productivity features in an assortment of runner bean (Phaseolus coccineus L.) in the environmental conditions from NE Romania".* Lucrări științifice, seria Agronomie, editura Ion Ionescu de la Brad, Iași. 56 (1), pp. 159-162.
9. **Panin I., 1951** – *Determinatorul Coleopterelor dăunătoare şi folositoare din Republica Populară Română (Determinator of damaging and useful coleoptera from Romania)*. Edit. de Stat, Bucureşti.
10. **Panin I., 1952** – *Fauna Republicii Populare Române. Insecta (Fauna from Romania. Insects), vol. X, fasc. 1, Ord. Coleoptera, Fam. Carabidae.* Edit. Academiei R.P.R., Bucureşti.
11. **Popa Lorena Diana, 2010** - *Cercetări privind agrobiologia speciei Phaseolus coccineus L. în vederea optimizării cultivării*. Teză de doctorat. USAMV Iaşi. 232 p.
12. **Reitter E., 1908** – *Fauna Germanica. Die Kafer des Detschen Reiches*. Band I, Stuttgart.
13. **Rogojanu V., Perju T., 1979** – *Determinator pentru recunoaşterea dăunătorilor plantelor cultivate (Determinator for knowing the crop pests).* Edit. Ceres, Bucureşti.
14. **Southwood T.R.E. and Way M. I., 1970** - *Ecological background to pest management.* pp 6-29. In Concepts of Pests Management. Ed. Rabb, R.L. and Guthrie, F. E. North Carolina State University, Raleigh, North Carolina.
15. **Tălmaciu M., 2003** - *Protecția plantelor. Entomologie*. Editura "Ion Ionescu de la Brad", Iași.
16. **Vandermeer, J.H., 1989** - *The ecology of intercropping.* Cambridge University Press, Cambridge, U.K.

EVALUATION OF THE POSSIBILITIES OF USING RUNNER BEAN (*PHASEOLUS COCCINEUS* L.) IN LANDSCAPING DESIGN

EVALUAREA POSIBILITĂȚILOR DE FOLOSIRE A FASOLEI MARI (*PHASEOLUS COCCINEUS* L.) ÎN DESIGN-UL PEISAGER

HAMBURDĂ Silvia Brîndușa[1], MUNTEANU N.[1], STOLERU V.[1], TELIBAN G. C.[1], BUTNARIU Gianina[1], POPA Lorena Diana[1]
e-mail: silvia_hamburda@yahoo.com

Abstract. *The paper presents an overview of the specialized literature on runner bean decorative valences, determined by the main morphological, physiological and technological characteristics of this species. The paper aims is to promote the species as an ornamental plant in the specific conditions of our Romania. The main morphological characteristics refer to growth mode, habitus and plant strenght, foliage color, flower color, inflorescence number and its dynamics, shape, size and color of pods, number of pods per plant, shape, size and color of seeds. Physiological particularities refer to the main culture phenophases, their duration, the duration of the ornamental and vegetation period. Technological particularities relate to the time and manner of establishing a culture, different plant layout devices, using the support system and some specific care works.*
Key words: *mixed gardens, decorative valences, support system*

Rezumat. *Lucrarea prezintă o sinteză a literaturii de specialitate referitoare la valențele decorative ale fasolei mari, determinate de principalele caracteristici morfologice, fiziologice și tehnologice ale acestei specii. Lucrarea are ca scop promovarea acestei specii ca plantă ornamentală în condițiile din România. Principalele caracteristici morfologice fac referire la modul de creștere, habitusul și vigoarea plantei, culoarea foliajului, culoarea florilor, numărul de inflorescențe și dinamica acestuia, forma, mărimea și culoarea păstăilor, numărul de păstăi pe plantă, forma, mărimea și culoarea semințelor. Particularitățile fiziologice fac referire la principalele fenofaze în cultură, durata acestora, durata perioadei ornamentale și durata perioadei de vegetație. Particularitățile tehnologice se referă la epoca și modul de înființare a culturii, diferite dispozitive de aranjare a plantelor, folosirea sistemului de susținere și unele lucrări de îngrijire specifice.*
Cuvinte cheie: *grădini mixte, valențe decorative, suport de susținere*

INTRODUCTION

The cultivation of vegetables with ornamental value has a long tradition since ancient times, the vegetable crop marked by a great diversity of taxons (species, subspecies, varieties and forms), cultural practices and traditions of use. The huge biodiversity of vegetable species and their great movement across the globe have allowed the evidence of many uses, besides the nutritive value, such as

[1] University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

in landscaping, phytotherapy, cosmetics etc. Are known many taxons of vegetable plants what can successfully replace a series of ornamental plants within a landscaping, because of their decorative appearance. Vegetables can be placed alongside other vocation ornamental plants (flower and deontological), arranged in clusters and distributed according to size or habitus. Also, there might be places in the landscape to be used only for this type of vegetable plant with ornamental features. Decorative vegetables have a dual role, that of decorating a space in the garden or on the terrace of the house, but also to ensure vegetable products, food value recognized, combining harmoniously with pleasure (http://www.flori-cultura.ro/includerea-plantelor-leguminoase-in-amenajarile-peisagere/).

The ornamental value of vegetable plants results from their own morphological characteristics: size and general habitus of plants, shape, appearance, size and color of the leaves, flowers and fruits (Muntean *et al*., 2011). The ornamental value also results from some phenological features (vegetation period, ornamental period, the foliage, flowers, fruit etc.). Of great interest and importance is the arrangement, the alignment of these vegetable plants, ornamental features even lower, such as running groups and even some artistics, in field, garden spaces, terraces, balconies, pergolas, large or small pots, jardiniere etc. The importance of growing vegetables with ornamental value arising from permanent human need for more opportunities to create beauty. Has ever since been, man has been attracted to nature. Vegetable plants with ornamental value can be considered today as an opportunity to increase the diversity of landscape (Iliescu, 2008). Garden art provides a shining example of the use of vegetable plants in decorative arrangement – the park from Villandry castle, in France, in the Renaissance manner (Iliescu, 2008).The vegetables with ornamental value along with other ornamental plants contribute to combate air pollution and to establish an equilibrium in environment. Lately there is a particular focus on achieving a healthy gardens and decorative at the same time. Also are trying to achieve mixed gardens where vegetables and flowers are harmoniously associated, taking into account the allelopathic relations (http://www.gradinavesela.ro/2014/02/gradini-de-legume decorative/).

Cultivated in the green spaces they extend their utility through social utility character that it acquires. The vegetation in parks, gardens, squares and roads besides visible influence the microclimate of population centers. It helps purify the air and is an absolute necessity of modern life, springing from the unprecedented development of the industry through residues resulting from processes, vitiating the environment. The use and knowing of differential habitus, forms and colors, flowers, leaves and fruits, while that can easily adapt to specific environmental conditions of a site, constitutes the starting point in approaching the creation of landscape architecture (Mărgărit et al. , 2004).

In this paper we propose an overview of runner bean (*Phaseolus coccineus* L.), with emphasis on ornamental value. Ornamental value analysis is made with special reference to biological characteristics, ecophysiological and ornamental, as well as how to use in landscaping.

MATERIAL AND METHOD

Biological material to which evaluations are made on the vocation ornamental is runner bean species (*Phaseolus coccineus* L.) and some of its subspecies and varieties. Runner bean is a herbaceous annual species which normally is multiplied by seed, but in some cases vegetative with tuberous roots (Munteanu, 1985). In this study we propose to consider as many subspecies of *P. coccineus* species such as *P.c.* ssp. *formosus* (Kunth) Mare, Masch. & Stain, *P. c.* ssp. *glabellus* (Piper) A. Delgado, *P. c.* ssp. *griseus* (Piper) A. Delgado, *P. c.* ssp. *coccineus* L. and *P. c.* ssp. *darwinianus* Hdez. X. & Miranda C.

Achievement of the aim and targets is made on the basis of a literature review, systematized on groups of botanical, physiological and technological features which confers ornamental and / or landscape value. As a basic research methods have been used observation, case study and statistics grouping.

RESULTS AND DISCUSSIONS

The runner bean is a vegetable species with a broad interest to growers, both in terms of food and ornamental. In terms of food, runner bean is grown for **green pods**, **immature seeds** and **dry seeds** (Munteanu, 2005). In Central America are use in food also **the tuberous roots** (from ssp. *darwinianus*), rich in starch, after being cooked, and the boiling liquid has been removed (Kay, 1979). In Mexico, **the leaves and young shoots**, as well **the flowers**, are boiled and then fried, consumed as they are, after seasoning with garlic and onions in the form of various meat trimmings (Popa, 2010). The flowers can be used for flowers or fried pancake (http://www.petitchef.ro/retete/clatite-cu-flori-de-salcam-fid-859027).

Native of Mexico and Central America, the species prefers a cool and moist climate, unlike the common bean (*P. vulgaris* L.) showing a high degree of rusticity and ecological plasticity, being resistant or tolerant to pathogens. Of great importance is that this rusticity assures high suitability of sustainable agriculture systems, including biological / organic / ecological. The runner bean`s ornamental value is conferred by the appearance of the entire plant, from seed germination mode, hypogeal, and continuing with every stage of plant growth and development, ending with the end of the vegetation period (Munteanu, 1985a).

The harmony of colours in landscape composition is determined by the combination of several colors, being found that one color stands out in opposition to another, so that instead of exclusion, they place more value (Şelaru, 2004). Runner bean attract more eyes in the landscape and it imposes especially during flowering. Through this work, the landscape composition is based on the dominant tunner bean effects, achieving a harmonious landscape, renneted and aesthetically. For a good and quality landscaping, it is necessary to know the landscape qualities of plants and also their biological characters and their ecological requirements.

The stem is herbaceous, slightly twisted, fine-edged, more vigorous compared to that of the common bean. Voluble stem forms are known as well as on common bean, with few branches or dwarf stem (bush or determined) (Kay, 1979).

At the bush bean, the main stem along with branches of higher order is right and at the climbing bean is straight in the young stage until it reaches a

height of 15-20 cm, after which it becomes voluble. The strain, at bush bean, has a height of 30-40 cm and has many branches, giving the plant a bushy shape. Typically, the bean has a relatively thick stem on the base without being rigid. It has a cylindrical shape, edged or slightly flattened at the top and on its surface are numerous bristles.

At the runner bean, the stem has very less branches (the lower half), with 3-5 branches on average, but indeterminate length is increased, peaking at 3-4 m volubility is achieved by twisting the stem and the branches thereof the support, counterclockwise. Near the stem appear the flowers, and the other are successively formed as soons as they form new nodes. The number of branches, length and diameter of the stem are in a continuously growing from emergence until to the pods formation.

Usually, the stem color is green during the vegetation period and yellow-brown at maturity. The leaves are trifoliate, with ovate or broadly-ovate leaflets, acuminate to long-acuminate at apex and rounded to truncated at the base (Salinas, 1988); the first two true leaves are simple and opposite. The buds are formed in the armpit leaves, ones at the base evolv in the ramifications of the stem and ones from the top becoming the inflorescences.

The flower is typical of *Phaseolus* genus, zygomorphic and hermaphrodite. The flowers are grouped in multiflorous bundles located on pseudoracemes (60 cm long). The inflorescence is terminal at dwarf forms (which makes the stem and its ramifications have a determinate growth habit) and axillary at vining forms (which ensures an indeterminate growth of the main stem and all branches). The corolla color can be white, gray and white to yellow, red, red with white wings, purple or lilac. In Romania, the runner bean due to their abundance of flowers and coloring, is known as "the bean of flower" (Munteanu, 1985a).

The fruits are pods typical of the *Papilionaceae* family. They are, in fact, the modified carpels which are closed more seeds, disposed on the dorsal welding line. These are large, with length between 10 - 26 cm, width 1.5-2.5 cm and thickness from 1.3 to 1.9 cm, linear-oblong in shape, slightly curved, ending with a distinct rostrum and are coarse to the touch (Olaru, 1982; Munteanu, 1985b).

The pods contains 4-6 seed. Usually, they are dehiscent, but many of the cultivated forms for green pods are indehiscent (Kaloo, 1995). Initially, all the pods are green, and as they approach the edible maturity becomes pale green, yellow or dark green, and finally, beige or brown-gray, typical of the variety (Olaru, 1982; Munteanu, 1985b).

The seeds are round, almost spherical, oblong or slightly kidney-shaped (Munteanu, 2005), big or very big, 15-22 mm long, 10-15 mm wide, 7-10 mm thick (Munteanu, 2005; Popa, 2010) and a mass of 1000 seeds of 950-1250 g (Munteanu, 2005), white colour, black, light brown or purple or beige or purple with a punctiform drawing or as an darker arabesque. Just like flowers, the seeds of runner bean have their decorative valences which are highlighted in different ways.

The phenology of the plant in culture, in conditions of our country, varies depending on the variety (cultivar) and climatic conditions of the area. The period from sowing to the springing of the runner bean is between seven and 10 days, the

period from the springing to the first flowers appearance range from 30-36 days, the period from the springing to the first pods appearance have values between 60 and 70 days and the period from springing to the seed maturation is between 107 and 122 days (Hamburdă *et al*., 2014; Munteanu *et al*.,2013; Popa,2010). The flowering lasts about 20-25 days at the dwarf varieties and is taking place in July-September and more than 60 days at the climbing varieties. The flowers in a raceme lasts 10-15 days and begin to open itself from the base to the top (Olaru, 1982).

As a **specific care works** are watering, the construction of a support system and the pinching. How to use the runner bean crop is the fact that it, through habitus, leaves, flowers and fruits, forms a temporary setting. Considering the climbing port of the species, this can be used as a hedge that can mask unsightly various areas of the gardens. Also, the base unit of the plant is extremely diverse and inventive. The support can be made of wood, sugar cane, plastic products, metal, sizes and different types, but it is considered that they are not exaggerated or unaesthetic. The plants can be supported through the wire mesh (nylon and string). As a support can also be used the stems of the associated plants, for example, those of sunflower, maize, Jerusalem artichoke etc. (Hamburdă et al., 2013).

In the landscape of a garden, runner bean can be used both in simple compositions and in the mixed compositions, in splashes of color, and because it can be used as a climbing plant, it can grow easily on pergolas, trellis, columns and archways. The runner bean is grown in pots, possibly in decorative vases, embellish the spaces where are located. The crop can be achieved, with good success, also in greenhouses or in polytunnels, but usually, to obtain green pods is mandatory the presence of pollinators.

CONCLUSIONS

1. The runner bean is a vegetable species that deserves more attention, given the importance of food, and the importance of decorative value. The botanical and ecological peculiarities show that the runner bean is a species with hypogeic germination, is prefering a cool and moist climate, with a high degree of rusticity and ecological plasticity, being resistant or tolerant to pathogens.

2. The remarkable decorative value is conferred by the size and general habitus of the plant, shape, appearance, size and color of the leaves, flowers, fruits and seeds.

3. The runner bean beautify by flowers, a period of about 60 days, from July to September; the period from springing to the appearance of the first flower is 30-36 days and the flowers in a raceme lasts 10-15 days and begin to open itself from the base to the top; the colour of the corolla can be white, gray and white to yellow, red, red with white wings, purple or lilac.

4. The ornamental value is also conferred by the shape, size and color of the seeds, with the mass of 1000 seeds of 950-1250 grams, white colour, black, light brown or purple or beige or purple with a punctiform drawing or as an darker arabesque.

5. This species is mostly used in mixed gardens, aiming at obtaining a healthy and decorative garden at the same time.

6. The runner bean are among the species chosen by those who grow vegetables as an extra-activity (hobby); in this case, the support system of the plant is extremely varied and inventive by placing it at the expense of achieving beauty combined with the creation of favorable conditions for the growth and the development of the plant.

***Acknowledgments**: This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. Hamburdă Silvia Brîndușa, Dascălu T., Munteanu N., **2013** – *Preliminary studies for new cultivation systems of runner bean (Phaseolus coccineus L.).* Lucrări științifice, seria Horticultură, USAMV Iași. 56 (2), pp. 167-172.
2. Hamburdă, Silvia Brîndușa, Munteanu, N., Popa, Lorena Diana, 2014 - *Evaluarea unui sortiment de fasole mare (Phaseolus coccineus L.) în condițiile din județul Iaşi.* Știința Agricolă. Universitatea Agrară de Stat din Moldova, Chișinău. 1, pp. 38-41.
3. Iliescu Ana-Felicia, 2008 – *Arhitectură peisageră*. Editura Ceres, București, 328 p.
4. Kalloo G., 1993 – *Runner bean – Phaseolus coccineus* L. In „Genetic Improvement of Vegetable Crops" (editors Kalloo G., Bergh B. O.). Pergamon Press Ltd. Published by Elsevier Ltd., pp. 405-407.
5. Kay E. Daisy, 1979 – *Food Legumes*. Tropical Products Institute, London, 435 p.
6. Mărgărit A., Mărgărit Ana, 2004 – *Arta peisageră între pasiune și profesie. Metode de concepere a unei grădini.* Editura Cetatea de Scaun, Târgoviște, 130 p.
7. Muntean Delia, Munteanu N., Hobincu Marlena, Avasiloaiei D. I., 2011 – *Valoarea ornamentală a unor specii legumicole cultivate*. Lucrări Ştiințifice, Seria Horticultură, vol. 55, nr. 2, pp. 193-198.
8. Munteanu N., 1985 a – *Phaseolus coccineus L. – o specie legumicolă care merită mai multă atenție*. Producția Vegetală, Horticultura, nr. 4/1985, pp. 17-19.
9. Munteanu N., 1985 b – *Câteva aprecieri asupra unor populații locale de fasole de grădină*. Cercetări Agronomice în Moldova, vol. 4, pp. 33-36.
10. Munteanu N., 2005 – *Studii preliminare privind biodiversitatea speciei fasole mare* (*Phaseolus coccineus* L.). Lucrări științifice, UȘAMV, Iași, seria Horticultură, pp. 83-92.
11. Munteanu, N., Hamburdă, Silvia Brîndușa, Popa, Lorena Diana, 2013 - *Research on the main productivity features in an assortment of runner bean (Phaseolus coccineus L.) in the environmental conditions from NE Romania.* Lucrări științifice, seria Agronomie, editura Ion Ionescu de la Brad, Iași. 56 (1), pp. 159-162.
12. Olaru C., 1982 – *Fasolea – Biologia și tehnologia culturii*. Editura Scrisul românesc, Craiova, 268 p.
13. Popa Lorena-Diana, 2010 – *Cercetări privind agrobiologia speciei Phaseolus coccineus L. în vederea optimizării cultivării*. Teză de doctorat. UŞAMV Iaşi, 232 p.
14. Salinas A. D., 1988 – *Variation, taxonomy domestication and germplasm potentialities in Phaseolus coccineus*. In „Genetic Resources of Phaseolus Beans" (edited by Gepts, P.), Kluver Academic Publishers, Dordrecht/Boston/London, pp. 441 – 463.
15. Șelaru Elena, 2004 – *Artă florală*, Editura Ceres, București, 157 p.
16. http://www.flori-cultura.ro/includerea-plantelor-leguminoase-in-amenajarile-peisagere/
17. http://www.gradinavesela.ro/2014/02/gradini-de-legume-decorative/
19. http://www.petitchef.ro/retete/clatite-cu-flori-de-salcam-fid-859027
20. http://www.silvic.usv.ro/cursuri/arhitectura_peisagera.pdf

RESEARCH ON ENTOMOFAUNA OF THE RUNNER BEAN CULTURE (*PHASEOLUS COCCINEUS* L.) IN POLYTUNNELS

CERCETĂRI CU PRIVIRE LA PRINCIPALII DĂUNĂTORI DIN CULTURA DE FASOLE MARE (*PHASEOLUS COCCINEUS* L.) ÎN SOLAR

BUTNARIU Gianina[1], TELIBAN G.C.[1], HAMBURDĂ Silvia Brînduşa[1], POPA Lorena Diana[1], TĂLMACIU M.[1], MUNTEANU N.[1]
e-mail: gianina.butnariu@yahoo.com

Abstract. *This paper presents a study on the main runner bean crop insects (Phaseolus coccineus L.) cultivated in polytunnels, in order to determine present entomofauna in the conditions of the "V.Adamachi" farm,University of Agricultural Sciences and Veretinary Medicine of Iasi. The collection was performed using Barber insect traps. The results revealed a great diversity of species.*
Key words: *aphids, Barber traps, chemical treatments*

Rezumat. *Lucrarea prezintă un studiu asupra principalelor insecte din cultura de fasole mare (Phaseolus coccineus L.) cultivată în solar, cu scopul de a stabili entomofauna prezentă, în condițiile din ferma "V. Adamachi" a Universității de Științe Agricole și Medicină Veterinară, Iași. Colectarea insectelor a fost realizată folosind capcane de tip Barber. Rezultatele au pus în evidență o diversitate de specii de insecte.*
Cuvinte cheie: *afide, capcane Barber, tratamente chimice*

INTRODUCTION

In Romania, the culture of runner bean (*Phaseolus coccineus* L.) pods in protected areas is not known, although the results from other countries recommend this culture system (Laitenberger, 2013; Popa, 2010, Ruști, 2007).

Research focused on this topic have been conducted worldwide, but in our country there were no concerns in this regard, one of the reasons is probably represented by the confusion between runner bean and common bean (Hamburdă et al., 2014; Munteanu, 1985 , Popa and Munteanu, 2009).

The culture used different varieties being found both climbing as Apollo White, Celebration, Desiree, Polestar, Scarlet Empire, Moonlight Lady Di Firestorm etc. and as well as dwarf varieties, including Jackpot and Hestia.

Our research presents a study on the main runner bean crop insects cultivated in polytunnels, in order to determine present entomofauna in the conditions of the "V.Adamachi" farm, University of Agricultural Sciences and Veretinary Medicine of Iaşi.

[1]University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

MATERIAL AND METHOD

The experiment was organized in a polytunne, with dimensions of 40 x 10 m, in the experimental farm "V. Adamachi "University of Agricultural Sciences and Veretinary Medicine of Iasi, in 2014. The biological material was represented by four runner bean pods varieties, from the United Kingdom: Lady Di, Desiree, Polestar, White Apollo. The experiment was organized in a device plots with three repetitions, each plot was planted with six nests. Experience is a two-factor type corresponding to the two factors studied:

- Factor A - range used four graduations: a1 = Lady Dy; a2 = Desiree;a3 = Polestar; a4 = White Apollo.

- Factor B - density culture expressed through three graduations:b1 = 33000 plants / ha (100 x 30 cm); b2 = 25000 plants / ha (100 x 40 cm); b3 = 20000 plants / ha (100 x 50 cm).

Culture was established by planting seedlings in nests, on June 3, in rows spaced at 100 cm, two plants in the nest, the distances determined by the experimental protocol. To collect and determine entomofauna from this experimental field, Barber traps were used and were installed on 07/01/2014. Were placed two such traps for each variant. Approximately every three weeks samples were collected on data 19/07/2014, 08/14/2014, 12/09/2014. The main research method used was observation. Determination of species and their systematic classification was carried out in the Laboratory of Entomology of University of Agricultural Sciences and Veretinary Medicine of Iasi,according to the literature (Gaetan du Chatenet, 1990; Panin, 1951; Panin, 1952; Reitter, 1908; Rogojanu and Perju, 1979).

RESULTS AND DISCUSSIONS

Entomofauna collected from runner bean crop grown in polytunne, belongs to nine orders: *Coleoptera, Diptera, Hymenoptera, Homoptera, Isopoda, Lepidoptera, Heteroptera,Pulmonata and Rodentia* and the highest number of insects, 661, was recorded in order *Homoptera* (tab.1; fig. 2).

Order *Homoptera*, *Aphididae* family - In this order will rank aphids or plant lice. Widespread species, almost cosmopolitan, though most are found in temperate zones. Are phytophagous species, which feed with plant juices, reinforced with a pierced mouth conformed to sting and suck. They had a parallel development with gymnosperms and angiosperms, which commonly they attack. Their attack is so diverse that it can say that there are no plants in these groups that are not attacked by aphids. Insects are small, ranging in size from 0.5 to 8 mm. The body is oval, globular or ellipsoidal. Body color is variable: green, brown, black, or bright colors (orange, red), etc. The skin is weak, so that, normally, the body is easy. The skin may have some decorations (pear, spines, scales) or is covered with a waxy secretion. Cerigene glands are grouped and metameric arranged (fig. 1).

Black bean aphid, *Aphis fabae* Scop., attacks the leaves, flowers and pods that sting and they suck sap. They are twisted and dried and appears the honeydew which favors the develop of bacteria and fungi. Wet and cold seasons intensifies attack. Pest overwinters as eggs on various shrubs and spring wings

appearing form which attack plants are visible (had 2 mm long, black legs and short antennae) and they also are vectors for mosaic virus (Tălmaciu, 2003).

Fig. 1 - Aphids attack (original photo)

Table 1

Entomofauna collected, number of individuals belonging to each order

Orders	19.07.2014	14.08.2014	12.09.2014	Total
Coleoptera	3	4	1	8
Diptera	98	54	214	393
Hymenoptera	38	15	12	65
Homoptera	52	215	394	661
Isopoda	6	-	12	18
Lepidoptera	4	-	9	13
Heteroptera	-	-	2	2
Pulmonata	36	13	18	67
Rodentia	-	1	-	1

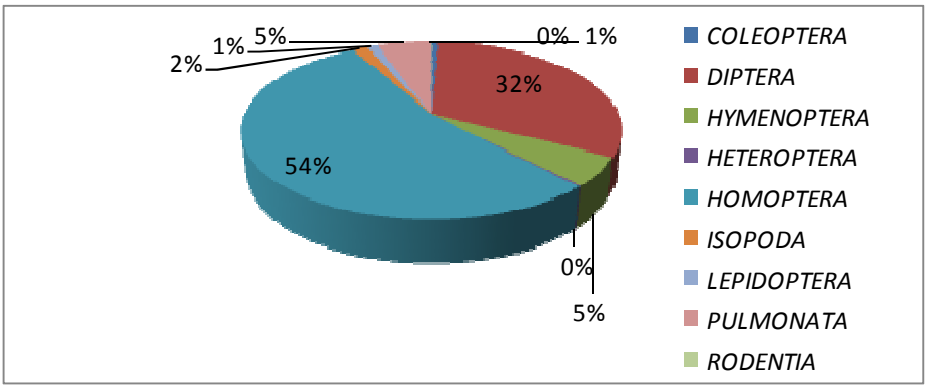


Fig.2 - Graphic representation of data collected

During the growing season following treatments were applied *(tab. 2):*

Table 2

Chemical treatmets applied

NAME OF PRODUCT	CONCENTRATION	APPLICATION DATE
Actara	0.02%	18.06.2014
Vertimec	0.08%	25.06.2014
Calypso	0.02%	18.07.2014
Actara	0.02%	15.09.2014
Faster	0.03%	15.09.2014

CONCLUSIONS

1. Runner bean cultivated in polytunne presented a diverse entomofauna.

2. Runner bean grown in popytunne is prone to attack by a large number of pests.

3. Stage of growth and development of culture is directly proportional to entomofauna collected, the number of insects belonging to each order.

Acknowledgments*: This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. Chatenet du Gaetan, 1990 – *Guide des Coléoptères d'Europe*. Delacrois et Niestlé, Paris.

2. Hamburdă Silvia Brîndușa, Munteanu N, Popa Lorena Diana, 2014 - *Evaluarea unui sortiment de fasole mare (Phaseolus coccineus L.) în condițiile din județul Iaşi*. Știința Agricolă. Universitatea Agrară de Stat din Moldova, Chișinău. 1: 38-41.

3. Laitenberger Klaus, 2013 – *Vegetables and herbs for the greenhouse and polytunnel*. London.

4. Munteanu N., 1985 – *Phaseolus coccineus L. - o specie legumicolă care merită mai multă atenție*. Producția vegetală, Horticultura, nr. 4, Bucureşti.

5. Panin I., 1951 – *Determinatorul Coleopterelor dăunătoare şi folositoare din Republica Populară Română (Determinator of damaging and useful coleoptera from Romania).* Edit. de Stat, Bucureşti.

6. Panin I., 1952 – *Fauna Republicii Populare Române. Insecta (Fauna from Romania. Insects),* vol. X, fasc. 1, Ord. Coleoptera, Fam. Carabidae. Edit. Academiei R.P.R., Bucureşti.

7. Popa Lorena-Diana, 2010 - *Cercetări privind agrobiologia speciei Phaseolus coccineus L. în vederea optimizării cultivării*. Teză de doctorat, USAMV Iași.

8. Popa Lorena-Diana, Munteanu N., 2009 – *Yield study on some runner bean (Phaseolus Coccineus L.) local populations depending of the establishment date*. Lucrări științifice USAMV Iași, seria Horticultură, anul LII-vol. 52, pp.. 465-470.

9. Reitter E., 1908 – *Fauna Germanica*. Die Kafer des Detschen Reiches. Band I, Stuttgart.

10. Rogojanu V., Perju T., 1979 – *Determinator pentru recunoaşterea dăunătorilor plantelor cultivate (Determinator for knowing the crop pests).* Edit. Ceres, Bucureşti.

11. Ruști G., 2007 – *Cercetări privind îmbunătățirea tehnologiei de cultură a fasolei de grădină urcătoare (Phaseolus vulgaris L. var. communis)*. Teză de doctorat, USAMV Iaşi.

SALINITY EFFECT ON POTATO (*SOLANUM TUBEROSUM* L.) MICROPROPAGATION

EFECTUL SALINITĂȚII ASUPRA MICROPROPAGĂRII CARTOFULUI (*SOLANUM TUBEROSUM* L.)

***NISTOR Andreea*[1], *CIOLOCA Mihaela*[1], *CHIRU Nicoleta*[1], *POPA Monica*[1], *BADARAU Carmen*[1]**
e-mail: tican_andreea@yahoo.com

Abstract: *The effect of salinity on plantlets growth was determined under saline medium and non-saline at five cultivars of potato (Christian, Roclas, Marfona, Riviera, Tresor). Plantlets belonging to selected cultivars, were propagated through single nodal culture. To study the effect of salinity (NaCl) on the growth of single nodal explants, they were cultured on MS media with different concentrations of NaCl, including 0, 25, 50, 75 and 100 m mol l*$^{-1}$*. Growth of single nodal explants on the media with NaCl indicated that all the characters differed significantly according to salinity levels. By increasing salinity level the values for all the parameters decreased.*
Key words: *salinity tolerance, in vitro multiplication, concentration of NaCl*

Rezumat: *Efectul salinității asupra creşterii plantulelor a fost determinat în mediu salin și în mediu a cărui concentrație a NaCl a fost 0 m mol l*$^{-1}$*, utilizând cinci cultivare de cartof (Christian, Roclas, Marfona, Riviera, Tresor). Pentru a studia efectul salinității asupra creşterii minibutaşilor, aceştia au fost inoculați pe mediul nutritiv MS cu diferite concentrații ale NaCl (0, 25, 50, 75 și 100 m mol l*$^{-1}$*). Creştere minibutaşilor pe un mediu cu NaCl a indicat că toți parametrii a diferit semnificativ în funcție de concentrația salinității. Prin creşterea nivelului salinității valorile pentru toți parametrii au scăzut.*
Cuvinte cheie: *tolereanța salină, multiplicarea in vitro, concentrații de NaCl*

INTRODUCTION

Salinity is one of the most serious factors limiting the productivity of agricultural crops, with adverse effects on plant vigour and crop yield (Munns and Tester, 2008).

High salinity affects plants in several ways: water stress, ion toxicity, nutritional disorders, oxidative stress, alteration of metabolic processes, membrane disorganization, reduction of cell division and expansion, genotoxicity (Hasegawa *et at*., 2000; Munns, 2002; Zhu, 2007). These effects reduce plant growth, development and survival. During initial exposure to salinity, plants experience water stress, which in turn reduces leaf expansion. The osmotic effects of salinity stress can be observed immediately after salt application and it is posible to continue for the duration of exposure, resulting in inhibited cell expansion and cell division (Flowers, 2004; Munns, 2002). Plant growth reduction is commonly

[1] National Institute of Research and Development for Potato and Sugar Beet of Brasov, România

correlated either to ion toxicity or to water deficit. Heuer and Nadler (1995) observed a significant decline in leaf water and osmotic potential under intensified salt stress conditions while studying the physiological response of potato plants to soil salinity and water deficit.

Sodium excess and, more importantly, chloride excess have the potential to affect plant enzymes and cause cell swelling, resulting in reduced energy production and other physiological changes (Larcher 1980). Ionic stress results in premature senescence of older leaves and in toxicity symptoms (chlorosis, necrosis) in mature leaves due to high Na which affects plants by disrupting protein synthesis and interfering with enzyme activity (Hasegaw *et al*., 2000; Munns, 2002; Munns and Termaat, 1986).

Salinity stress is a critical environmental constraint to crop productivity especially in arid and semiarid regions. The most of the crop plants is intolerable to high salinity conditions resulting decreased yield. Generally, plants are stressed in next ways in saline soils a) low water potential of the root medium leads water deficit, b) the toxic effects of the Na^+ and Cl^- nutrient imbalance by depression in uptake and/or shoot transport (Munns and Termaat 1986, Chapin 1991, Marschner 1995). Toxic accumulation of Na^+ and Cl^- in leaves has also been correlated with reduction of total chlorophyll content in leaves both of which limit the amount of photosynthetic production (Romero-Aranda and Syvertsen 1996).

Potato (*Solanum tuberosum* L.), a vegetative plant cultivated for its starch-rich tubers, is the fourth most important agricultural crop after rice, wheat, and corn (Byun et al., 2007, Nhut et al., 2006, cited by Aycili and Alikamanoğlu, 2012). Economically, it is the most important tuberous plant, and potato plant cultivars are usually very sensitive to environmental stresses such as temperature changes, drought, and salinity due to their sparse and short root systems. There is significant loss in plant growth and product yields when potato is grown in soil that contains 20-35 mM concentrations of NaCl. When compared to other agricultural plants such as pepper and corn, the potato plant is more resistant to salinity; however, it is less resistant than tomato, rice, soy and barley (Byun *et al*., 2007, Manrique, 2000, cited by Aycili and Alikamanoğlu, 2012).

The selection of crop varieties for greater tolerance to saline environment will allow greater productivity from large saline lands.

In this paper our aim was to follow the growing and development *in vitro* plantlets from different potato varieties and the tolerance to NaCl

MATERIAL AND METHOD

Research was conducted at Laboratory of Vegetal Tissue Culture from National Institute of Research and Development for Potato and Sugar Beet Brasov, to record the effect of various concentration of NaCl on potato varieties Christian, Roclas, Marfona, Riviera, Tresor. Nodal cuttings were used as explants.

A medium MS (1962), with the amount of 20 g sucrose and agar concentrations of 9 g were used, as well as different concentrations of salt were applied. The pH was adjusted at 5.7 with HCl and NaOH. After 30 day, the plantlets were harvested and four vegetative growth parameters were measured. The experience was of type 5*5,

made by combining two experimental factors; the number of studied variants was 25 (Table 1), set in three relicates.

Table 1

Experimental variants according to the NaCl concentration

Variants	Cultivar	NaCl concentration (mmol/l)
V_1	Christian	0
V_2		25
V_3		50
V_4		75
V_5		100
V_1	Roclas	0
V_2		25
V_3		50
V_4		75
V_5		100
V_1	Marfona	0
V_2		25
V_3		50
V_4		75
V_5		100
V_1	Riviera	0
V_2		25
V_3		50
V_4		75
V_5		100
V_1	Tresor	0
V_2		25
V_3		50
V_4		75
V_5		100

- Experimental factor A-cultivar has five graduations:
- a_1- Christian;
- a_2- Roclas;
- a_3- Marfona;
- a_4- Riviera;
- a_5- Tresor.
- Experimental factor B – concentration of NaCl, has five graduations:
- b_1 - MS medium and 0 Mm NaCl (control);
- b_2 - MS medium and 1 Mm NaCl;
- b_3 - MS medium and 2 Mm NaCl;
- b_4 - MS medium and 3 Mm NaCl;
- b_5 - MS medium and 4 Mm NaCl.

The proposed objective of this research is to determine the influence of salinity in the culture medium *in vitro* over different potato cultivars.

The experience was mounted in the laboratory using conditions required by „in vitro" technology; experimental conditions were those specific to growth chamber of plantlets, provided in the working protocol, sterilization of culture vessels was performed in a drying chamber at 180^0C and culture media was sterilized by autoclaving at 121^0C for 20 minutes at pressure of 1.25 atmospheres.

Cultures were transferred to growth chamber under conditions of darkness; after crossing this period light regime is 4000 lux, with a period of 16 hours light and eight hours dark at a temperature of 20^0C.

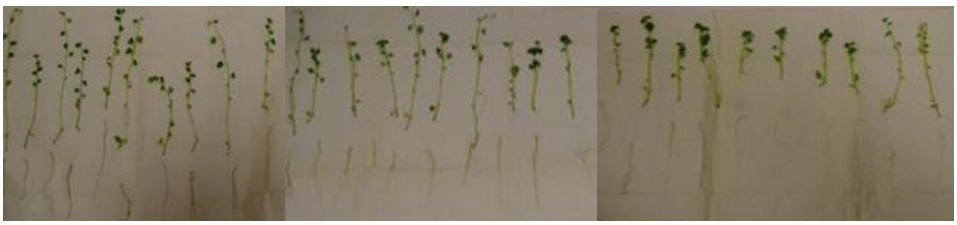
This experience, as shown in Figure 1, included 25 variants. The experimental conditions were the specific in growth room of plantlets.

	a_1					a_2					a_3					a_4					a_5				
r1	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5
	a_1					a_2					a_3					a_4					a_5				
r2	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5
	a_1					a_2					a_3					a_4					a_5				
r3	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5	b_1	b_2	b_3	b_4	b_5

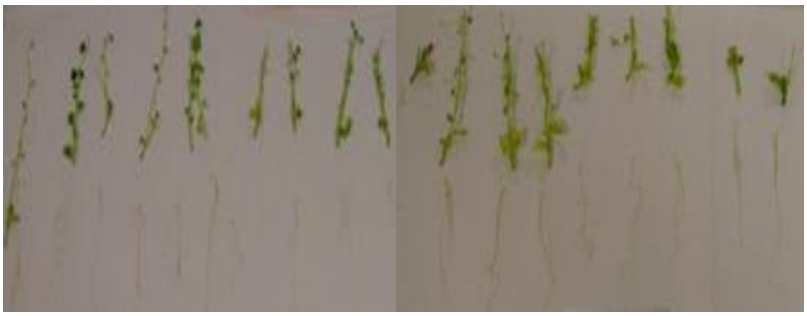
Fig. 1 - Location sketch of the experimental variants made to the five varieties and five NaCl concentration

Legend:
- a – cultivar;
- b – NaCl concentration;
- r – replicates.

RESULTS AND DISCUSSION:



0 m mol/l NaCl 25 m mol/l NaCl 50 m mol/l NaCl



75 m mol/l NaCl 100 m mol/l NaCl

Fig. 2 - Plantlets from control medium and from different NaCl concentrations, with reduced height and reduced number of leaves

Saline stress induces several alterations on growth, cell division and metabolic activities (Wincov, 1993). The results showed that the presence of NaCl in the media affects the growth of single nodal explants (fig. 2). The increase of NaCl concentration in the media is proportional to the decrease of the measured parameters.

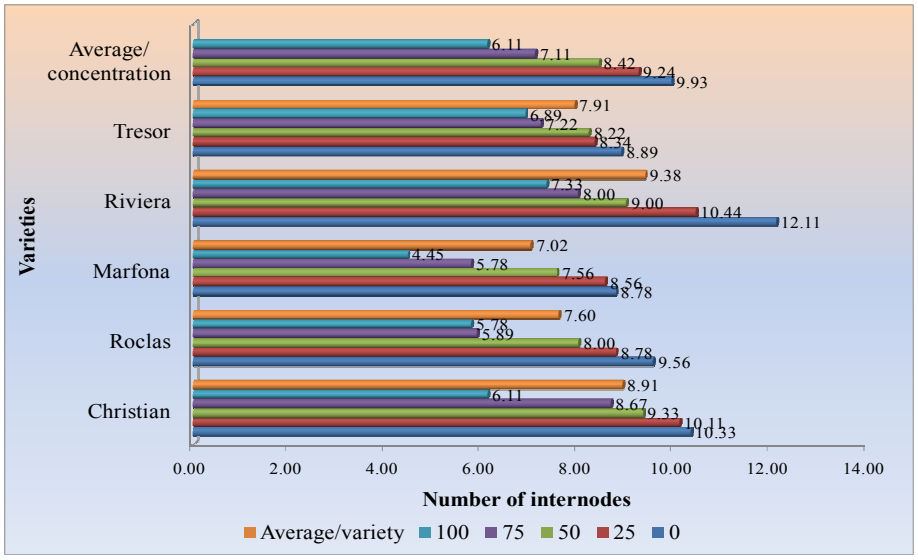


Fig.3 - Variation of number of internodes/plantlets for tested cultivars

If we compare the five cultivars in terms of number of internodes can say that the cultivar Riviera responded the best showing the highest average value (9.38 internodes) and the lowest average value is at Marfona variety (7.02 internodes) (Fig. 3).

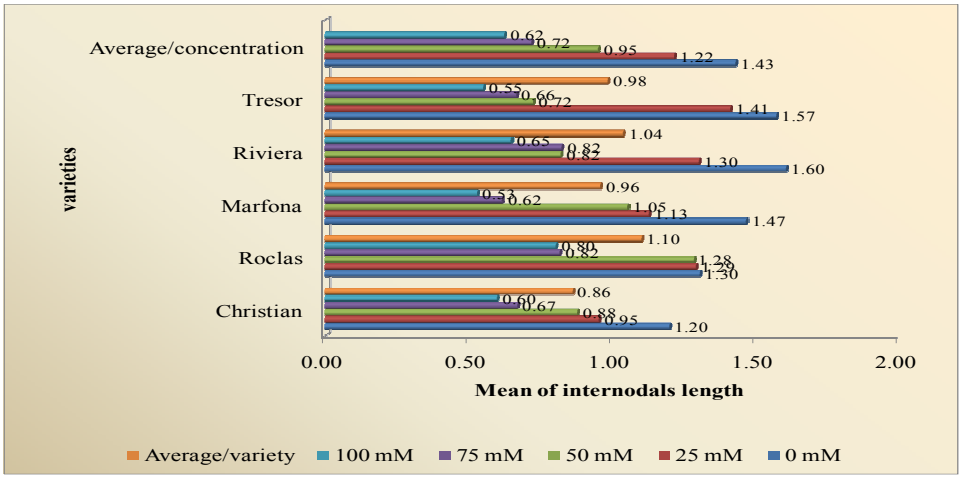


Fig. 4 - Variation of mean of internodes length for tested cultivars

The effect of salinity on mean of internodals length (Fig. 4) was least accentuated for Roclas and Riviera (hey had an average value / concentration 1.10 and 1.04 cm.

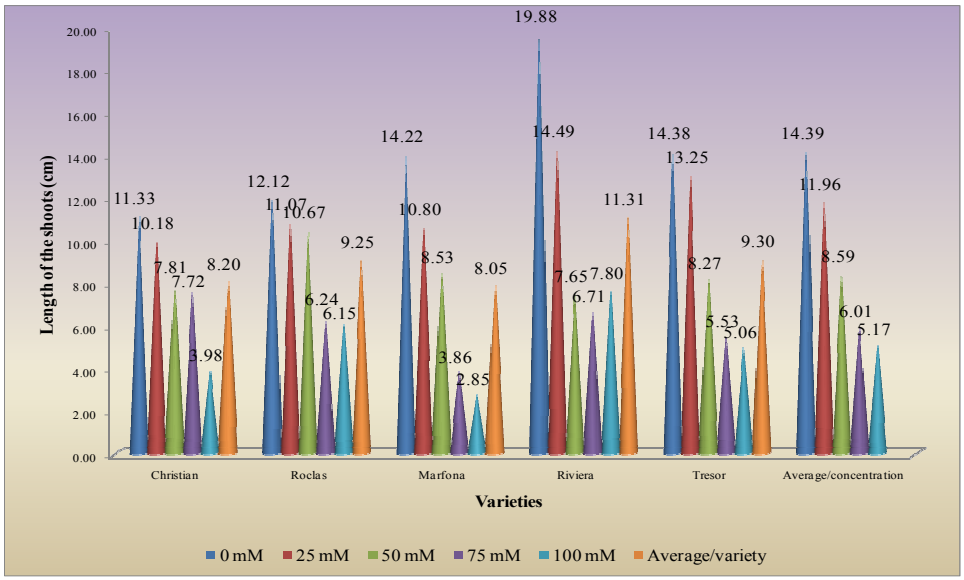


Fig. 5 - Variation of shoot length for tested cultivars

Reffering the shoot length (Figure 5), the cultivar with the least damaged because of salinity level is Riviera that recorded at concentration at 100 mmol/l the higher value (7.80 cm); shoot length decreased with increasing of salt concentration in culture medium.

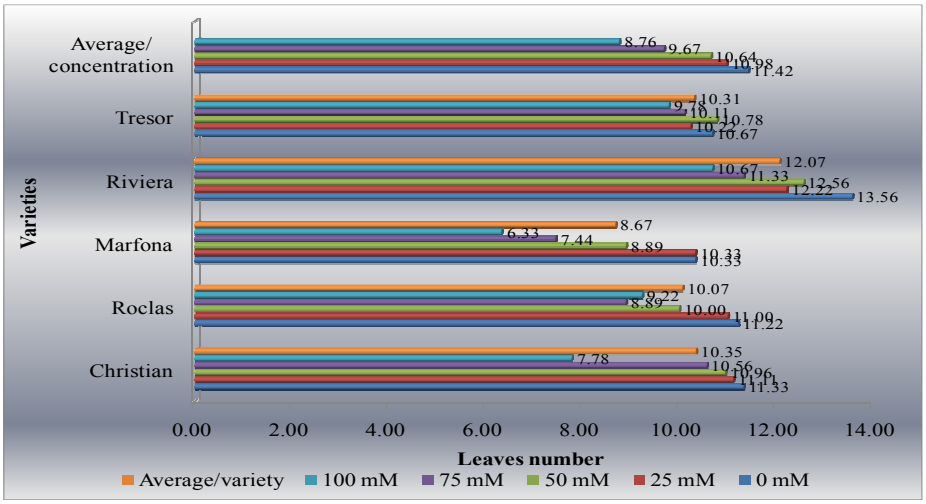


Fig. 6 - Variation of leaves number for tested cultivars

From the point of view the number of leaves, for concentration of 100 mmol/l, Riviera cultivar present the highest value (10.67 leaves/plant) (Fig. 6).

Statistical interpretation:

In table 2 is present the statistical analysis of varieties. The most tolerant variety to salinity stress is Riviera cultivar. On the opposite side is Marfona cultivar which shows for almost all parameters studied (except - mean of internodes length) the lowest values.

Table 2

Salinity effect on the studied potato cultivars

Varieties	Internodes number	Mean of internodes length (cm)	Shoot length (cm)	Leaves number
Christian	8.91a	0.86b	8.20b	10.35b
Roclas	7.96bc	1.1a	9.25ab	10.07b
Marfona	7.02c	0.96ab	8.05b	8.67c
Riviera	9.38a	1.04ab	11.31a	12.07a
Tresor	7.91b	0.98ab	9.30ab	10.31b

Means within same column followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$).

In table 3 is shown the statistical analysis of NaCl concentration. The variant 2 of concentrations (25 m mol/l NaCl) did not lead in significantly different values for the internodes number (compared to the variant 1 - concentration 0 m mol/l NaCl), showing that these are tolerant to salt at this concentration. Additions of 100 m mol/l of NaCl to the medium significantly decreased the internodes number. Shoot length was also negatively affected (inverse-relation) by salinity for concentrations 50, 75, 100 m mol/l.

In this study, the values of leaves number are not significantly different for concentrations 25, 50, 50, 75 m mol/l.

Table 3

Salinity effect on different characters measured

Variants	Salinity concentration (m mol/l)	Internodes number	Mean of internodes length (cm)	Shoot length (cm)	Leaves number
V_1	0	9.93a	1.43a	14.39a	11.42a
V_2	25	9.24ab	1.22a	11.96a	10.96a
V_3	50	8.42bc	0.95b	8.59b	10.66a
V_4	75	7.11cd	0.72c	6.01bc	9.66ab
V_5	100	6.11d	0.62c	5.16c	8.76b

Means within same column followed by the same letter are not significantly different according to Duncan ($p \leq 0.05$).

CONCLUSIONS

The effect of all treatments was very drastic on 100 m mol/l NaCl. The addition of salinity to the culture media decreased the osmotic potential of the

media inducing salinity stress and affected the plants growth of potato cultivars. The results indicate that the "Marfona" cultivar is not salt tolerant, while “Riviera” cultivar present a tolerance to NaCl. On concentration 25 m mol/l, all studied parameters was not affected (the values were not significantly different, compared to concentration 0 mol/l).

REFERENCES

1. **Chapin E S, 1991** - *Integrated responses of plants to stress*. A centralized system of physiological responses. Bioscience 40, pp. 29-31.
2. **Flowers T. J**., **2004** - *Improving crop salt tolerance*. Journal of Experimental Botany, 55 (396), pp. 307-319.
3. **Hasegawa P. M**., **Bressan R. A**., **Zhu J. K., Bohnert H**. **J**., **2000** - *Plant cellular and molecular responses to high salinity*. Annual Review of Plant Physiology and Plant Molecular Biology, 51, pp. 463-499.
4. **Heuer B**., **Nadler A**., **1995** - *Growth and development of potato under salinity and water deficit*. Australian Journal of Agricultural Research 46:, pp.1477-1486.
5. **Larcher W**., **1980** - *Physiological plant ecology*. In 2nd totally rev. edition ed., (pp. 303). Berlin and New York: Springer-Verlag.
6. **Marschner H**., **1995** - *Saline soils*. In: *Mineral Nutrition of higher plants*. Academic Press, New York. pp. 657-680.
7. **Munns R**., **2002** - *Comparative physiology of salt and water stress*. Plant, Cell and Environment, 25 (2), pp. 239-250.
8. **Munns R**., **Termaat A., 1986** - *Whole-Plant Responses to Salinity.* Functional Plant Biology, 13 (1), pp. 143-160.
9. **Munns R**., **Tester**, **M**., **2008** - *Mechanisms of salinity tolerance*. Annual Review of Plant Biology, 59, pp. 651-681.
10. **Murashige T., Skoog F**, **1962** - *A revised medium for rapid growth and bio-assays with tobacco tissue cultures*. Physiol Plant 15(3), pp. 473-497.
11. **Orkun Yaycili, Sema Alikamanoğlu**, **2012** - *Induction of salt-tolerant potato (Solanum tuberosum L.) mutants with gamma irradiation and characterization of genetic variations via RAPD-PCR analysis*. Turk J. Biol. 36., pp. 405-412.
12, **Romero-Aranda R., Syvertsen J. P.**, **1996** - *The influence of foliar applied urea nitrogen and saline solutions on net gas exchange of Citrus leaves*. J. Amer. Soc. Hort. Sci. 121, pp. 501-506.
13. **Wincov I**., **1993** - *Gene expression in relation to salt tolerance.* In: Basra, A. S. (Eds.), Stress-induced Gene Expression in plants. Hardwood Academic Publishers, Switzerland, pp: 61-130
14. **Zhu J. K**., **2007 -** *Plant Salt Stress:* John Wiley and Sons, Ltd. Environment, 25 (2), pp. 239-250.

THE STUDY OF THE INFLUENCE OF THE PLANTING DISTANCE ON THE EARLY PRODUCTION OF CERTAIN RUNNER BEAN CULTIVARS (*PHASEOLUS COCCINEUS L.)* FOR PODS, IN THE ENVIRONMENT OF THE POLYTUNNEL

STUDIUL INFLUENȚEI DISTANȚEI DE PLANTARE ASUPRA PRODUCȚIEI TIMPURII LA UNELE CULTIVARE DE FASOLE MARE (*PHASEOLUS COCCINEUS* L.) PENTRU PĂSTĂI, ÎN CONDIȚII DE SOLAR

TELIBAN G.C.[1], MUNTEANU N.[1], POPA Lorena-Diana[2], STOLERU V.[1], STAN T.[1], HAMBURDĂ Silvia Brîndușa[1]
e-mail: teliban_gabriel_ciprian@yahoo.com

Abstract. *The objective of this paper is to evaluate the cultivation possibilities of the runner bean for pods in the polytunnel, in the Romanian conditions and taking into account different planting distances. This factor has a major influence on the production of the runner bean culture, the species being sensitive to light. Research was performed within the Teaching Facility of USAMV (University of Agronomic Sciences and Veterinary Medicine) Iasi in 2014, on a culture of runner bean (Phaseolus coccineus L.), created in the polytunnel by planting the seedlings in nests, with three different densities (20, 25 and 33 thousand nests/ha), using four cultivars from Great Britain (Lady Di, Desiree, Polestar and White Apollo).The obtained results indicate to us the fact that the highest early productions for the culture of runner bean created in the polytunnel by planting the seedling are obtained at lower densities of 20 and 25 thousand nests/ha.*
Key words*: runner bean for pods, planting distances, polytunnel*

Rezumat. *Lucrarea îşi propune evaluarea posibilităților de cultivare a fasolei mari pentru păstăi în solarii, în condițiile din România, luându-se în considerare distanțe diferite de plantare. Acest factor prezintă o influență majoră asupra producției la cultura de fasole mare, specia fiind pretențioasă la lumină. Cercetările au fost realizate la Stațiunea Didactică a USAMV Iaşi, în anul 2014, la o cultură de fasole mare (Phaseolus coccineus L.), înființată în solar prin plantarea răsadului în cuiburi, câte două plante la cuib, cu trei densități diferite (20, 25 şi 33 mii cuiburi/ha), utilizând patru cultivare provenite din Marea Britanie (Lady Di, Desiree, Polestar şi White Apollo). Rezultatele obținute ne indică faptul că cele mai ridicate producții timpurii pentru cultura de fasole mare înființată în solar prin plantarea răsadului se obțin la densități de 20 şi de 25 mii cuiburi/ha.*
Cuvinte cheie: *fasole mare pentru păstăi, distanțe de plantare, solar.*

INTRODUCTION

The culture of runner bean for pods in protected environments is not known

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
[2] Agricultural Research and Development Station Secuieni-Neamt, Romania

in Romania, although the results from other countries recommend this culture system (Klaus L., 2013). Research focused on this topic have been conducted worldwide, in Great Britain, Netherlands, France, etc., but in our country there were no concerns in this regard, one of the reasons being probably the confusion between the runner bean and the common bean, as well as the lack of an adequate cultivation assortment (Munteanu N., 1985, Popa Lorena-Diana, 2010, Popa Lorena-Diana and Munteanu N., 2009).

The distributing companies and/or the producers of seeds for this culture also make some recommendations for the species that they promote, according to the existing specialized literature worldwide. The creation of the culture in the field can be achieved by seedling, its production beginning from the second decade of April, or by direct sowing, the period for the creation of the culture being divided from May up to July. The sowing depth is of 5 cm, at a distance of 30 cm between the seeds/ nests on a row, sowing being done in lanes of 45 + 105 cm (http://www.marshalls-seeds.co.uk/, http://www.thompson-morgan.com/, http://www.tozerseeds.com/eu/en/).

The assortment for the polytunnels generally comprises climbing cultivars (indeterminate growth), in consequence the culture of runner bean for pods requiring support systems. As assortment, different cultivars are used in the culture, finding both climbing varieties such as White Apollo, Lady Di, Polestar, Desiree, Scarlet Empire, Celebration, Firestorm, Moonlight etc. and some dwarf cultivars, of which we mention Hestia, Jackpot Mixed (http://www.marshalls-seeds.co.uk/, http://www.thompson-morgan.com/).

Because the need to diversify the range of vegetables is well known, as well as some technological features of this culture (Munteanu N., 1985, Hidalgo R. et al., 1986), our research aim at assessing the cultivation possibilities of this species in polytunnels, in the conditions of Romania, establishing as main objectives the study of the influence of the cultivar and of the density on the culture and on its harvest.

MATERIAL AND METHOD

The experiment was organized in a covered polytunnel, with dimensions of 40x10 m in the experimental conditions from the Teaching Facility of USAMV Iasi, in 2014. The experimental conditions are determined by a soil of medium-levigated chernozem type (cambic), environment stocked with nutrients.

The biological material was represented by for varieties of runner bean for pods from Great Britain (Lady Di, Desiree, Polestar and White Apollo). A brief description of the cultivars from the study is presented in table 1.

The experiment is of bifactorial type, according to the two studied factors:

- factor A – the assortment used with four gradations: a1 = Lady Dy; a2 = Desiree; a3 = Polestar; a4 = White Apollo.
- factor B – the density of the culture, expressed by three gradations: b1 = 33000 nests/ha (100 x 30 cm); b2 = 25000 nests/ha (100 x 40 cm); b3 = 20000 nests/ha (100 x 50 cm).

The experiment was organized in a device of subdivided parcels, with three replications and in each replication of the parcel six nests were planted.

The culture was created by planting the seedling in nests on June 3rd. In order to obtain the seedling, three seeds in nutritious pots (9 x 9 x 10 cm) were sowed on May 20th, the substrate consisting of peat. The obtained seedling was planted in equidistant rows of 100 cm, two plants in the nest, at the distances determined by the experimental protocol.

Table 1

Type of runner bean used in the experiment

Type		Source	Reference Data		Presence/absence of threads in the pod
No. crt.	Cultivar		Color of the flowers	Color of the seeds	
1.	Lady Di	Great Britain	Red	Violet with black arabesque	No threads
2.	Desiree	Great Britain	White	White	No threads
3.	Polestar	Great Britain	Red	Violet with black arabesque	No threads
4.	White Apollo	Great Britain	White	White	No threads

During the vegetation period care works consisted of hand and mechanical weeding, phytosanitary treatments, radicular and foliar fertilization, drip irrigation, trellising and pinching the plants.

During the experiment, determinations of the amount of the early harvest were done, the data being processed by appropriate statistical and mathematical methods. The harvesting of the pods in order to determine early production was performed at three different dates, July 21st, July 26th and July 31st.

RESULTS AND DISCUSSIONS

The experimental results obtained during the experiment from 2014 are shown in the table below:

Table 2

Production results for the three planting distances (kg/ha)

No. crt.	Cultivar	30 cm	40 cm	50 cm	Average ($\bar{x}$)
1.	Lady Di	7264	6240	8250	7251
2.	Desiree	1651	3684	3468	2934
3.	Polestar	5514	8177	6683	6791
4.	White Apollo	875	2654	3069	2199
Average ($\bar{x}$)		3826	5189	5368	4794

In 2014, the early production of pods varied in extremely high limits, between 875 kg/ha to 8250 kg/ha, while the experimental average was 4794 kg/ha.

Compared to the experimental average, the highest production increase of 12% was recorded at a distance of 50 cm. The distance of 40 cm determined a production increase of 8%, both variants being assured at a statistically significant level.

It is important to note that the distance of 30 cm between pods determined productions under the level of the experimental average, thus being able to appreciate that such distance is too small, resulting in a density that is too high, where plant "inconvenience" each other in what concerns the nutritious space and the light space.

The comparative analysis of the production results for the three experimental distances is shown in Table 3.

Table 3

The comparative analysis of the early production according to the distances between nests

No. crt.	Planting distance	Production		Production differences between distances and their significance			
		t/ha	% compared to $\bar{x}$	D1=30 cm	D2=40 cm	D3=50 cm	average ($\bar{x}$)
1.	D1=30 cm	3826	80	-	-1363^{0}	-1542^{0}	-968
2.	D2=40 cm	5189	108	1363^{x}	-	-179	395
3.	D3=50 cm	5368	112	1542^{x}	179	-	574
Average ($\bar{x}$)		4794	100	968	-395	-574	-

LSD5% = 1290,36 kg/ha
LSD1% = 1757,88 kg/ha
LSD0,1% = 2362,55 kg/ha

For the distance of 30 cm between nests on a row (table 4), the Lady Di variety (7264 kg/ha) has made statistically assured productions at a significant level compared to the experimental average (3826 kg/ha). Positive differences distinctly significant compared to the average were also recorded in the case of the Polestar variety, obtaining a production increase of 1688 kg/ha.

Table 4

Production results for the distance of 30 cm

No. crt.	Cultivar	Production		Difference compared to $\bar{x}$	Significance of the differences
		t/ha	% compared to $\bar{x}$		
1.	Lady Di	7264	190	3438	xxx
2.	Desiree	1651	43	-2175	00
3.	Polestar	5514	144	1688	x
4.	White Apollo	875	23	-2951	000
Average ($\bar{x}$)		3826	100	0	

LSD5% = 1290,36 kg/ha
LSD1% = 1757,88 kg/ha
LSD0,1% = 2362,55 kg/ha

The results regarding the distance of 40 cm are presented in table 5.

Table 5

Production results for the distance of 40 cm

No. crt.	Cultivar	Production		Difference compared to $\bar{x}$	Significance of the differences
		t/ha	% compared to $\bar{x}$		
1.	Lady Di	6240	120	1051	-
2.	Desiree	3684	71	-1505	0
3.	Polestar	8177	158	2988	xxx
4.	White Apollo	2654	51	-2535	000
Average ($\bar{x}$)		5189	100	0	

LSD5% = 1290,36 kg/ha
LSD1% = 1757,88 kg/ha
LSD0,1% = 2362,55 kg/ha

In the case of the distance between nests of 40 cm, the early production of pods ranged from 2654 kg/ha (White Apollo) to 8177 kg/ha (Polestar). In these conditions, the average of the experiment was 5189 kg/ha.

The Polestar variety recorded productions superior to the average level, with very significant positive differences towards it.

In the case of the distance between nests of 50 cm, productions ranged from 3069 kg/ha (White Apollo) and 8250 kg/ha (Lady Di) (Table 6). The highest production, Lady Di - 8250 kg/ha, assures a production increase of 54% compared to the average of the experiment (5368 kg/ha).

Table 6

Production results for the distance of 50 cm

No. crt.	Cultivar	Production		Difference compared to $\bar{x}$	Significance of the differences
		t/ha	% compared to $\bar{x}$		
1.	Lady Di	8250	154	2882	xxx
2.	Desiree	3468	65	-1900	00
3.	Polestar	6683	124	1315	x
4.	White Apollo	3069	57	-2299	00
Average ($\bar{x}$)		5368	100	0	

LSD5% = 1290,36 kg/ha
LSD1% = 1757,88 kg/ha
LSD0,1% = 2362,55 kg/ha

In the case of the interaction cultivar x distance (table 7), the early production from 2014 varied from 875 kg/ha (White Apollo x 30 cm) to 8250 kg/ha (Lady Di x 50 cm). The difference between the combinations of the two factors varied between -3919 kg/ha (a_4b_1 with average) and 3456 kg/ha (a_1b_3 with average).

Highly significant production increase has been recorded in the case of the comparison of the following combinations with the average: a_1b_1 (2470 kg/ha), a_1b_3 (3456 kg/ha) and a_3b_2 (3383 kg/ha). Highly significant negative differences were recorded after comparing the two combinations of factors with the average: a_2b_1 (-3143 kg/ha) and a_4b_1 (-3919 kg/ha).

Table 7

Comparative results between the combinations cultivar x distance between nests on a row (AxB)

No. crt.	Studied factors	Production		Difference compared to $\bar{x}$	Significance of the differences
		t/ha	% compared to $\bar{x}$		
1.	a_1b_1	7264	152	2470	xxx
2.	a_1b_2	6240	130	1446	x
3.	a_1b_3	8250	172	3456	xxx
4.	a_2b_1	1651	34	-3143	000
5.	a_2b_2	3684	77	-1110	-
6.	a_2b_3	3468	72	-1326	0
7.	a_3b_1	5514	115	720	-
8.	a_3b_2	8177	171	3383	xxx
9.	a_3b_3	6683	139	1889	xx
10.	a_4b_1	875	18	-3919	000

11.	a_4b_2	2654	55	-2140	00
12.	a_4b_3	3069	64	-1725	0
Average ($\bar{x}$)		4794	100	0	

LSD5% = 1290,36 kg/ha
LSD1% = 1757,88 kg/ha
LSD0,1% = 2362,55 kg/ha

CONCLUSIONS

1. The highest production increase (12%), compared to the average, was recorded for the 50 cm distance between the nests, while a second gradation of factor B (distance of 40 cm) resulted in a production increase of 8%, both variants being statistically assured at a significant level compared to the average.

2. The Lady Di variety recorded the highest production values for two of the three gradations of factor B (D1 = 30 cm, D2 = 50 cm), respectively 7264 kg/ha and 8250 kg/ha, resulting in production increase statistically assured compared to the average.

3. In the experiment for the distance of 40 cm the Polestar variety was noticed, with a production of 8177 kg /ha, value provided at a very significant level from the average.

4. Through the variation of the distance in 2014, the White Apollo variety has determined the lowest productions, with negative differences compared to the average.

5. In case of the combination cultivar x distance between nests/rows the best results were recorded by the varieties: Lady Di x 50 cm (8250 kg/ha), Polestar x 40 cm (8177 kg/ha) and Lady Di x 30 cm (7264 kg/ha). Also, statistically assured high productions were obtained for the combinations Polestar x 50 cm (6683 kg/ha) and Lady Di x 40 cm (6240 kg/ha).

Acknowledgments: *This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. Laitenberger K., 2013 – *Vegetables and herbs for the greenhouse and polytunnel*. Constable and Robinson Ltd., London;
2. Munteanu N., 1985 – *Phaseolus coccineus L. - o specie legumicolă care merită mai multă atenție*. Producția vegetală, Horticultura, nr. 4, Bucureşti;
3. Popa Lorena-Diana, 2010 - *Cercetări privind agrobiologia speciei Phaseolus coccineus L. în vederea optimizării cultivării*. Teză de doctorat, USAMV Iași;
4. Popa Lorena-Diana, Munteanu N., 2009 – *Yield study on some runner bean (Phaseolus coccineus L.) local populations depending of the establishment date*. Lucrări științifice, seria Horticultură, anul LII-vol. 52, pp. 465-470, USAMV Iași;
5. Rigoberto H. et al., 1986 – *The cultivated species of Phaseolus*. Centro Internacional de Agricultura Tropical CIAT, Cali, Columbia;
6. http://www.marshalls-seeds.co.uk/ ;
7. http://www.thompson-morgan.com/ ;
8. http://www.tozerseeds.com/eu/en/ .

AGRONOMIC PERFORMANCE OF SEVERAL CAVENDISH CULTIVARS (*Musa* spp. AAA) UNDER PLASTIC GREENHOUSE

PERFORMANȚELE AGRONOMICE ALE CÂTORVA CULTIVARE DE BANANE CAVENDISH (*Musa* spp. AAA) ÎN SERELE ACOPERİTE CU PLASTİC

GÜVEN Dilek[1], GÜBBÜK Hamide[2]
e-mail: ygdilek@gmail.com

Abstract: *Banana is grown both in open-field and protected cultivation in Turkey. However, protected cultivation is very popular due to the high yield and quality. The present study was carried out to evaluate agronomic performance of four new banana cultivars under plastic greenhouse. Cultivars'Williams', 'MA 13', 'Jobo','CV 902' and 'Dwarf Cavendish' (control) were used as experimental material. Pseudostem circumference, pseudostem height, total number of leaves, days from shooting to harvest, bunch stalk circumference, hand and finger numbers, finger circumference and length and also bunch weight were determined according to cultivars. The lowest pseudostem circumference, pseudostem height and bunch stalk circumference were found in 'Dwarf Cavendish' and the highest in 'Williams'. Total number of leaves varied between 27 and 32. Number of days from shooting to harvest was the shortest (141 days)in 'Williams' and the longest (164 days) in 'MA 13'. The highest bunch weight (57 kg)wasobserved in'MA 13'and the lowest (42 kg) in 'Dwarf Cavendish'.In conclusion, we found that all tested cultivars were superior to 'Dwarf Cavendish' underunheated plastic greenhouse.*
Key words: *Banana, protected cultivation, cultivar, adaptation, yield, quality.*

Rezumat: *Bananele sunt cultivate atât în câmp deschis cât și în mod protejat, în Turcia. Cu toate acestea, cultivarea protejată este utilizată în mod frecvent datorită randamentului ridicat și calității ftuctelor obținute. Prezentul studiu a fost efectuat pentru a evalua performanțele agronomice a patru noi soiuri de banane, în sere tip tunel, din plastic. Cultivarele 'Williams',' MA 13',' Jobo',' CV 902 "și" Dwarf Cavendish"(martor) au fost utilizate ca material experimental. Circumferința și înălțimea, pseudotulpinii, numărul total de frunze, numărul de zile de la apariția florilor până la recoltare, circumferința mănunchiului de banane , numărul de banane intr-un mănunchi, circumferința și lungimea unei banane, dar și greutatea unui mănunchi fost determinate în funcție de soiuri. Cele mai mici valori ale circumferințe și înălțimii pseudotulpinii și a circumferinței mănunchiului de banane au fost găsite în cazul cultivarului "Dwarf Cavendish", iar cele mai mari în cazul cultivarului "Williams". Numărul total de frunze a variat între 27 și 32. Cel mai scurt număr de zile de la apariția florilor până la recoltare a fost de 141 zile, în cazul cultivarului "Williams" iar cel mai lung (164 zile), în cazul cultivarului "MA 13". Masa cea mai mare a mănunchiului (57 kg) a fost identificată în cazul 'MA 13' iar cea mai*

[1] Bati Akdeniz Agricultural Research Institute, Antalya, Turkey
[2] Akdeniz Universty, Faculty of Agriculture, Depertmant of Horticulture, Antalya,Turkey

mică (42 kg), în cazul cultivarului "Cavendish'. In concluzie, s-a constatat că toate soiurile testate au fost superioare cultivarului martor "Dwarf Cavendish" în sere tip tunel, de plastic, neîncălzite.
Cuvinte cheie: *Banana, cultivare protejată, cultivar, adaptare, recoltă, calitate.*

INTRODUCTION

Banana is grown mainly under tropical conditions. India, Philippines and Ecuador are the major banana producer in the world. However, banana is also grown in Egypt, South Africa, Spain, Morocco, Australia, Turkey and Israel under subtropical conditions.

Banana is the only tropical fruit grown economically in Turkey. It is grown in both open-field and protected cultivation. However, protected cultivation is more popular in Turkey as it results in high yield. Presently, banana production in Turkey is around 206.00 tonnes, whereas the total consumption is over 400.000 tonnes (FAO, 2012).

Subtropical conditions are characterized by low winter temperatures, a large difference between day and night temperatures, high and low temperature extremes in summer and winter, and low rainfall which is often poorly distributed (Smith, M.K. et. al., 1998). Therefore, protected cultivation can be recommended in subtropical conditions to prevent temperature fluctuation, to reduce frost and wind damage to banana. Besides temperature, cultivar used has a profound effect on yield and quality in banana production. The main cultivar grown in Turkey, like other subtropical regions in the world, is Dwarf Cavendish. However, bunch shape and short fingers limit potential production of this cultivar in subtropical areas. There are many studies on evaluation of cultivars for their performance in different subtropical conditions. Galan Sauco. et. al. (1998) evaluated five banana cultivars (Eylon, Zelig, Gal, 19-39, and Chinese Cavendish) in Canary Islands which foundZelig and Chinese Cavendish suitable for commercial cultivation in the Canary Islands. Ecksteinet al. (1998), examined the performance of different banana cultivars such as Williams, Chinese Cavendish and Grand Nain Israel, which are a subgroup of Cavendish grown in both open-field and greenhouse cultivation in South Africa. Grand Nain Israel and Williams were comparatively superior to Chinese Cavendish in terms of cultivar performance in both open-field and greenhouse cultivation. Ribeira, L.V.N.P. and Alcino de Silva, J. (1998) evaluated the adaptation of cultivars, Grand Nain, Williams, Zelig, Eylon and Chinese Cavendish in Madeira Island and found the cultivar Grand Nain with highest yield and pseudostem. A similar study on evaluation of performance of five banana cultivars (Valery, Lacatan, Giant Cavendish, Dwarf Cavendish, Green Red) in South Western Kenya found Valery and Giant Cavendish best suitable for cultivation due to the their high yield, good taste and strong pseudostems (Kwach, et. al., 2000). Previously, our group compared the performence of some Cavendishcultivars (AAA) under open-field and plastic greenhouse conditions in terms of yield and quality(Gubbuk et al. (2004), andwefound Williams and Grand Nainwere superior to Dwarf Cavendish for greenhouse cultivation in terms of

their yield and quality. All tested cultivars, except 'Poyo', were recommended for open field cultivation. Cabrera Cabrera and Galan Sauco (2012) compared the cultivars Gruesa and Grand Nain under protected cultivation showed that the Gruesa plants in comparison to Grand Nain plants resulting in a higher humber of leaves until bunch emerge, lower height, smaller width and a lower pseudostem height width ratio, and longer cycle producing shorter fingers.

The objective of the present study was to evaluate the agronomic performance of some of the newly introduced banana cultivars under plastic greenhouse condition.

MATERIAL AND METHOD

The study was carried out in between 2006 to 2008 in Kargıcak-Alanya (altitude 10 m, latitude 36°28' N) in the province of Antalya. Average mean yearly minimum/maximum temperatures under protected cultivation were 13.51- 27.37^{0}C and relative humidity 70-90%.

The greenhouse structure was consisted with iron structure covered with plastic and top height and height from the gutter were around 7.5 meters and 5 meters, respectively. The greenhouse was ventilated from all the sides and the top. The greenhouse was not heated during two cycles. The experimental materials were introduced from Vitropic (French Tissue Culture Company).

All plant materials propagated via meristem culture. Cultivar Williams, MA13, Jobo, CV 902 and Dwarf Cavendish (control) were used as experimental material. Plant spacing was 3 x 1.8 m (1850 plant/ha).

The soil condition consisted of pH 7.7, 1.7% lime content, loamy texture, with 2% organic matter. Irrigation and fertilization were uniformly applied as per normal recommended practices (Pekmezci et. al., 1998).

Pseudostem circumference (20 cm above the soil level), pseudostem height, total leaf number (at shooting stage), days from shooting to harvest, bunch stalk circumference (5 cm above the first hand), hand and finger numbers, finger circumference (at the center of first, middle and last hands of fingers), finger length (from end to end in a straight line) and bunch weight, were determined at harvest time according to Pekmezci et. al.(1998) and Mendez Hernandez (1998). Trials were laid out with three replications and 3 plants in each replicate in a completely randomized experimental design.

The experiment results were analyzed using analysis of variance (ANOVA). Means were separated using LSD multiple range test at 0.05 levels.

RESULT AND DISCUSSION

Pseudostem circumference and height, total leaf number and days from shooting to harvest are presented in tab. 1. The least pseudostem circumference and height (71 cm - 196 cm) was found in Dwarf Cavendish, whereas the greatest (92 cm - 314 cm) in Williams. Total leaf number was highly variable among cultivars and was on an average more than 27 per plant. Number of days from shooting to harvest differed among cultivars and was statistically significant (tab. 1). Shooting to harvest was longest in MA 13 and the shortest in Williams. The values obtained for all cultivar regarding pseudostem circumference, pseudostem height, total leaves number and shooting to harvest varied according to cultivars.

Similar results were also obtained in previousstudies (Galán Saúco et al., 1998; Eckstein et al., 1998; Kwach et al. 2000; Gubbuk et al., 2004). The outcome of the present studies clearly suggests that genotype affects morphological features and also shooting to harvest. Our results are concomitant with previous studies.

Table 1.

Pseudostem circumference, pseudostem height and total leaves number, shooting to harvest of different banana cultivar grown in plastic greenhouse in Alanya

Cultivars	Pseudostem circumference (cm)	Pseudostem height (cm)	Total leaves number (number/plant)	Shooting to harvest (day)
Williams	92.06 a	313.83 a	32.14 a	140.67 d
MA 13	88.58 b	310.16 a	29.16 b	164.00 a
Jobo	86.58 b	300.50 a	28.61bc	147.50 c
CV 902	85.07 c	282.83 b	32.00 a	159.33 b
DwarfCavendish	71.37 d	195.83 c	27.06 c	159.66 b
LSD %5	*3.12*	*14.86*	*2.27*	*1.61*

The cultivar, Williams showed the higher bunch stalk circumference including other features(tab. 2). Hand number per bunch varied among 11 (Dwarf Cavendish) and 13 (Williams) depending on the cultivars. Finger number was also statistically different and CV 902 had the highest mean finger number (258 per bunch) followed by MA 13 (255 per bunch). Our results showed that yield component such as hand and finger numbers per bunch varied within cultivars. Compared to Dwarf Cavendish, all cultivars showed the best result in yield parameters. Similar results have been obtained in other cultivars in previous works (Galán Saúco et al., 1998; Eckstein et. al., 1998; Kwach et al., 2000; Gubbuk et. al., 2004).

Table 2

Bunch stalk circumference, hand and finger numbers of different banana cultivars grown in plastic greenhouse in Alanya

Cultivars	Bunch stalk circumference (cm)	Hand number (number/bunch)	Finger number (number/bunch)
Williams	34.52 a	*13.33 a*	251.42 b
MA 13	29.75 bc	12.16 b	255.40 a
Jobo	27.83 c	11.83 b	251.70 b
CV 902	30.76 b	12.50 b	257.57 a
DwarfCavendish	24.90 d	10.67 c	224.76 c
LSD %5	***2.07***	***0.68***	***3.54***

Finger circumference and length differed significantly among the cultivars (tab. 3). Finger circumference was the highest (14) in Jobo and the lowest (12) in 'Dwarf Cavendish'. On the other hand, finger length was highest (25 cm) in MA 13 and the lowest in Dwarf Cavendish (21 cm). A significant (P <0.05) difference was also found in bunch weight among cultivars. The highest bunch weight was obtained in MA13 (57 kg) and the lowest in Dwarf Cavendish (42 kg). According to experimental results, yield parameters (finger circumference, finger length and

bunch weight) varied within cultivars. Dwarf Cavendish gave the lowest yield parameters. These results are in agreement with those obtained in our previous studies in other cultivars (Gubbuk et.al., 2004).

Table 3

Fingercircumference, fingerlengthandbunchweightof different banana cultivars grown in plastic greenhouse in Alanya

Cultivars	Fingercircumference (cm)	Finger length (cm)	Bunch weight (kg)
Williams	12.83 b	22.92 b	55.97 b
MA 13	13.41 a	24.58 a	57.42 a
Jobo	13.66 a	23.67 ab	45.92 d
CV 902	12.33 b	23.83 ab	52.00 c
Dwarfcavendish	11.00 c	21.00 c	41.78 e
LSD %5	*0.57*	*1.08*	*1.97*

CONCLUSIONS

Our studies on evaluation of the agronomic performance of the banana cultivars grown in plastic greenhouse showed the Cultivars MA 13 and Williams resulting in the best yield parameters and fruit quality, followed by CV. 902 and Jobo. Our results recommend, cultivation of all cultivars as a replacement to the main cultivar Dwarf Cavendish.

Acknowledgement: *This was supported by the project unit of Akdeniz University. The authors thanks Dr. Frederic Bakry from CIRAD/France and Vitropic Company for providing plant materials.*

REFERENCES

1. **Cabrera Cabrera J.,Galán Saúco V., 2012** *Evaluation of different covers used in greenhouse cultivation of Cavendish bananas (Musa acuminata colla AAA) in the Canary Islands*. Acta Horticulturae, 928, pp. 131-138.
2. **Eckstein K., Joubert W., Fraser C., 1998** -*Greenhouse cultivation of banana in South Africa.* Acta Horticulturae, 490, pp.135-145.
3. **Galan Sauco V., Cabrera Cabrera J., Hernandez Delgado P.M., Rodriguez Pastor M.C., 1998** -*Evaluation of medium-height Cavendish banana cultivars under the subtropical conditions of the Canary Islands.*Acta Horticulturae, 490, p. 103-113.
4. **Gubbuk H**., **Pekmezci M., Erkan M., 2004 -** *Production potential of Cavendish cultivars (Musa spp. AAA) under greenhouse and field conditions in subtropical areas of Turkey.* Acta Agriculturae Scandinavica, Section B - Plant Soil Science, 54 (4), pp. 249-253.
5. **Kwach J.K., Makworo S., Nasambu O., Onyango M., 2000** - *Performance of banana cultivars in south western Kenya*. Acta Horticulturae, 540, pp. 239-243.
6. **Mendez Hernandez C., 1998** - *A comparison of the parent crop of three cultivars of banana in the open air and under plastic mesh in the north of the Tenerife.* Acta Horticulturae, 490, pp. 97-101.
7. **Pekmezci M., Gübbük H., Erkan M., 1998** - *Investigation on growing possibilities of banana in Turkey*. Acta Horticulturae, 490, pp. 599-603.

8. Riberiro L.N.V.P., Alcino de Silva J., 1998 -*Preliminary studies of Cavendish banana cultivars under the edafoclimatic conditions of Madeira Islands.* Acta Horticulturae, 490, pp. 85-88.
9. Smith M.K., Hamill S.D.,Langdon P.W., Pegg K.G., 1998 - *Selection of new banana varieties for the cool subtropics in Australia.* Acta Horticulturae, 490, pp. 49-56.
10. FAO, 2012.-*www. fao.org.*

THE EVALUATION OF FRUITS PRODUCTION AND PHYSICO-CHEMICAL FEATURES FOR SOME CHERRY CULTIVARS CREATED AT S.C.D.P. IASI

EVALUAREA PRODUCȚIEI ŞI A ÎNSUŞIRILOR FIZICO-CHIMICE ALE FRUCTELOR LA UNELE SOIURI DE CIREŞ CREATE LA S.C.D.P. IAŞI

IUREA Elena[1], SÎRBU Sorina[1], CORNEANU G.[1]
e-mail: iurea_elena@yahoo.com

Abstract: *This paper presents some aspects concerning the influence of the environment factors between 2011-2013, registered in the Iaşi area, on the productivity and fruits quality for cherry species. In 2011 (432,4 mm) and 2012 (446,6 mm) there were registered quantities below the multiannual limit (524,6 mm), getting a deficit of 92,2 mm in 2011 and 78,0 mm in 2012 (this climatic variability influences negatively the fruit's growth) and in 2013 the multiannual was exceeded, being achieved 705,4 mm (a surplus of 180,8 mm). Analyzing the average productions on three years (the years XII-XIV from plantation), from the statistic point of view, we can see that the cultivars Margo (30,9 kg/tree) and Ludovic (19,9 kg/tree) registered positive production differences compared to the cultivars average. Under the aspect of the fruits weight (g) and of the equatorial diameter (mm), there were remarked the cultivars Alex (9,6 g and 24,8 mm), Ludovic (8,8 g and 24,1 mm) with positive significant differences compared to the witness and Paul (8,0 g with 22,9 mm), being significantly positive compared to the witness.*
Key words: *cultivars, cherry, production, fruits, features.*

Rezumat: *Această lucrare prezintă unele aspecte privind influența factorilor de mediu din anii 2011 – 2013, înregistrată în zona Iaşi, asupra productivității şi calității fructelor la specia cireş. În anii 2011 (432,4 mm) şi 2012 (446,6 mm) s-au înregistrat cantități sub limita multianualei (524,6 mm), realizându-se un deficit de 92,2 mm în anul 2011 şi 78,0 mm în anul 2012 (această variabilitate climatică influențează negativ creşterea fructului), iar în 2013 multianuala a fost depăşită realizându-se 705,4 mm (un excedent de 180,8 mm). Analizând producțiile medii pe trei ani (anii XII-XIV de la plantare), din punct de vedere statistic, se constată că soiurile Margo (30,9 kg/pom) şi Ludovic (19,9 kg/pom) au înregistrat diferențe de producție pozitive față de media soiurilor. Sub aspectul greutății fructelor (g) şi a diametrului ecuatorial (mm), s-au remarcat soiurile Alex (9,6 g şi 24,8 mm), Ludovic (8,8 g şi 24,1 mm) cu diferențe semnificativ pozitive față de martor şi Paul (8,0 g cu 22,9 mm) fiind semnificativ pozitiv față de martor.*
Cuvinte cheie: *soiuri, cireş, producție, fruct, însuşiri.*

[1] Research and Development Station for Fruit Tree Growing of Iaşi, Romania

INTRODUCTION

The cherry tree is a fruit-growing species with major economic importance, given by the fruits nutritive, technological and commercial features (Budan Gradinariu, 2000; Grădinariu Istrate, 2003; Petre, 2006).

It is a species with average claims to water. Thereby, it lives in the Iaşi area, with annual rainfall under 550 mm, being grafted on mahaleb (Dumitrescu et al., 1981; Grădinariu, 2002).

In 2011 (432,4 mm) and 2012 (446,6 mm) there were registered quantities under the multiannual limit (524,6 mm), getting a deficit of 92,2 mm in 2011 and 78,0 mm in 2012 (this climatic variability influences negatively the fruit's growing) and in 2013, the multiannual was exceeded, getting 705,4 mm (a surplus of 180,8 mm).

This paper presents some aspects concerning the influence of the environment factors from 2011-2013, registered in the Iaşi area, on the fruits productivity and quality for the cherry species.

MATERIAL AND METHOD

The researches were made during 2011-2013, having as research material four new cherry cultivars (Alex, Margo, Paul and Ludovic) grafted on mahaleb.

The fruit-growing trees are planted at a distance of 4 x 5 m with the shape of free flattened crown, being in the years XII-XIV from planting. The land where the planting was established is situated in Jijia-Bahlui depression, where the average annual temperature was of 9,8°C in 2011, of 10,6°C in 2012 and 10,2°C in 2013 (the multiannual average being of 9,6°C).

The meteorological factors were analyzed (during the three years), for each cultivar there was determined the fruits production (kg/tree), there were made measurements and determinations concerning the fruit's size (the equatorial diameter-mm), the fruit's weight (g), the soluble dry substance (SUS%), the titratable acidity (AT), the ratio between SUS and AT and the total content of polyphenols.

The fruits production and the fruit's weight was determined through weighing, the equatorial diameter of the fruit was determined by measuring with the electronic calipers, SUS was determined with the Zeiss refractometer, AT was determined with the potentiometric method and the determination of the total content of polyphenols was effectuated after the Folin-Ciocâlteu method.

The experimental data was statistically interpreted by the variance analysis and the correlation coefficient (r) was effectuated using Bravais's formula, 1978.

RESULTS AND DISCUSSIONS

For the studied cherry cultivars, the productions obtained in the XIIth year from planting were between 11,2 kg/tree (Alex) and 30,8 kg/tree (Margo), (tab. 1).

Analyzing the average production on three years (years XII-XIV from planting), from the statistical point of view, it can be said the cultivars Margo (30,9 kg/tree) and Ludovic (19,9 kg/tree) registered positive production differences in comparison with cultivars average.

Following the production in the three years of study, it can be seen that in 2011, there were registered the biggest values for the majority of the cultivars,

due to the fact that in August 2010, the rainfall quantity was sufficient (57,0 mm/m^2), thus the differentiation of the buds from fruits was performed in good conditions (tab. 1).

Table 1

The fruits production for four cherry cultivars in the years XII-XIV from planting

Cultivar	The average production kg/tree in the years:			Average production (2011-2013)		The difference in comparison with the variants average and the significance
	2011	2012	2013	kg/tree	t/ha	
Margo	30,8	30,0	32,0	30,9	15,5	+ 11,7
Ludovic	29,0	15,7	15,0	19,9	9,9	+ 0,7
Average (x)	23,7	16,7	17,0	19,2	9,6	0,0
Paul	23,7	12,6	12,0	16,1	8,1	- 3,1
Alex	11,2	8,8	9,0	9,7	4,8	- 9,5

LSD 5% = 15,2 kg/tree LSD 1% = 23,0 kg/tree LSD 0,1% = 36,9 kg/tree

The fruit's weight is a dimension that is influenced by the local climatic conditions and by each cultivar biological particularities.

From the statistical point of view, throughout the study, the Alex cultivar (9,6 g) registered very positive significant differences compared to the witness, the Ludovic cultivar registered distinct positive significant differences and the Paul cultivar registered positive significant differences compared to the witness (tab. 2).

Table 2

The fruit's weight registered during 2011-2013

Cultivar	Fruit's weight (g) in the years:			The average on the studied period (g)	The difference in comparison to the witness and the significance
	2011	2012	2013		
Alex	9,1	9,4	10,4	9,6	+ 2,9^{+++}
Ludovic	9,3	8,4	8,7	8,8	+ 2,1^{++}
Paul	8,6	7,4	8,0	8,0	+ 1,3^{+}
Margo	7,4	6,0	6,9	6,8	+ 0,1
Boambe de Cotnari (mt)	**6,5**	**6,6**	**7,0**	**6,7**	0,0

LSD 5% = 1,0 g LSD 1% = 1,5 g LSD 0,1% = 2,4 g

Under the aspect of the equatorial diameter (mm), there got remarked the cultivars Alex (24,8 mm), Ludovic (24,1 mm) and Paul (22,9 mm) with distinct positive significant differences compared to the witness (tab. 3).

Analyzing the values of the fruit's size registered in 2012, droughty year which influenced negatively the fruits growth, the Alex cultivar got remarked as being the most resistant to drought registering the biggest equatorial diameter (24,4 mm) (tab. 3).

Table 3

The equatorial diameter of the fruit registered during 2011-2013

Cultivar	The fruit's equatorial diameter (mm) in the years:			The average on the studied period (mm)	The difference compared to the witness and the significance
	2011	2012	2013		
Alex	22,7	24,4	27,3	24,8	+ 3,6^{++}
Ludovic	23,8	22,2	26,3	24,1	+ 2,9^{++}
Paul	23,1	21,9	23,8	22,9	+ 1,7^{++}
Margo	21,1	20,2	23,2	21,5	+ 0,3
Boambe de Cotnari (mt)	**20,5**	**20,2**	**23,0**	**21,2**	0,0

LSD 5% = 1,0 mm **LSD 1% = 1,5 mm** **LSD 0,1% = 4,7 mm**

A continuous growing of the fruit's weight determines a correspondent growth of the equatorial diameter (D) (fig. 1).

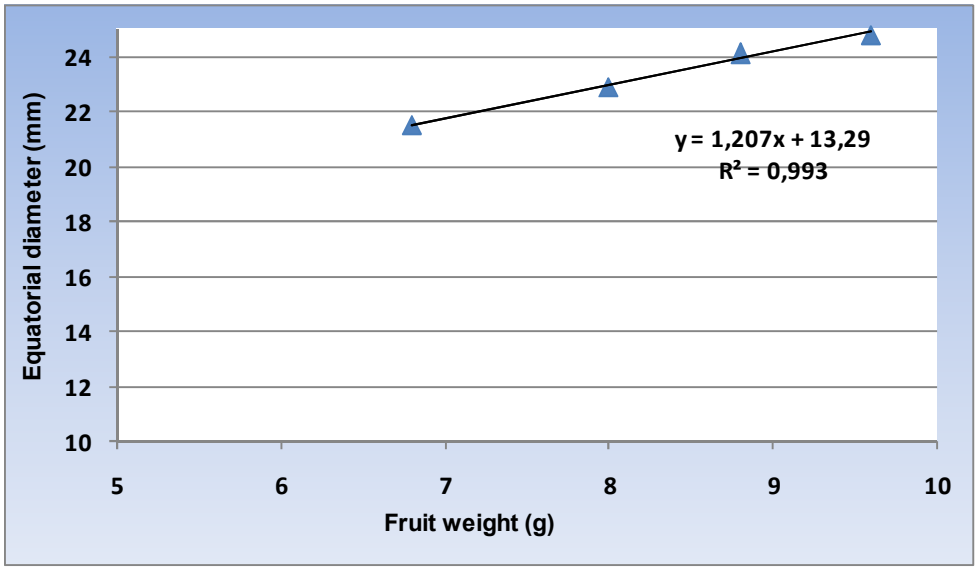


Fig. 1 – The correlation between the weight and the equatorial diameter of the fruit for the cherry cultivars taken in study

The cherries are characterized through a specific organoleptic harmony and that is why the taste and flavor remain the tangible effects of the moment when the fruits are consumed (Tomás-Barberán et. al., 2001; Crisosto et al., 2002).

In the studied genotypes, the SUS values were between 14,8% (Paul) and 17,6% (Alex), the titratable acidity (AT) of the fruits varied in a wide range with values between 0,4 - 0,7 mg malic acid/100 ml juice, with a ratio between SUS and AT framed in the interval 20-39%. Also the values of the total polyphenols

varied from a genotype to another, this index being situated in the interval 104,93 – 336,28 mg galic acid/100 ml fresh juice (tab. 4).

Table 4

Physical and biochemical features of the fruits for the cherry cultivars

Cultivar	The epidermis color	The biochemical features of the fruits			
		SUS -%-	AT - mg Malic acid/100 ml -	Ratio SUS and AT -%-	The total content of - mg GAE/100 ml-
Alex	Dark red	17,6	0,447	39,328	336,28
Ludovic	Dark red	16,4	0,497	32,951	205,36
Paul	Double colored	14,8	0,722	20,458	104,93
Margo	Whitish yellow	15,8	0,643	24,513	326,88

A high content of polyphenols is associated with an intense color of the fruits, with a big content of dry substance, but also with a more intense flavor (fig. 2).

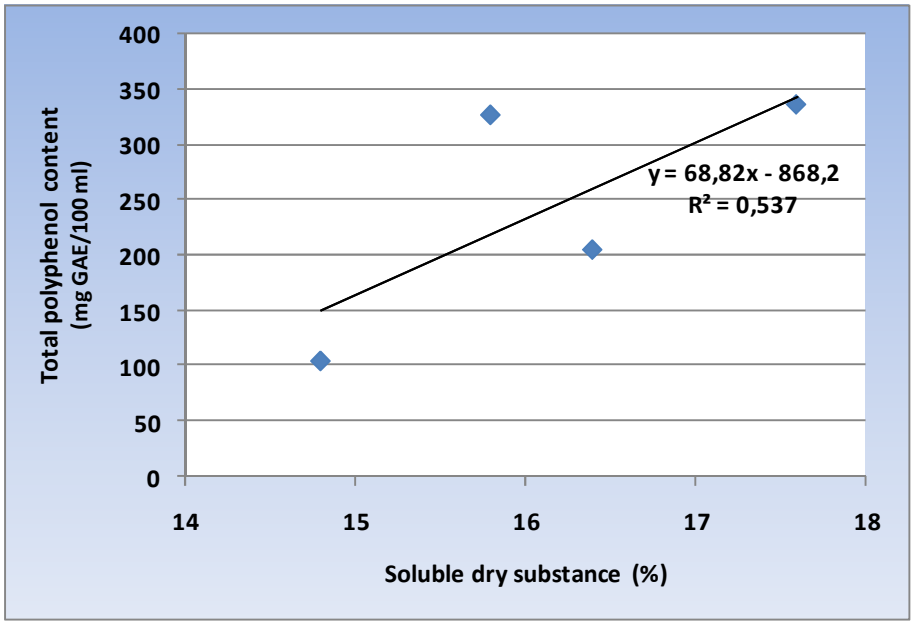


Fig. 2 – The correlation between SUS and the total content of polyphenols for the cherry cultivars taken in study

Following the obtained results, the Paul cultivar registered the lowest values for SUS (14,8%), for the ratio SUS and AT (20,458%) as well as for the total content of polyphenols (104,93 mg GAE/100 ml of fresh juice) (tab. 4).

CONCLUSIONS

1. In the conditions of the years 2011, 2012 (droughty years) and 2013(rainy year), in the Iaşi area, under the productivity aspect, there got remarked the cultivars Margo and Ludovic, registering the biggest annual productions.

2. Analyzing the values of the fruit's size, registered in 2012, droughty year that influenced negatively the fruits growth, the Alex cultivar got remarked as the most resistant to drought, registering the biggest equatorial diameter (24,4 mm) followed then by the Ludovic cultivar (22,2 mm).

3. The fruits of the cultivars Alex, Ludovic and Margo can be considered as having a very appreciated taste by the consumers because there exists a balance between the sweet taste (the content of SUS) and sour (AT) and it presents a high content of polyphenols, giving them a more intense flavor.

REFERENCES

1. **Budan S., Grădinariu G., 2000 –** *Cireşul*, Editura Ion Ionescu de la Brad, Iaşi, 262 p.
2. **Crisosto C.H., Crisosto G.M., Ritenour M.A., 2002 –** *Testing the reliability of skin color as an indicator of quality forearly season 'Brooks' (Prunus avium L) cherry*. Postharv est Biol. Technol, 24, pp. 147-154.
3. **Dumitrescu Gh., Hrițcu C., Frunză P., Bazgan C., 1981** – *Studiu privind cultura cireşului şi vişinului în județul Iaşi,* Cercetări Agronimice în Moldova, vol. 3, Iaşi., pp. 77-82.
4. **Grădinariu G., Istrate M., 2003** - *Pomicultură Generală şi Specială*, Editura Moldova Iasi, 627 p.
5. **Petre L., 2006 –** *Rezultate obținute în ameliorarea sortimentului de cireş, vişin şi nuc la SCDP Iaşi,* Lucr. Şt. ICDP Piteşti-Mărăcineni, vol. XXII, Piteşti, pp. 45-49.
6. **Tomás-Barberán F.A., Espin J.C., 2001 –** *Phenolic compounds and related enzymes as determinants of quality in friuts and vegetables,* Journal of Science and Food Agricultural, 81, pp. 853-876.

EFFECT OF NAPHTHALENEACETIC ACID (NAA) ON PREHAVEST DROP OF GALA MUST APPLE VARIETY

EFECTUL ACIDULUI ALFANAFTILACETIC (ANA) ASUPRA CĂDERII PREMATURE A FRUCTELOR DIN SOIUL DE MĂR GALA MUST

PESTEANU A.[1]
e-mail: a.pesteanu@uasm.md

Abstract. *The experimental plot is placed in the orchard "Dacfruct" Ltd. founded in 2006. The study subject of the experience was Gala Must apple variety grafted on M 9. The trees were trained as slender spindles. The distance of plantation is 3.5 x 1.2 m. The research was conducted during the period of 2013 year. The aim of this study was to evaluate the effectiveness of grower regulator Obsthormon 24a in reducing fruit drop of apple trees. The tested agents were NAA, which was sprayed in one time in preharvest period. During the research, it was studied the quantity of dropped fruits under the trees and percentage of total fruit yield. It was established that, one treatment with Obsthormon 24a in dose of 300 ml/ha have a significant effect on fruit drop and there quality.*
Key words: *fruit drop, quality, NAA, preharvest spray*

Rezumat. *Livada experimental a fost fondată în anul 2006 în întreprinderea SRL „Dacfruct". Ca obiect de studiu în experiment a fost soiul Gala Must altoit pe portaltoiul M9. Pomii au fost conduşi ca fus subţire ameliorat. Distanţa de plantare 3,5x1,2 m. Cercetările s-au efectuat pe parcursul anului 2013. Scopul acestor cercetări au fost de a evalua eficacitatea regulatorului de creştere Obsthormon 24a la prevenirea căderii fructelor din coroană. Ingredientul activ a fost ANA, care s-a pulverizat o singură dată înainte de perioada de recoltare. În perioada cercetărilor s-a investigat cantitatea de fructe căzute sub pom şi ponderea lor la recolta de fructe. S-a stabilit că un tratament cu Obsthormon 24a în doza de 300 ml/ha are un efect pozitiv asupra căderii premature a fructelor şi calităţii lor.*
Cuvinte cheie: *căderea fructelor, calitate, ANA, tratare înainte de recoltare*

INTRODUCTION

Preharvest fruit drop can reduce production of apple cultivars in commercial orchards by as much as 30% (Babuc et al., 2013; Cimpoieş, 2012; Marini et al., 1993; Yuan and Carbaugh, 2007).

Application of synthetic auxins reduced preharvest fruit drop of apples (Marini et al., 1993). Naphthaleneacetic acid (NAA) is an auxin-type growth regulator that primarily is used to reduce preharvest drop. NAA does not strengthen up the fruit attachment, but only prevents further loosening from the fruit stem (Kvikliene et al., 2010; Schupp and Greene, 2004; Yuan and Li, 2008).

[1]State Agrarian University of Moldova, Republic of Moldova

One application of naphthalene acidic acid (NAA) may delay apple fruit drop for 10 to 14 days after treatment, and repeated applications of NAA delay fruit abscission more than single applications (Marini et al., 1993).

The aim of this study was to assess the effects of Obsthormon 24a (NAA) on the Gala Must variety used during the preharvest period, on fruit drop.

MATERIAL AND METHOD

The researches were made during the period of 2013 year in the apple superintensive orchard founded in autumn 2006 at the company "Dacfruct" Ltd. with "knip boom" type apple trees crown formation.

The study object of experience was Gala Must apple tree variety grafted on weak vigor M9 rootstock. The crown was conducted on ameliorated thin spindle system. Distance of plantation is 3.5 x 1.2 m.

The chemical growth regulator used was Obsthormon 24a, containing 84 g/l active ingredient NAA, the preparation by the „L. Gobbi Ltd." producer from Italy. To optimize the fruit dropping of the apple trees were experimented the following variants:

V_1 - Control variant- without chemical treatments;

V_2 - Obsthormon 24a - 300 ml/ha;

V_3 - Obsthormon 24a - 375 ml/ha.

On experimental section in accordance with the experiences scheme in the second and in the third variants were made a single treatment (09/15/13) with a dose of 300 ml/ha and 375 ml/ha.

Fruit drop was evaluated one time, in harvest period. Dropping fruit was expressed as percentage of total fruit yield.

Firmness was measured with a penetrometer (FT- 327) with 11 mm diameter probe. Soluble solids content was measured with a refractometer.

RESULTS AND DISCUSSIONS

The apple orchard frequently in the fall before harvest register prematurely fruit dropping. To exclude this phenomenon or decrease of dropped fruits is advisable to treat the trees with NAA 1-3 weeks before harvest. Of these preparations widespread in the European Community have become Hergon L, Obsthormon 24a etc.

The conducted investigation, demonstrate that during treatment (15/08/2013) the amount of fruit in apple trees of Gala Must variety constituted 87 - 90 pcs (tab. 1). This amount of fruit in the trees crown is considered optimal to achieve consistent and quality productions annually. If, in the control variant, the total amount of fruit from the tree crown was 88 pcs, then in the variants where was used the growth regulator Obsthormon 24a, it ranged from 87 - 90 pcs/tree. A slight increase in the amount of fruit was recorded from variant 2, where planned treatment with Obsthormon 24a in dose of 300 ml/ha - 90 pcs/tree, compared to variant Obsthormon 24a in dose of 375 ml/ha - 87 pcs/tree.

This slight difference between variants in study, 3 pcs/tree, allowed us to mount the experiment for testing the growth regulator Obsthormon 24a to prevent premature fruit dropping in the autumn before harvest.

On the day of harvest (29.08.2013) was registered, the amount of fruit in the studied variants was 76 to 88 pcs. The small amount of fruit in the trees crown was recorded for control variant - no treatment, where the studied index was 76 pcs/tree.

In the variants treated with the growth regulator Obsthormon 24a the amount of fruit in the crown of trees was placed between 86-88 pcs. In the variant Obsthormon 24a in dose of 300 ml/ha the amount of fruit recorded maximal values 88 pcs/tree, and when the dose of treatment was 375 ml/ha - 86 pcs/tree. The difference between the amount of fruit in variant Obsthormon 24a in dose of 300 ml/ha and control variant without treatment was 8 pcs/tree, and between Obsthormon 24a in dose of 375 ml/ha and control variant - 10 pcs/tree.

Table 1

The influence of growth regulator Obsthormon 24a on the quantity of fruits before and after treatment in the trees of the variety Gala Must, average fruit weight and diameter

Variants	Quantity of fruits, pieces			The average weight, g	The average diameter, mm
	total per tree at treatment	on trees at harvest	dropped under the trees		
Control, without treatment	88	76	12	148.0	69.7
Obsthormon 24a, 300 ml/ha	90	88	2	148.5	70.8
Obsthormon 24a, 375 ml/ha	87	86	1	149.0	71.0

The most important index of apple plantation treatment with growth regulator to prevent fruit dropping is the amount of fruits on the ground. The conducted investigations show that in the control variant is recorded the largest amount of dropped fruit - 12 pcs/tree.

The treatments with growth regulator Obsthormon 24a essentially decreased premature fruit dropping before harvest. In the variant Obsthormon 24a dose 300 ml/ha the amount of dropped fruit was 2 pieces and in the variant Obsthormon 24a in dose of 375 ml/ha was only a fruit. So, treating trees with growth regulator Obsthormon 24a improved physiological processes taking place in plants didn't allow forming suber layer between stalk and fruit bearing formations and prevent premature fruit drop.

The use of growth regulators Obsthormon 24a influenced insignificantly and on the average fruit weight and diameter. If the average weight of fruits in the control variant was 148.0 g and diameter was 69.7 mm, in the variant of treatment Obsthormon 24a in dose of 300 ml/ha the studied indexes were respectively increased by 3.4% and 15.7% and in the variant Obsthormon 24a in dose of 375 ml/ha with 6.7% and 18.6%.

Studying the influence of the dose treatment on analyzed indicators, we noticed an essential difference between variant Obsthormon 24a in dose of 300 ml/ha and in dose of 375 ml/ha. In the variant Obsthormon 24a in dose of 300 ml/ha the amount of dropped fruits were 2 pcs/tree and in the variant Obsthormon 24a in dose of 375 ml/ha was 1 pcs/tree. The average diameter of the fruit weight was the same.

Fruit production is one of the major indexes to assess the technological elements that were conducted in apple plantation.

The data of table 2 shows that growth regulator Obsthormon 24a did not influence the production of fruit and was placed at the same level as in the control variant, without treatment. In the control variant, fruit production amounted to 29.76 t/ha, in the variant Obsthormon 24a in dose of 300 ml/ha – 30.33 t/ha and Obsthormon 24a in dose of 375 ml/ha - 29.62 t/ha.

Table 2

The influence of Obsthormon 24a growth regulators on fruit production obtained from a unit area of the variety Gala Must at harvest t/ha

Variants	Production, t/ha			The difference between the production left and control
	total	dropped under the tree	in the tree	
Control, without treatment	29.76	4.21	25.55	-
Obsthormon 24a, 300 ml/ha	30.33	0.78	29.55	+ 4.00
Obsthormon 24a, 375 ml/ha	29.62	0.36	29.26	+ 3.71
LSD 5%	1.21	-	1.18	-

It is obvious that the amount of total production, some fell on the ground until the harvest, and the other was collected from the crown of trees. The amount of production collected from the ground and from the crown of the tree is different and has been influenced by treatments with Obsthormon 24a whose active ingredient is naphthaleneacetic acid.

In the control variant, production collected from trees crown was 25.55 t/ha and harvested from the ground for industrialization 4.21 t/ha, or 16.48% of the qualitative one.

The treatment with growth regulator Obsthormon 24a positively influenced on maintaining maximum production in the crown of apple trees. When the treatment was with Obsthormon 24 in dose of 300 ml/ha production collected from crown was 29.55 t/ha and from the soil 0.78 t/ha, which constituted 2.64% of the share of quality. In the variant Obsthormon 24a in dose of 375 ml/ha lawfulness exposed to the previous variant is available, and the share of

production of poor quality picked from the ground was 1.23% compared to that harvested from crown.

The effectiveness of treatment with growth regulator Obsthormon 24a is demonstrated by the production difference collected in these variants and control variant. In variant treatment with growth regulator Obsthormon 24a in dose of 300 ml/ha, the difference between output collected from the crown in time variant and control variant was 4.0 t/ha and in the variant Obsthormon 24a in dose of 375 ml/ha - 3 71 t/ha.

The conducted investigations (tab. 3) demonstrates that fruit pulp firmness on Gala Must apple variety 15 days before harvest variants in the study was 8.3 to 8.4 kg/cm^2.

At the time of harvest (15 days after treatment) Gala Must variety flesh firmness decreased to a value of 6.5 to 6.9 kg/cm^2. The small farm has been in version control - 6.5 kg cm^2. When treating with growth regulator Obsthormon 24a, pulp firmness was 6.8 to 6.9 kg/cm^2 which demonstrates that treatments with product based on naphthaleneacetic acid inhibit physiological processes of formation of ethylene and increases fruit firmness.

Table 3

The influence of growth regulators Obsthormon 24 on firmness and soluble solids content of fruits of Gala Must apple variety

Variants	Firmness, kg/cm^2			Soluble solids (% harvest)	
	at treatment	at harvest	dropped fruits	fruits of the tree	dropped fruits
Control, without treatment	8.3	6.5	5.6	13.2	14.9
Obsthormon 24a, 300 ml/ha	8.4	6.8	5.7	12.8	14.7
Obsthormon 24a, 375 ml/ha	8.4	6.9	5.6	12.8	14.9

Dropped fruits on the ground have a much lower firmness compared to the ones in the crown of the tree. The firmness of the fruit of the studied variants ranged from 5.6 -5.7 kg/cm^2. Therefore, the difference in firmness of dropped fruits on the ground and the ones in the tree crown that were collected were 0.9 - 1.3 kg/cm^2.

The fruit soluble solids determine the optimal harvest time. As harvest time approaching, the intensity of accumulation of soluble dry substances is increased.

The obtained data demonstrates that the amount of fruit soluble dry substances of the tree, at Gala Must variety, in the study variants was 12.8 to 13.2%.

The highest value of the weight soluble dry substance was obtained in the control variant - 13.2%. When treating with growth regulator Obsthormon 24a, we notice a decline by 0.4% compared to the control variant where the studied

index is 12.8%. The latest number demonstrates that growth regulator Obsthormon 24a increased fruit firmness and decreased the amount of soluble solids.

Dropped fruits have a smaller firm and a high content of soluble solids, representing in the study variations from 14.7 to 14.9%. The amount of soluble solids demonstrates that fruit drop to the ground before maturation, and can only be used for industrialization.

Conducted investigations demonstrates that convincing results on quality index record the variant where was used the growth regulator Obsthormon 24a in dose 300 ml/ha.

CONCLUSIONS

The treatments made with growth regulator Obstnormon 24a based of NAA (84 g/l) 15 days before harvesting decreased essentially the amount of dropped fruits.

The fruit firmness and soluble solids content recorded higher values in variants treated with Obstnormon 24a then in the control variant.

Based on the experimental results obtained, the growth regulator Obsthormon 24a can be included in the technological system to prevent premature fruit dropping before harvest ripening apple production to Gala Must variety in dose 300 ml/ha, applied by means of the spray by 15 days before harvest.

REFERENCES

1. **Babuc V., Peşteanu A., Gudumac E., Cumpanici A., 2013** - *Producerea merelor*. Ed. Bons Offices, Chişinău, 240 p.
2. **Cimpoieş Gh., 2012** - *Cultura mărului.* Ed. Bons Offices, Chişinău, 380 p.
3. **Marini R. P., Byers R .E., Sowers D. L., 1993** - *Repeated applications of NAA control preharvest drop of 'Delicious' apples.* J. Hort. Sci. Biotech. 68(2), pp. 247–253.
4. **Kvikliene N., Kviklys D., Sasnauskas A., 2010** - *Effect of plant growth regulators on apple fruit preharvest drop and quality*. J. Fruit Ornam. Plant Res. 18(2), pp. 79-84.
5. **Schupp J.R., Greene D.W., 2004** - *Effect of aminoethoxyvinylglycine (AVG) on preharvest drop, fruit quality, and maturation of 'McIntosh' apples. I. Concentration and timing of dilute applications of AVG.* HortScience 39, pp. 1030–1035.
6. **Yuan R., Carbaugh D., 2007** - *Effects of NAA, AVG, and 1-MCP on ethylene biosynthesis, preharvest fruit drop, fruit maturity, and quality of 'Golden Supreme' and'Golden Delicious' apples*. HortScience 42(1), pp. 101–105.
7. **Yuan R., Li J., 2008** - *Effect of sprayable 1-MCP, AVG, and NAA on ethylene biosynthesis, preharvest fruit drop, fruit maturity, and quality of 'Delicious'apples.* HortScience 43, pp. 1454–1460.

STUDY ON IMPROVING THE TRAINING TECHNOLOGY ON ARTISTIC CROWNS SHAPE AT FRUIT TREES AND HOW TO USE THEIR IN LANDSCAPING

STUDIU PRIVIND ÎMBUNĂTĂȚIREA TEHNOLOGIEI DE FORMARE A COROANELOR ARTISTICE LA POMII FRUCTIFERI SI FOLOSIREA LOR ÎN PEISAGISTICĂ

SILIVĂȘAN M.[1], BERAR C.[1], MERGHEŞ P.[1], BĂLA Maria[1]
e-mail: marius_silivasan@yahoo.com

Abstract: *The research was conducted over six years of USAMVB Didactic Timişoara. The experimental plot established in 2004 were studied with two apple varieties, Generos and Florina, each drove in five crown systems. The five crown systems studied were: vertical cordon, "U" simple "U" double, palmet chandelier with six arms and Belgian fence system. Based on the results it is observed that both the crown system and variety, respectively years had a significantly distinct real influence on trunk diameter apple on a background of trees experimental uniformity in the study. The research had several objectives: the influence of crown shape on the trunk diameter apple and the influence of crown shape on vigor index.*
Key words: *apple trees, landscape, pear trees, espaliered fruit trees*

Rezumat: *Cercetările s-au efectuat pe parcursul a şase ani la Staţiunea Didactică a U.S.A.M.V.B. Timişoara. În parcela experimentală înfiinţată în anul 2004 au fost luate în studiu cu 2 soiuri de măr, Generos şi Florina, fiecare fiind condus in 5 sisteme de coroana. Cele cinci sisteme de coroana studiate au fost: cordon vertical, "U"simplu, "U"dublu, palmeta candelabru cu 6 braţe şi sistemul gard Belgian. Pe baza datelor rezultate se observă că atât sistemul de coroană cât şi soiul, respectiv anii, au avut o influenţă reală distinct semnificativă asupra diametrului trunchiului la măr, pe fondul unei uniformităţi a condiţiilor experimentale pentru pomii cuprinşi în studiu. Principalele obiective urmărite au fost: influenţa formei de coroana asupra diametrului trunchiului la măr şi păr şi influenţa formei de coroana asupra indicelui de vigurozitate;*
Cuvinte cheie: *măr, amenajare peisageră, păr, forme artistice de pomi fructifer*

INTRODUCTION

In the experimental plot established in 2004 in Didactic of U.S.A.M.V.B. Timişoara was studied two varieties of apple, Generos, and Florina. The purpose of the research concern the improvement sequences from technology training, artistic crown fruit trees and the possibility of using them in green spaces.

Crown systems chosen for conducting the two varieties of fruit trees are Vertical Cordon, "U" simple, "U" double, Candelabra Palmette with 6 branches

[1] Banat University of Agricultural Sciences and Veterinary Medicine of Timişoara, Romania

and Belgian Fence system.

MATERIAL AND METHOD

To achieve the desired crown shapes, and a balance of vertically (between the arms of the stem spindle) and a horizontal balance (between branches belonging to the same storey), take 3-4 years, during which are executed 2 groups of technical operations:

- operations for modified the position of the branches and shoots growing (Manageable, bending, bending, twisting, etc.);
- Cutting operations.

1. Method of operation for directing pear varieties in crown system vertically Cordon (Baciu, 2005).

This system has a short trunk, the extension of which will form a ax with a length of about 2 m, on it will find inserted only semischelet branches and fruit, which will not exceed 20-40 cm in length.

2. Working method for directing pear varieties in crown system "U" simple

Planting distances are of 1 meter between trees in a row and 3 meters between rows, with a height of 2.5 meters the final shape. Beginning in the spring of February, extending of the 2 arms was shortened to 30-40 cm above the curve for a uniform garnish. In the the years to come, crown forming operations will follow the shortening to 30-40 cm arm extensions and during the growing season will continue directing it in a vertical direction, it will suppress the horizontal portion shoots (Drăgănescu and Mihuț, 2005).

3. Working method for directing pear varieties in crown system "U" double

When planting trees, were shortened to a dwarf tree, 40-50 cm above two buds, directed by one. Of those two buds emerged from the first year, each a root length of 50-60 cm, which were curved and were directed vertically. The distance between the two extensions of the arms is 80 cm.

4. Working method for directing pear varieties in crown system Candelabra Palmette with 6 branches (Iordănescu and Micu, 2011).

For training system palmetto wreath chandelier with six arms, rods were planted at least 3.5 meters and 3.5 meters between rows. The height, which was shortened the rod to form the trunk is 40-50 cm above the 2 row-oriented buds.

5. Working method for directing apple varieties Gard Belgian crown system

Shaped rods trees were planted at a distance of 80 cm in the row and 3.5 m between rows. Shortening yardarm was done immediately after planting at a high trunk 60-65 cm above 2 buds sit opposite and the row.

RESULTS AND DISCUSSION

A. Data concerning the influence of the crown on the growth of some varieties of apple trees

Considering the unilateral influence of the type of cut, were recorded during the study average trunk diameter, with values ranging from 21.57 mm vertical cordon, and 34.02 mm in Candelabra Palmette with 6 branches, with an amplitude of 12.45 mm, and medium variability (17.80%) between crown systems

Regarding the effects of crown system in Table 1, show that the system "U" simple trees of this species showed a significantly lower increase in diameter, to

the "U" double Candelabra Palmette with 6 branches, and Belgian Fence with relative deviations between 29-58%.

Table 1

The effect of crown system on pear foliar surface

Crown systems	Trunk diameter (mm)		Relative values (%)	Difference / significance
U simplu – Cordon vertical	23,56	21,57	109,23	1,99
U dublu – Cordon vertical	27,83	21,57	129,02	6,26*
Palmeta candelab. 6 br. – Cordon vertical	34,02	21,57	157,72	12,45***
Gard belgian – Cordon vertical	28,14	21,57	130,46	6,57*
U dublu – U simplu	27,83	23,56	118,12	4,27
Palmeta candelab. 6 br. – U simplu	34,02	23,56	144,40	10,46***
Gard belgian – U simplu	28,14	23,56	119,44	4,58
Palmeta candelab. 6 br. – U dublu	34,02	27,83	122,24	6,19*
Gard belgian – U dublu	28,14	27,83	101,11	0,31
Gard belgian – Palmeta candelab. 6 br.	28,14	34,02	82,72	$-5,88^{0}$

$LSD_{5\%}$=4,70 mm $LSD_{1\%}$=6,84 mm $LSD_{0,1\%}$=10,27 mm

In the case of Generous variety (tab. 2), the use of different types of cuttinghave allowed obtaining of diameter limits between 21.66 mm vertical cordon, and 39.34 mm in palmette candelabra with six arms, as a variability higher than 60%.

Vertical Cordon system, trees of this variety showed a significantly lower increase in diameter of 17-82% compared to other systems.

Also, under the influence of the cutting in the system "U" simple trunk diameter was significantly lower compared to other systems from 19 to 56%, with the exception of "U" simple.

Table 2

Effect of crown system on trunk diameter of apple tree varieties

Crown systems x Generos	Trunk diameter (mm)		Relative values (%)	Difference / significance
U simplu – Cordon vertical	25,28	21,66	116,71	3,62*
U dublu – Cordon vertical	30,13	21,66	139,10	8,47***
Palmeta candelab. 6 br. – Cordon vertical	39,34	21,66	181,63	17,68***
Gard belgian – Cordon vertical	31,01	21,66	143,17	9,35***
U dublu – U simplu	30,13	25,28	119,19	4,85**
Palmeta candelab. 6 br. – U simplu	39,34	25,28	155,62	14,06***

Gard belgian – U simplu	31,01	25,28	122,67	5,73**
Palmeta candelab. 6 br. – U dublu	39,34	30,13	130,57	9,21***
Gard belgian – U dublu	31,01	30,13	102,92	0,88
Gard belgian – Palmeta candelab. 6 br.	31,01	39,34	78,83	-8,33^{000}
Crown systems x Florina	**Trunk diameter (mm)**		**Relative values (%)**	**Difference / significance**
U simplu – Cordon vertical	21,84	21,48	101,68	0,36
U dublu – Cordon vertical	25,53	21,48	118,85	4,05*
Palmeta candelab. 6 br. – Cordon vertical	28,69	21,48	133,57	7,21***
Gard belgian – Cordon vertical	25,27	21,48	117,64	3,79*
U dublu – U simplu	25,53	21,84	116,90	3,69*
Palmeta candelab. 6 br. – U simplu	28,69	21,84	131,36	6,85***
Gard belgian – U simplu	25,27	21,84	115,71	3,43*
Palmeta candelab. 6 br. – U dublu	28,69	25,53	112,38	3,16
Gard belgian – U dublu	25,27	25,53	98,98	-0,26
Gard belgian – Palmeta candelab. 6 br.	25,27	28,69	88,08	-3,42^{0}

$LSD_{5\%}$=3,28 mm $LSD_{1\%}$=4,66 mm $LSD_{0,1\%}$=6,74 mm

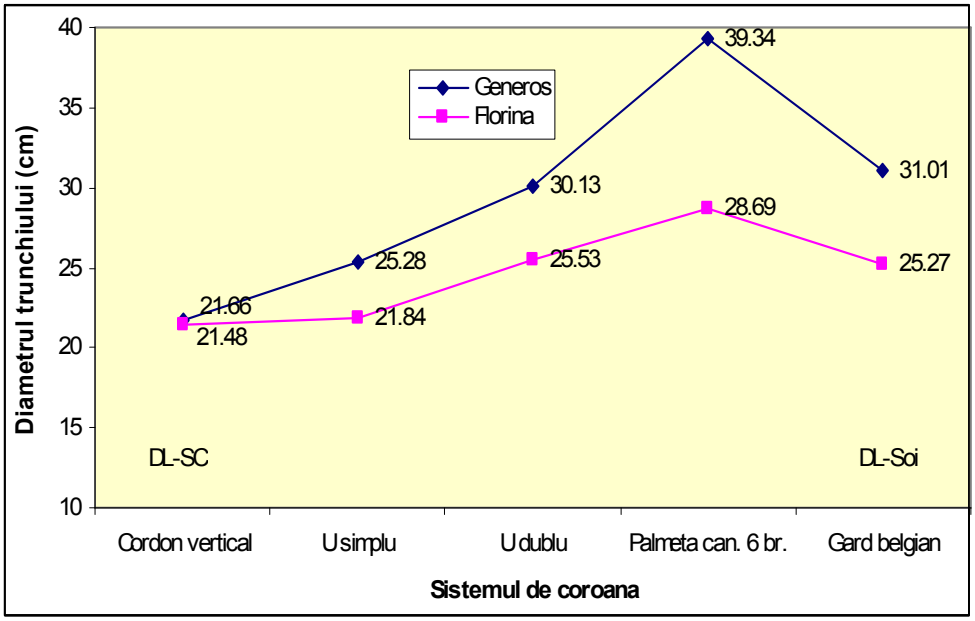


Fig. 1 - The trunk diameter for the varieties of apple under the influence of different systems of crown

B. Data concerning the influence of the crown on the vigor index of some varieties of apple trees

Regarding the effects of crown system, in Table 3, show that systems Cordon vertical crown and "U" simple recorded values were lower than the other forms.

There were values that ranged between 21.42 cm vertical Cordon system,, and 26.00 cm to the "U" simple, with the highest values in the system palmette candelabra with six branches of 62.13 cm (Ciulcă, 2002).

Table 3

The effect of crown system on apple vigor index (cm)

Crown systems	Vigor index		Relative values (%)	Difference / significance
U simplu – Cordon vertical	26,00	21,42	121,38	4,58
U dublu – Cordon vertical	35,96	21,42	167,88	14,54*
Palmeta candelab. 6 br. – Cordon vertical	62,13	21,42	290,06	40,71***
Gard belgian – Cordon vertical	36,22	21,42	169,09	14,80*
U dublu – U simplu	35,96	26,00	138,31	9,96
Palmeta candelab. 6 br. – U simplu	62,13	26,00	238,96	36,13***
Gard belgian – U simplu	36,22	26,00	139,31	10,22
Palmeta candelab. 6 br. – U dublu	62,13	35,96	172,78	26,17***
Gard belgian – U dublu	36,22	35,96	100,72	0,26
Gard belgian – Palmeta candelab. 6 br.	36,22	62,13	58,30	-25,91^{000}

$LSD_{5\%}$=11,36 $LSD_{1\%}$=16,53 $LSD_{0,1\%}$=24,83

Regarding the effects of crown system, on vigor index from variety Generos, it appears that the crown forms of a "U" simple and vertical Cordon (Table 4) recorded the lowest values of 22.18 cm and 23.23 cm, and the highest values recorded it the systems "U" double, fence Belgian and palmette candelabra with six branches, with the values of 29,97cm, 28,82cm and 44,08cm.

Table 4

Effect of crown system, on the vigor index of apple varieties studied (cm)

Crown systems x Generos	Vigor index		Relative values (%)	Difference / significance
U simplu – Cordon vertical	22,18	23,23	95,48	-1,05
U dublu – Cordon vertical	29,97	23,23	129,01	6,74
Palmeta candelab. 6 br. – Cordon vertical	44,08	23,23	189,75	20,85**
Gard belgian – Cordon vertical	28,82	23,23	124,06	5,59
U dublu – U simplu	29,97	22,18	135,12	7,79
Palmeta candelab. 6 br. – U simplu	44,08	22,18	198,74	21,90**

Gard belgian – U simplu	28,82	22,18	129,94	6,64
Palmeta candelab. 6 br. – U dublu	44,08	29,97	147,08	14,11*
Gard belgian – U dublu	28,82	29,97	96,16	-1,15
Gard belgian – Palmeta candelab. 6 br.	28,82	44,08	65,38	-15,26^{0}
Crown systems x Florina	**Vigor index**		**Relative values (%)**	**Difference / significance**
U simplu – Cordon vertical	29,82	19,61	152,07	10,21
U dublu – Cordon vertical	41,94	19,61	213,87	22,33**
Palmeta candelab. 6 br. – Cordon vertical	80,18	19,61	408,87	60,57***
Gard belgian – Cordon vertical	43,63	19,61	222,49	24,02***
U dublu – U simplu	41,94	29,82	140,64	12,12
Palmeta candelab. 6 br. – U simplu	80,18	29,82	268,88	50,36***
Gard belgian – U simplu	43,63	29,82	146,31	13,81*
Palmeta candelab. 6 br. – U dublu	80,18	41,94	191,18	38,24***
Gard belgian – U dublu	43,63	41,94	104,03	1,69
Gard belgian – Palmeta candelab. 6 br.	43,63	80,18	54,42	-36,55^{000}

$LSD_{5\%}$=12,19 $LSD_{1\%}$=17,02 $LSD_{0,1\%}$=23,91

Florina variety lowest vigor index was recorded in the form of a crown, vertical Cordon of 19.61 cm, whit average values from the form of "U" simple, 29,82cm, and the highest values were recorded from the systems Belgian fence 43,63cm "U" double 41,94cm and from palmette candelabra with six branches of 80,18cm.

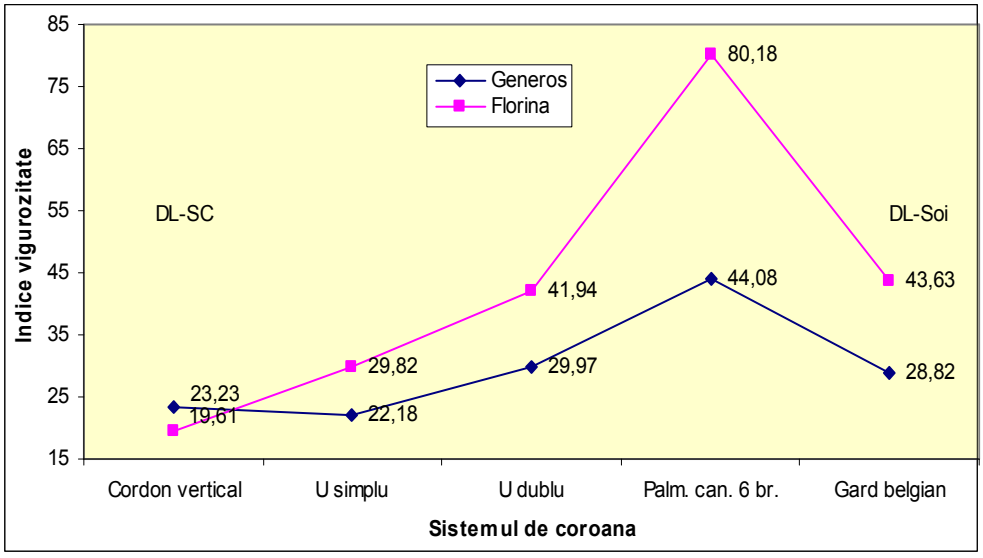


Fig. 2 - Index vigor for the varieties of apple under the influence of different systems of crown

CONCLUSIONS

When referring to the effect of crown system, on the diameter of the trunk at each variety is noted that the highest amplitude of variation (17.68 mm) was registered at the variety generous in while the variety Florina the amplitude was significantly reduced (7.21 mm).

The influence of crown, on the diameter of the trunk leads to the following conclusion: the variety Generous using different types of cutting have led to the limits within 21.66 mm diameters with vertical cordon, and 39.34 mm palmette candelabra with six branches.

Also, under the influence of cutting in the "U" simply the diameter of the trunk, was significantly lower with 19.56% as compared to the other systems, other than the vertical Cordon

Cuts carried out to the system palmette candelabra with six branches have had the highest influence, contributing this way significantly an increase in trunk diameter crown superior to other systems while Belgian fence and "U" double were not significantly differentiated regarding their effect on the character to the Generos variety trees.

The trees of variety Florina, under the effect of different types of cuttings, the trunk diameter, showed values between 21.48 mm on system vertical cordon, and 28.69 mm palmette candelabra with six branches, and the variability of approximately 53%.

Regarding the effects of crown system results that the crown systems vertical Cordon, and "U" simple recorded values were lower than the other forms. There were values that ranged from vertical Cordon to the system 21,42cm and 26.00 cm at the "U" simple, with the highest values at the system palmetto chandelier with six branches of 62.13 cm.

Generos variety lowest values were recorded at Cordon vertical crown system, and "U" simply by 23.23 cm respectively 22.18 cm and the highest values were recorded to the crown system "U" double, Belgian fence and palmetto chandelier with six branches exactly 29.97 cm, 28.82 cm and 44.08 cm.

Florina variety lowest values were recorded on vertical Cordon and "U" simple, 19.61 cm and 29.82 cm and the higher vigor index was recorded in systems 'U' double Belgian fence and palmetto chandelier with six branches 41.94 cm, 43.63 cm and 80.18 cm.

For the variety Generos the vigor index increased progressively from 4.19 cm in 2004 to 56.87 cm in 2009.

For the variety, Florina, the vigor index increased from 5.81 cm in 2004 to 82.88 cm in 2009. For the variety, Florina vigor index increased progressively from 5.81 cm in 2004 to 82.88 cm in 2009. For variety generous the vigor index increased from 4.19 cm in 2004 to 56 , 87 cm in 2009.

REFERENCES

1. Baciu A., 2005 - *Pomicultură generală*, Editura Universitaria, Craiova.
2. **Ciulcă S., 2002** – *Tehnică experimentală*, Ed. Mirton, Timişoara;
3. Drăgănescu E., Mihuț E., 2005 – *Cultura speciilor pomicole*, Colecția revistei Ferma, Ed. Waldpress, Timişoara;
4. Iordănescu O.A., Micu R. E., 2011 – *Pomicultură generală și specială*, Editura Eurobit, Timişoara.

STUDY OF PHYSIOLOGICAL INDICES ON THE NEW VARIETIES OF VINE GRAPES GROWN IN THE WINE-GROWING CENTER COPOU IAȘI

STUDIUL UNOR INDICI FIZIOLOGICI LA SOIURILE NOI DE VIȚĂ DE VIE PENTRU STRUGURI DE MASĂ CULTIVATE ÎN CENTRUL VITICOL COPOU IAȘI

ALEXANDRU L.C.[1], ROTARU Liliana[1], DAMIAN Doina[2], ZAMFIRACHE Maria Magdalena[3], OLTEANU Zenovia[3], NECHITA Ancuța[2]
e-mail: lulu75cata@yahoo.com

Abstract. *In the process of photosynthesis an important role have chlorophyll assimilators pigments a and b, which comprise two photosynthetic systems involved in photosynthesis. The purpose of this study was the quantitative determination of chlorophyll and carotenoids pigments in leaves of new table grape varieties Paula and Gelu compared to control variety "Aromat de Iaşi", all our creations. The results obtained after processing data using formulas proposed by Lichtenhaler (1987), showed a higher chlorophyll content on variety Paula (4.057 mg/g), followed by Gelu (3.057 mg/g) both higher than Iaşi (2.441 mg/g). The same trend was recorded for chlorophyll b and carotenoids -ratio 3 /1 or 4/1. The content of photosynthetic pigments (mg/g) as compared to the reference values was influenced primarily by the grape variety: the anatomical structure and morphology of the leaves.*
Key words: *carotenoids, chlorophyll, new varieties*

Rezumat. *În procesul de fotosinteză un rol important revine pigmenților asimilatori clorofilieni (a și b) care intră în alcătuirea celor două sisteme fotosintetice care participă la procesul de fotosinteză. Scopul acestui studiu l-a constituit determinarea cantitativă a pigmenților clorofilieni și carotenoizi, din frunzele soiurilor noi pentru struguri de masă Paula și Gelu, comparativ cu soiul martor Aromat de Iași, toate fiind creații proprii. Rezultatele obținute după prelucrarea și calcularea datelor utilizând formulele propuse de Lichtenhaler (1987) au arătat un conținut în clorofilă mai mare la soiul Paula (4,057 mg/g), urmat de soiul Gelu (3,057 mg/g), superioare soiului de comparație Aromat de Iași (2,441 mg/g). Aceiași tendință s-a înregistrat în cazul clorofilei b și a carotenoizilor păstrându-se raportul de 3/1, respectiv 4/1. Conținutul în pigmenți fotosintetici (mg/g) față de valorile de referință a fost influențat în primul rând de factorul soi și anume de structura anatomică și morfologică a frunzei.*
Cuvinte cheie: *compuși carotenoizi, clorofila, soiuri noi*

INTRODUCTION

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
[2] Reasearch and Development Station for Viticulture and Winemaking Iasi, Romania
[3] "Al. I. Cuza" University of Iasi, Romania

During the growing season the vines carryout a number of physiological processes (fluid and mineral absorbtion, photosynthesis, respiration, transpiration) vital to achieve growth and development. They are closely related to a number of factors, such as: genetic (variety), ecological (light, temperature, air humidity and soil) as well as the agrotechnical measures.

An important role in the development of the plant physiological processes lies to the leaf by focusing all of these factors and affecting their activity, ultimately conditioning the phenomen of development, production and quality (Stoev, 1979). The leafis the only organ of the vine that has ability to synthesize organic substances from inorganic under the influence of solar energy. In the leaves there are important changes regarding dry matter and water content, especially quantitative changes in the assimilatorschlorophyll and carotenoid pigments involved in photosynthesis.

Research conducted to date on the chlorophyll pigments into the vine leaf shows that Vitis vinifera varieties, reach a maximum at Incept vegetation period when the ratios chlorophyll a/chlorophyll b is 3/1, decreasing during ripening grapes and the ratio chlorophyll/carotenoids can record values 1.4 (Toma and Jităreanu, 2007). Generally for the vines total amount of chlorophyll and carotenoid pigments may have values between 1.05 and 1.58 mg/g leaf, and from 0.33 to 0.65 mg/g leaf (Burzo et al. 2005; Acatrinei and Andor, 2006).

These values are mainly influenced by the climatic factors mostly atmospheric and soil moisture, temperature and light. Thus, the results obtained in controlled environmental conditions Cabernet Sauvignon grafted on five rootstocks showed a significant reduction in the content of chlorophyll pigments in leaves under water stress, both pedological and atmospheric, and from 11.66 mg chlorophyll/1 g d.s. at 7.56 mg/1 g d.s. Chlorophyll content lower deficit is caused not only fluid but also the effect of high temperatures producing a general disturbance of the physical condition of the colloids in the leaves, where the water contained in the leaves becomes physiologically inactive in the formation of chlorophyll pigments (Şerdinescu et al, 1994). The favorable climatic conditions allow the formation of assimilating pigments (chlorophylls and carotenoids), differences arise between the amount of chlorophyll or carotenoids and is due to genetic factors or variety, the morphological and anatomical leaf (Stoev, 1979).

MATERIAL AND METHOD

The biological material for research has been the new varieties of Paula and Gelu table grapes, created in the Station of Research and Development for Winegrowing and Wine Production of Iaşi, which are compared to another new variety – Aromat de Iaşi used as control.

Thevarieties mentioned here are the research plantations - being grown on stems 70 cm tall, the governance bilateral cordon system, short cut, ensuring 35-45 buds / vine. The applied technology is recommended by agrotechnics viticulture, all varieties benefiting from the same ecopedoclimatic conditions.

The research was conducted in 2014, before flowering phenophase in terms of average daily temperatures of 22.7 to 25.1°C and air humidity of 51-56%. To conduct

experiments were harvested leaves from the middle third of the shoot (internodes 5-8) in the same time for all the varieties analyzed.

For the quantitative determination of the assimilating pigments in leaves varieties studied using the method of solvent extraction. From the leaves were harvested weighed 0.5 g plant material which has been milled in the presence of $CaCO_3$ and quartz sand. Quartz sand has the ability to prevent distortion by transforming them into Pheophytin chlorophylls. The milled was washed with 25 ml of acetone 80% by half, to the bleaching plant material. The extract obtained was filtered with suction, and the filtrate was passed quantitatively a 25 ml volumetric flask. The extract was spectro photocolorimetere done a Shimadzu UV-VIS spectro photocolorimeter. Reading was done at three wavelengths: 663 mm, 646 mm and 470 mm, compared to 80% acetone. The values obtained were introduced into the corresponding equations that calculate assimilating pigments developed by Lichtenhaler (1987):

chlorophyll a = (11,24 x A663) - (2,04 x A646);

chlorophyll b = (20,13 x A646) - (4,19 x 663);

carotenoids = (1000 x A470) - (1,90 x clorofila a) - (63,14 x clorofila b / 214)

In order to obtain conclusive results for each variety were made 10 determinations that allowed statistical and mathematical interpretation of experimental data. The results were expressed in mg / g leaf pigment to three decimal places.

RESULTS AND DISCUSSIONS

After reading the spectrophotometer and after the calculation performed by using the equations above were foundlarge highlighted differences both between the two varieties studied and the control variety. The Paula leaf variety with large, trisectat tongue with thick mesophyll and epidermis was recorded the highest quantitative values both chlorophyll a and b and the carotenoids.

The data demonstrates the ability of this kind to accumulate a larger amount of assimilating pigments than the other species studied (Table 1). The leaves analyzed were recorded 4,056 mg / g plant material chlorophyll a, 1.273 mg / g chlorophyll b and 2.051 mg / g carotenoids, almost double to the values of control - Aromat de Iaşi.

Table 1

The content in assimilating pigments

Variety	Chlorophyll a			Chlorophyll b			Carotenoids		
	mg/1 g leaf	Diff.	Means.	mg/1 g leaf	Diff.	Means	mg/1 g leaf	Diff.	Means.
Paula	4,056	+1,612	***	1,273	+0,448	**	2,051	+0,703	**
Gelu	3,058	+0,614	***	0,934	+0,109		1,610	+0,262	
Aromat de Iaşi	2,444	-	-	0,825	-	-	1,348	-	-
LSD 5%	0,030			0,150			0,372		
LSD 1%	0,050			0,248			0,616		
LSD 0,1%	0,094			0,464			1,153		

Differences from the control were significant for chlorophyll a and chlorophyll b significant distinct and carotenoids, keeping the ratio of 1.3 for chlorophyll and b but not in the case report of chlorophylls and carotenoids 4.1

indicated Burzo et al., 2005 in terms of the total content of assimilatory pigments (chlorophyll a and b + carotenoids) found that all sorts Paula synthesized largest assimilating pigments content in leaves 7.380 mg / g plant material and the offset to the variety of compared Aromat de Iaşi (4.617 mg / g leaf) is significant, and hence this variety has high capacity to synthesize chlorophyll pigments organic substances necessary for growth, development and fructification (table 2).

Table 2

The total content of assimilatory pigments and the relations between them(mg/g)

Variety	chlorophyll a / Chlorophyll b	Chlorophylls (a + b) / carotenoids	Total pigments (mg/1g leaf)	Diff.	Means
Paula	3,186	2,598	7,380	+2,763	***
Gelu	3,274	2,544	5,602	0,985	*
Aromat de Iaşi	2,962	2,425	4,617	-	-
LSD 5%			0,739		
LSD 1%			1,223		
LSD 0,1%			2,229		

According to the specialty literature it is considered that chlorophyll pigments in the leaves of the vine are the only components that have the ability to create organic matter from the inorganic. Regarding Gelu variety, in the same climate and soil conditions, and the same applied agricultural technology, the amount of assimilating pigments in leaves was lower than the previous variety, but higher than the control - Aromat de Iaşi. This difference is attributed to the size of the leaf and its morphological structure (lower leaf mesophyll and epidermis with thick middle).

The amount of synthesized chlorophyll pigments in the leaves of the Gelu variety was 3.058 mg / g leaf chlorophyll a, 0.934 mg / g leaf chlorophyll b and 1.610 mg / g carotenoid pigments. The differences from the control are evident, but only in chlorophyll a case, they are assured of statistical and mathematical point of view as significant.

Report of the two clorolofiles had a value of 3,274 and 2,574 between chlorophylls and carotenoids, lower than that found in the literature, probably due to situation higher carotenoid pigments content. Determined total assimilating pigments content in Gelu leaves variety had a value of 5,602 provided statistically significant.

A study on the variety Gelu on its ability to assimilate photosynthetic pigments in the presence of stimulators show their positive influence on the growth of chlorophyll content (from 1,36mg / g to 1.65 mg / g) and carotenoids (from 0,43mg / g to 050 mg / g) but not chlorophyll b thatpositively reacted only on an average concentrations of stimulators (Cotovanu, 2014).

Regarding on the Aromat de Iaşi variety wich is also SCDVV Iaşi creation, the content noticeably decreased in chlorophyll and carotenoid pigments can be explained by anatomical and morphological structure of the leaves that are smaller, sections, with smooth and thin mesophilic.

An overall analysis of the results obtained on some physiological indices on varieties studied , points out that they have the ability to synthesize large amounts of chlorophyll and carotenoid pigments (beyond the limits found in the literature) and the ability to intensify work accumulation of photosynthetic and biological processes and metabolic equivalents needed during the growing season.

For that the degree of hydration of leaf assimilating pigments analyzed,the research has been complemented by measurements on the amount of water contained in them (Table 3).

Table 3

The dry substance and the free water content in the leaves of the varieties studied

Variety	Free Water %	Dry substance %
Paula	77,35	22,07
Gelu	80,45	19,55
Aromat de Iaşi	79,35	20,65

The results obtained after drying at 105 ° C and repeatedweighings revealed a free water content between 77.93% (Paula) and 80.45% (Gelu). These values indicate a degree of leaf hydration favorable to assimilation of photosynthetic pigments wich in the literature is stated as being between 80-82%.

CONCLUSIONS

1. The studied biological material has been the new varieties of Paula and Gelu table grapes, created in the Station of Research and Development for Winegrowing and Wine Production of Iaşi, which are compared to another new variety – Aromat de Iaşi.

2 .The method used for quantitative evidence of chlorophyll and carotenoid pigments was the extracting solvent and the measurements were performed spectrophotometrically (Shimadzu UV-VIS) with readings at wavelengths of 663 nm, 646 nm and 470 nm.

3. The results obtained by calculating the experimental data revealed Paula variety that has higher potential for accumulation of chlorophyll pigments (chlorophyll a 4,056 mg/1 g leaf, chlorophyll b 1.273 mg/1 g leaf and carotenoids 2,051 mg/1 g leaf) values significant and distinctly significant in terms of statistical and mathematical.

4. The chlorophyll ratio a and b was within the limit of 1.3 in both varieties while the ratio between chlorophylls and carotenoids was less than 1.4, and this is due to the higher content of carotenoid pigments.

5.O n both varieties studied,the anatomical and morphological leaf is capable of assimilating pigments as required to support an intense photosynthesis and thus the biochemical and metabolic processes that promote growth, development and fruition.

Acknowledgments. This paper was published under the frame of the European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.

REFERENCES

1. **Acatrinei Angela, Andor I., 2006** - *Research physiological varieties of vines in vineyard Cotnari under the effect of pesticide treatments*. Scientific Works of the University of Agricultural Sciences and Veterinary Medicine, Horticulture Series;
2. **Burzo I., Dejeu L., Şerdinescu A., Bădulescu Liliana, 2005** - *Crop physiology*, vol. III. *Physiology vine*. Elisavanos Publishing, Bucharest, pp. 80-127;
3. **Cotovanu Filimon Roxana, 2014** - *Research on the influence of harsh treatments from biostimulating substances on production and quality for some varieties of table grapes grown in Iasi Vineyard conditions*. PhD Thesis, USAMV Iasi;
4. **Stoev K., 1979** - Physiology of grapevine, Ceres Publishing House, Bucharest, pp. 9-49 and 188-212;
5. **Şerdinescu A., Gavrilescu Elena, Simu N., 1994** - *The effect of atmospheric and soil drought on key physiological processes vines.* Annals I.C.D.V.V. Călugărească Valley, vol. XIV, Bucharest, pp.177 - 196.

STUDY CONCERNING THE INVOLVEMENT OF GUAIACOL PEROXIDASE – PHENOLIC COMPOUNDS RELATIONSHIP ON ASSIMILATORY PIGMENT DEGRADATION IN *VITIS VINIFERA* L. LEAVES

STUDIU PRIVIND IMPLICAREA RELAȚIEI GUAIACOL PEROXIDAZA-COMPUŞI FENOLICI ÎN DEGRADAREA PIGMENȚILOR ASIMILATORI DIN FRUNZELE *VITIS VINIFERA* L.

FILIMON V.R.[1], ROTARU Liliana[1], PATRAŞ Antoanela[1], FILIMON Roxana[1]
e-mail: razvan_f80@yahoo.com

Abstract. *Previous research highlighted that chlorophyll and some carotenoids are bleached by the action of oxidative enzymes such as peroxidases in the presence of phenolic compounds. In the present investigation, leaves of 11 Vitis vinifera L. varieties (in blooming phenophase), were assayed for their chlorophyll (a+b) and carotenoid (x+c) concentration and their degradation after 30 days of cold (6°C) and dark storage. Peroxidase activity (EC 1.11.1.7) and total phenolic content (TP) of leaves were initially determined. We found an important linear correlation between peroxidase activity (PA) and the percentage of total chlorophyll (Chl) degraded (R^2=0.9243; p<0.05), and also a positive statistically significant relationship between PA and TP concentration (R^2=0.8389; p<0.05). Analyzing by fractions, the most important coefficient of determination was registered for the relationship PA – % of Chl a degraded (R^2=0.8389; p<0.05), with a poor correlation for PA – % of Chl b degraded and PA – % of carotenoid degraded relationships. Experimental data obtained indicates that peroxidase might be involved in chlorophyll bleaching in the presence of phenolic compounds, and might mediate in a lesser extent leaf carotenoids degradation.*
Key words: *peroxidase, chlorophyll, carotenoids, phenolic compounds, Vitis vinifera L. leaves*

Rezumat. *Cercetări anterioare au subliniat faptul că degradarea clorofilei şi a carotenoizilor este mediată de acțiunea unor enzime oxidative de tipul peroxidazei, în prezența compuşilor fenolici. În prezentul studiu, frunzele a 11 soiuri Vitis vinifera L. (în fenofaza de înflorire) au fost analizate în ceea ce priveşte concentrația de clorofilă (a+b) şi carotenoizi (x+c) şi degradarea acestor compuşi după 30 de zile de păstrare la rece (6°C) şi la întuneric. Activitatea peroxidazei (CE 1.11.1.7) şi conținutul de compuşi fenolici (CF) al frunzelor au fost de asemenea determinate. Astfel, a fost identificată o corelație liniară importantă între activitatea peroxidazei (AP) şi procentul de clorofilă (Cl.) degradat (R^2=0,9243; p<0,05) şi o relație pozitivă statistic semnificativă între AP şi concentrația de CF (R^2=0,8389; p<0,05). Analizând individual fracțiunile rezultate, cel mai important coeficient de determinare a fost înregistrat în cazul relației AP – % de Cl. a degradat (R^2=0,8389; p<0,05), cu o valoare mai redusă a acestuia în cazul relațiilor AP – % de Cl. b degradat şi AP – % de carotenoizi degradat. Datele experimentale obținute indică faptul că peroxidaza poate fi implicată în degradarea clorofilei în prezența*

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

compuşilor fenolici şi poate media într-o măsură redusă degradarea carotenoizilor din frunzele soiurilor V. vinifera L.
Cuvinte cheie: *peroxidaza, clorofilă, carotenoizi, compuşi fenolici, frunze Vitis vinifera L.*

INTRODUCTION

Guaiacol (o-methoxyphenol) peroxidase (E.C. 1.11.1.7) is widely distributed in plants (chloroplasts, vacuoles, and cell walls) where they catalyze the reduction of hydrogen peroxide (H_2O_2) to water, rendering it harmless (Yamasaki et al., 1997; Bania and Mahanta, 2012), and that have been thought to be involved in the biodegradation pathway(s) of chlorophylls during leaf senescence (Matile and Hortensteiner, 1999; Yamauchi et al., 2004).

In the earlier literature was shown that chlorophylls and its derivatives are oxidized *in vitro* by the peroxidase-H_2O_2 system in the presence of a kind of phenolic compound (Kato and Shimizu, 1985), flavonols being able to act as electron donors for peroxidase (Miller and Schreier, 1985). Was also suggested that the phenolic compounds involved in chlorophyll degradation could be monophenols with a hydroxyl group at the *p*-position (Whitaker, 1994).

More recently, several studies have concluded that peroxidases can catalyze the type II degradation of chlorophylls („bleaching" or „photobleaching"), as an alternative route for chlorophyll biodegradation (Hynninen et al., 2010). According to Yamauchi et al. (2004), peroxidase oxidizes the phenolic compounds (which have the hydroxyl group at the *p*-position), to form the phenoxy radical and superoxide anion, that attacks chlorophyll *a*, which is degraded to colorless low molecular weight compounds. Data on the degradation of carotenoids by peroxidases are quite poor. Partial degradation of carotene by peroxidase was reported (Gelinas et al., 1998). Also, Matile and Martinoia (1982), highlighted that commercial peroxidase catalizes the oxidation of lutein to unknown colorless products.

However, the mechanism of chlorophyll and carotenoid degradation with the oxidation of the phenolic compounds is still unclear. Given the importance of peroxidases (in biological processes) and of chlorophyll and carotenoids, both as photosynthetic pigments and nutraceuticals, relationships that occur in their degradation must be known and understood.

MATERIAL AND METHOD

The research has been carried out on the leaves of 11 *Vitis vinifera* L. indigenous varieties (Gelu, Milcov, Cetățuia, Napoca, Someşan, Splendid, Transilvania, Coarnă neagră, Coarnă neagră selecționată, Purpuriu and Radames), growing in the Ampelographic Collection of the University of Agricultural Sciences and Veterinary Medicine Iasi, Romania. Leaves were harvested at vine flowering (on the 3th day after the beginning of flowering), on ice, between the nodes 1 and 5 of vine shoots (Zapatta et al., 1995), rapidly frozen (10 min) and analyzed in same day.

Peroxidase (guaiacol units) assay procedure was based on that of Bergmeyer (1974), in which the rate of decomposition of hydrogen peroxide by peroxidase, with guaiacol as hydrogen donor, is determined by measuring the rate of colour development spectrophotometrically at 436 nm and at 25°C (UV-vis Spectrostar Nano microplates spectrophotometer). A peroxidase unit (U) represents the amount of enzyme which catalyses the conversion of one micromole of H_2O_2 per minute at 25°C.

For assimilatory pigment extraction, frozen leaf samples (0.5 g) were grinded and washed with 10 mL of 99,98% v:v acetone in order to extract the compounds from the leaf tissue. The extract was placed in the refrigerator overnight to minimize phototransformation of chlorophyll and to complete extraction, and subsequently centrifuged (refrigerated laboratory centrifuge Nahita 2816) 15 min, 3000 rpm (10 °C). The analytical determination was conducted using a UV-vis Shimadzu 1700 Pharmaspec Spectrophotometer at the following wavelengths: 662 and 645 nm, for chlorophyll a and b and 470 nm for carotenoids (xanthophylls and carotenes). Photosynthetic pigment content was calculated in mg/g fresh weight (f.w.), using the equations proposed by Carnegie Institution for Science through Spectranomics Protocol in 2011. After 30 days of cold (6°C) and dark storage, extracts were reevaluated regarding the chlorophyll and carotenoid concentration.

Total phenolic content was determined by Folin-Ciocâlteu method, measuring the absorbance at 750 nm (Singleton and Rossi, 1965). A calibration curve using different concentrations of gallic acid solutions was used for expressing the results as gallic acid equivalent (GAE), with the equation y=0.8757x+0.0438 (R^2=0.991).

A one-way Analysis of variance (ANOVA) test was initiated to investigate significant differences between data. The method used to discriminate among the means was Fischer's least significant difference procedure at 95% confidence level. Simple regression analysis was performed to look for relationships between data.

RESULTS AND DISCUSSION

Moisture content and total dry matter of leaf samples (4 h at 105°C) at harvest was specific to the flowering phenophase, and varying from 71.99% to 77.48% (with a mean of 75.02%), and from 22.52% to 26.43% (with a mean of 24.98%), respectively, being in accordance to the data presented for *V. vinifera* L. varieties by Mustea (2004). Total mineral content of leaves, represented by ash (4 h at 510 °C), was within the range of 1.43% – 2.32% (with a mean of 1.97%), in accordance with data presented by Burzo et al. (2005).

Chlorophyll (*a* and *b*) and carotenoid content of Romanian grapevine leaves, initially and after 30 days of cold storage, are shown in table 1.

Table 1

Chlorophyll and carotenoid concentration (mg/g) in *V. vinifera* L. leaf extract, Initially (In.) and after the storage period (A.)

Variety	Carotenoids		Chlorophyll a		Chlorophyll b		Total Chl	
	In.	A.	In.	A.	In.	A.	In.	A.
Purpuriu	0.34^{000}	0.31^{000}	0.69^{000}	0.54^{000}	0.31^{000}	0.27^{000}	1.00^{000}	0.80^{000}
Splendid	0.40^{NS}	0.37^{NS}	0.75^{NS}	0.59^{NS}	0.35^{000}	0.29^{000}	1.10^{000}	0.88^{000}
Coarnă neagră	0.41^{NS}	0.37^{NS}	0.80^{NS}	0.62^{NS}	0.50^{NS}	0.41^{NS}	1.30^{NS}	1.03^{NS}
Coarnă neagră selecționată	0.42^{NS}	0.39^{NS}	0.80^{NS}	0.62^{NS}	0.46^{NS}	0.38^{NS}	1.26^{NS}	1.00^{NS}
Cetățuia	0.42^{NS}	0.38^{NS}	0.72^{000}	0.56^{NS}	0.53^{NS}	0.42^{NS}	1.25^{NS}	0.98^{NS}
Someşan	0.42^{NS}	0.39^{NS}	0.78^{NS}	0.62^{NS}	0.47^{NS}	0.40^{NS}	1.26^{NS}	1.00^{NS}
Gelu	0.43^{NS}	0.40^{NS}	0.86^{***}	0.67^{***}	0.50^{NS}	0.43^{NS}	1.36^{***}	1.09^{NS}
Milcov	0.43^{NS}	0.40^{NS}	0.79^{NS}	0.63^{NS}	0.73^{***}	0.64^{***}	1.52^{***}	1.24^{***}
Radames	0.43^{NS}	0.41^{NS}	0.78^{NS}	0.62^{NS}	0.45^{NS}	0.38^{NS}	1.22^{000}	0.99^{NS}
Transilvania	0.44^{NS}	0.41^{NS}	0.80^{NS}	0.63^{NS}	0.46^{NS}	0.38^{NS}	1.26^{NS}	1.01^{NS}
Napoca	0.45^{***}	0.41^{NS}	0.76^{NS}	0.60^{NS}	0.59^{***}	0.48^{***}	1.35^{***}	1.08^{NS}
Mean	0.42	0.39	0.78	0.61	0.49	0.41	1.26	1.01
St. error	0.03	0.03	0.04	0.04	0.11	0.10	0.14	0.11

Note: Data expressed as mean values (n = 3). $^{NS, *, **, ***}$ - indicate nonsignificant and positive significant at $p \leq 0.05, 0.01, 0.001$, respectively; $^{o, oo, ooo}$ - negative significant at $p \leq 0.05, 0.01, 0.001$, respectively.

Statistical analysis of the data revealed a high positive significance ($p<0.001$), compared to the mean, at the variety Gelu (chlorophyll *a*), Milcov and Napoca (chlorophyll *b*). Total chlorophyll (Chl) content ranged initially from 1.00 mg/g f.w. to 1.52 mg/g f.w. (with a mean of 1.26 mg/g f.w.), and decreasing to the range of 0.80 mg/g f.w. – 1.24 mg/g f.w. after 30 days of extract storage. Both in the case of chlorophylls and carotenoids a high initial content resulted in a lower degradation of these compounds, as was mentioned previously by Wilows (2004).

Percentage of carotenoids and chlorophylls in grapevine leaf extracts degraded after the storage period, varied in low limits between samples, from 5.86% to 8.52% in the case of carotenoids (mean 7.28±0.83%), and from 18.38% to 21.77% (mean 20.00±0.94%) for total chlorophylls (fig. 1).

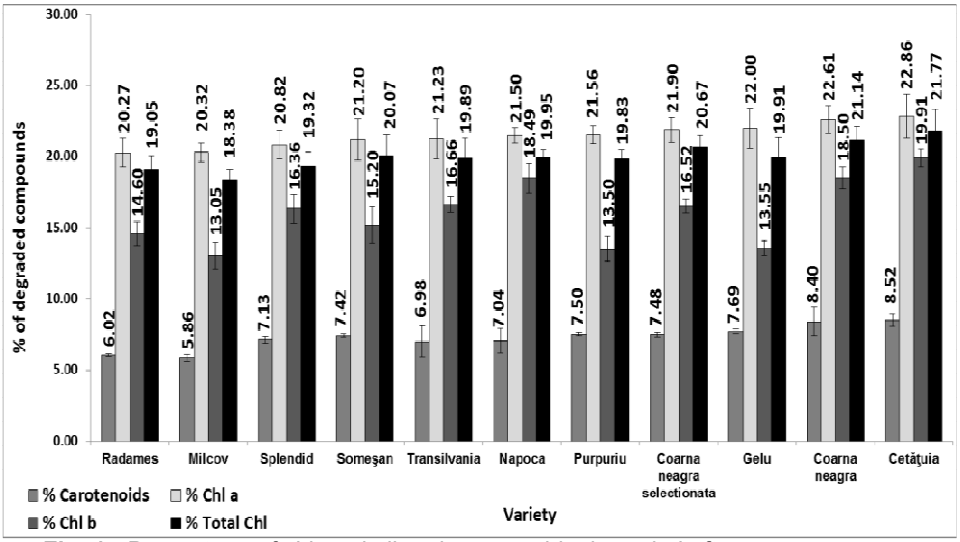


Fig. 1 - Percentage of chlorophyll and carotenoids degraded after extracts storage

Peroxidase activity (PA) in grapevine leaves ranging between 0.014 and 0.045 U/mg f.w., a higher phenolic concentration corresponding to a increased activity of the enzyme (fig. 2), as was earlier specified by Ghoul and Chebil (2012).

Involvement of peroxidase in assimilatory pigment degradation is demonstrated by the important coefficients of determination (R^2) registered for PA–TP relationship ($R^2=0.8389$; $p<0.05$), PA–% total Chl degraded ($R^2=0.9243$; $p<0.05$), and also for TP–% total Chl degraded ($R^2=0.8282$; $p<0.05$). Poor correlation of TP and % of Chl degraded after storage could be explain by the specificity of enzyme for certain phenolic compounds (with the hydroxyl group at the *p*-position) which might be not in adequate quantity in grapevine leaves. According to Katalinic et al. (2009), phenolic compounds in *V. vinifera* L. leaves were represented mainly by phenolic acids, flavonoids and stilbenes.

% of Chl *a* degraded was statistically significant correlated with the increase of PA ($R^2=0.8389$; $p<0.05$), while % of Chl *b* and carotenoids degraded after extract storage shown the same trend, but without a significant coefficient of determination ($R^2=0.7046$, and $R^2=0.7531$, respectively) (table 2).

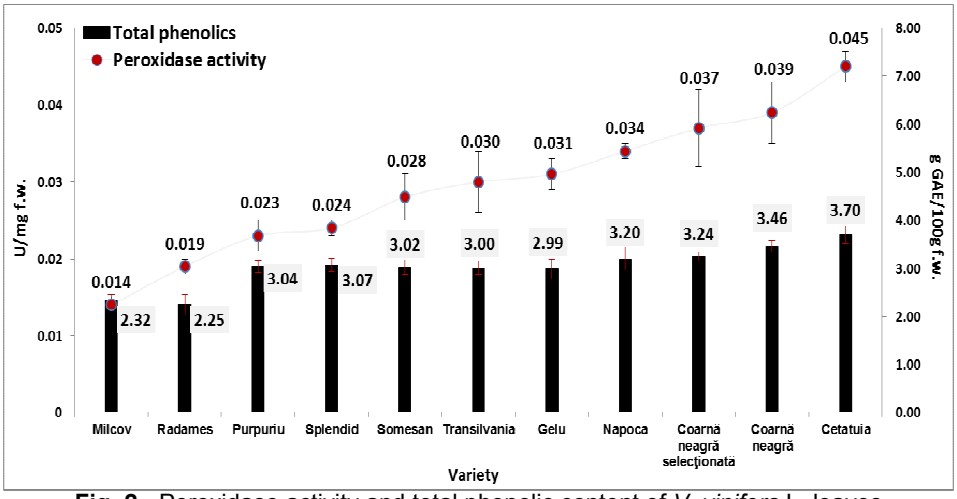


Fig. 2 - Peroxidase activity and total phenolic content of *V. vinifera* L. leaves

Table 2

Correlation (R^2) of phenolic content (TP) of leaves with peroxidase activity (PA) and the percentage of degraded pigments (%) after extract storage

V. vinifera L.	*TP*	*PA*	*% Carot.*	*% Chl a*	*% Chl b*	*% Total Chl*
TP	-					
PA	**0.8389**	-				
% Carot.	**0.8657**	0.7531	-			
% Chl a	**0.8162**	**0.8504**	**0.9058**	-		
% Chl b	0.6068	0.7046	0.3700	0.4128	-	
% Total Chl	**0.8282**	**0.9243**	**0.8453**	**0.8829**	0.5899	-

Note: Bolded values represent that correlation coefficients are statistically significant ($p<0.05$) in ANOVA test.

In the degradation process of assimilatory pigments, along with the activity of other specific enzyme (chlorophyllase, reductase) (Fang et al., 1998), Mg^{2+} degradation (Mg-dechelatase; phaeophytin formation) and the conversion of chlorophyll *b* to *a* (Matile and Hortensteiner, 1999), degradation of chlorophylls and carotenoids via peroxidase – phenolics– H_2O_2 system appears to be possible.

CONCLUSIONS

1. Percentage of carotenoids and chlorophylls in romanian *V. vinifera* L. varieties leaf extracts (at full flowering), degraded after 30 days of cold (6°C) and dark storage, varied in low limits, chlorophyll *a* being the most affected.

2. Experimental data obtained provide further evidence that peroxidase might be involved in chlorophyll bleaching in the presence of phenolic compounds, as an alternative route for the biodegradation of chlorophyll, and being able to mediate in a lesser extent leaf carotenoid degradation.

Acknowledgments: *This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. **Bania I., Mahanta R., 2012** - *Evaluation of peroxidases from various plant sources*. Int. J. of Scientific and Research Publications. Vol. 2 (5), pp. 1-5.
2. **Bergmeyer H.U., 1974** - *Methods of Enzymatic Analysis. Vol. 1*. Academic Press, New York. 2nd Edition, pp. 495.
3. **Burzo I., Dejeu L., Şerdinescu A., Bădulescu Liliana**, **2005** - *Fiziologia plantelor de cultură. Vol. III, Fiziologia viței de vie*. Edit. Elisavaros, Bucureşti.
4. **Carnegie Institution for Science, 2011** - *Spectranomics Protocol: Chlorophylls and Carotenoids*. http://spectranomics.stanford.edu/technical_information.
5. **Fang Z., Bouwkamp J.C., Solomos T., 1998** - *Chlorophyllase activities and chlorophyll degradation during leaf senescence in non-yellowing mutant and wild type of Phaseolus vulgaris L.* J. of Experimental Botany. Vol. 49 (320), pp. 503-510.
6. **Gelinas P., Poitras E., Mckinnon C.M., Morin A., 1998** - *Oxido-reductases and lipases as dough-bleaching agents.* Cereal chemistry. Vol. 75 (6), pp. 810-814.
7. **Ghoul M. and Chebil L., 2012** - *Enzymatic polymerization of phenolic compounds by oxidoreductases*. Springer briefs in molecular science Series. Springer Science.
8. **Hynninen P.H., Kaartinen V., Kolehmainen E., 2010** - *Horseradish peroxidase-catalyzed oxidation of chlorophyll a with hydrogen peroxide. Characterization of the products and mechanism of the reaction*. Bioch. et Bio. Acta. Vol. 1797, pp. 531-542.
9. **Kato M., Shimizu S., 1985** - *Chlorophyll metabolism in higher plants. VI. Involvement of peroxidase in chlorophyll degradation.* Plant Cell Phys. Vol. 26 (7), pp. 1291-1301.
10. **Katalinic V., Generalic I., Skroza D., Ljubenkov I., Teskera A., Konta I., Boban M., 2009** - *Insight in the phenolic composition and antioxidative properties of Vitis vinifera leaves extracts*. Croat. J. Food Sci. Technol. Vol. 1 (2), pp. 7-15.
11. **Matile P., Hortensteiner S., 1999** - *Chlorophyll degradation*. Annu. Rev. Plant Physiol. Plant Mol. Biol. Vol. 50, pp. 67-95.
12. **Matile P., Martinoia E., 1982** - *Catabolism of carotenoids: Involvement of peroxidase?* Plant Cell Rep. Vol. 1(6), pp. 244-246.
13. **Miller E., Schreier P., 1985** - *Studies on flavonol degradation by peroxidase (donor: H_2O_2-oxidoreductase, EC 1.11.1.7).* Food Chem. Vol. 17, pp. 143-154.
14. **Mustea M., 2004** - *Viticultură, bazele biologice, înființarea şi întreținerea plantațiilor tinere de vii roditoare*. Edit. Ion Ionescu de la Brad, Iaşi.
15. **Singleton V.L., Rossi J.A., 1965** - *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid*. Am. J. Enol. Vitic. Vol. 16, pp. 144-158.
16. **Zapata J.M., Calderon A.A., Ros Barcelo A., 1995** - *Peroxidase isoenzyme patterns in cell cultures derived from cotyledon, stem, leaf and fruit from grapevine (Vitis vinifera cv. Monastrell).* Annals of Botany. Vol. 75, pp. 443-448.
17. **Whitaker J.R., 1994** - *Principles of Enzymology for the Food Sciences*. Second Edition. Marcell Dekker, New York, USA.
18. **Willows R.D., 2004** - *Chlorophylls.* In Plant pigments and their manipulation. Annual plant reviews. Vol. 14 (ed. Davies K.M.). CRC Press, Boca Raton, USA, pp. 23-57.
19. **Yamasaki H., Sakihama Y., Ikehara N., 1997-** *Flavonoid-peroxidase reaction as a detoxification mechanism of plant cells against H_20_2*. Plant Physiol. Vol. 115, pp. 1405-1412.
20. **Yamauchi N., Funamoto Y., Shigyo M., 2004** - *Peroxidase - mediated chlorophyll degradation in horticultural crops.* Phytochemistry Reviews. Vol. 3, pp. 221–228.

VARIATION OF SOME BIOCHEMICAL CHARACTERISTICS OF *VITIS VINIFERA* L.GREEN PARTS IN RELATION TO GROWING HEIGHT

VARIAȚIA UNOR CARACTERISTICI BIOCHIMICE LA UNELE ORGANE VERZI ALE VIȚEI DE VIE (*VITIS VINIFERA* L.) ÎN FUNCȚIE DE ÎNĂLȚIMEA DE CREȘTERE

HARAS Diana Gabriela[1], ROTARU Liliana[1], FILIMON V.R.[1], ISTRATE A.[1]
e-mail: arasdia@yahoo.com

Abstract. *The purpose of the study was to evaluate and establish therelationships between some biochemical featrures of leaves collected from the top, middle and bottom of the vine stock in the phenophase of grapes tehnological mathurity. The determinations included analysis of the moisture and total dry matter, total polyphenolic compounds and peroxydase activity. The concentration of photosynthetic pigments and the ratios between them was also determinated. The varietys ana lysed were Fetească albă, Fetească Regală, Grasă de Cotnari, Tămâioasă românească and Frâncușă from Iași vineyard. The highest total polyphenolic content, was registrated in the top leaves while the middle collected leaves recorded a increased level of photosynthetic pigmentsdepending on variety and growing height.*
Key words: *leaves, phenolic compounds, assimilating pigments, growing height, Vitis vinifera L.*

Rezumat.*Scopul acestei lucrări a fost evaluarea si relaționarea unor caracteristici biochimice ale frunzelor prelevate din partea de superioară, partea mediană și partea inferioară a butucului de viță de vie, în fenofaza de maturare a strugurilor. Analizele efectuate au inclus determinarea conținutului total de umiditate și substanță uscată, a concentrației de compuși fenolici totali și a activității peroxidazei. A fost analizată, de asemenea, concentrația în pigmenți asimilatori, precum și raporturile dintre aceștia. Soiurile selectate pentru efectuarea determinărilor experimentale au fost Fetească albă, Fetească Regală, Grasă de Cotnari, Tămâioasă românească și Frâncușă, cultivate în arealul podgoriei Iași. Cel mai important conținut de compuși fenolici a fost identificat în cazul frunzelor din partea superioară a butucului, în timp ce concentrația de clorofile şi carotenoizi a variat semnificativ în funcție de soi şi înălțimea de creştere, frunzele din partea mediană înregistrând cele mai ridicate valori ale acestor parametri.*
Cuvinte cheie: *frunze, compuşi fenolici, pigmenți asimilatori, înălțime de creştere, Vitis vinifera L.*

INTRODUCTION

Climate change projections into the future suggest an increased variability of temperature and precipitation. Extreme climate conditions, such as dry spells,

[1] University of Agricultural Sciences and Veterinary Medicine of Iași, Romania

sustained drought, and heat waves can have large effects on vine. The timing of extreme events relative tosensitive phenological stages could affect growth and productivity of the vine.

The research conducted have shown only external effects of water and temperature stress, without going into the depths of physiological and biochemical changes that they induce in plants, and without considering the importance of the vine stock in ensuring resistance to grape vine.

Fewer studies were conducted on some variations of biochemical characteristics of vine leaves in grapes ripening phenophase and therefore this research produce a certain interest. During this stage the grapes are the main substances receptor and the vegetative organs reduce their growth, and also some of the some physiological and biochemical processes reduce their activity or even cease it (Mustea, 2004).

MATERIAL AND METHOD

The research has been carried out on the mature leaves of five*Vitis vinifera* L. indigenous grapes varieties for quality white wines (Fetească albă, Fetească Regală, Grasă de Cotnari, Tămâioasă românească and Frâncușă), growing in the Ampelographic Collection of the University of Agricultural Sciences and Veterinary Medicine Iasi, Romania, in the phenophase ofgrapes ripening.

Leaf samples were harvested manually, in the morning, from the top, middle, and bottomshoots of vine stock, rapidly frozen and analyzed in same day.

Peroxidase assay procedure was based on that of Bergmeyer (1974), in which the rate of decomposition of hydrogen peroxide by peroxidase, with guaiacol as hydrogen donor, is determined by measuring the rate of colour development spectrophotometrically at 436 nm and at 25°C (UV-vis Spectrostar Nano microplates spectrophotometer).

For assimilatory pigment extraction, frozenleaf samples (0.5 g) were grinded and washed with 10 mL of 99,98% acetone in order to extract the compounds from the leaf tissue. The extract was centrifugated (refrigerated laboratory centrifuge Nahita 2816) 15 min, 3000 rpm (10 °C).The analytical determination was conducted using a UV-vis Shimadzu 1700 Pharmaspec Spectrophotometer at the following wavelengths: 662 and 645 nm, for chlorophyll a and b and 470 nm for carotenoids. Photosynthetic pigment content was calculated in mg/g fresh weight (f.w.) according to the protocol presented by Lichtenthaler and Buschman, 2001.

Total phenolic content was determined by Folin-Ciocâlteu method, measuring the absorbance at 750 nm (Singleton and Rossi, 1965). A calibration curve using different concentrations of gallic acid solutions was used for expressing the results as gallic acid equivalent (GAE).

The method used to discriminate among the means was Fischer's least significant difference procedure at 95% confidence level. Simple regression analysis was performed to look for relationships between data registered by two independent methods. P values lower than 0.05 ($p<0.05$) were considered to be significant.

RESULTS AND DISCUSSION

According to Rotaru and Țârdea (2002), for leaves from the middle of the vinestock, the variability of ampelographic characters is the most reduced. Generally,leaves from the base and the top of vine stock have a lower content of assimilatory pigments and a lower photosynthetic activity, leaves between nodes 5–10 of shootsbeing the most photosynthetically active (Mustea, 2004).

Rate of photosynthesis, during grape ripeningis positively correlated with earliness or lateness character of grape maturation. Photosynthetic activity is favored by high temperatures in late August especially to middle maturing varieties (Ionescu and Condei, 1971).

Table 1

The content of chlorophyll a, chlorophyll b and carotenoids (mg/g f.w.) and the ratios between them in leaves of indigenous *V. vinifera* L. varieties

Variety	Position	Carot.	St. Dev.	Chl a	St. Dev.	Chl b	St. Dev.	Total chl	St. Dev.	Chl a/ Chl b	St. Dev.	Chl/ Carot.	St. Dev.
Fetească albă	T	0.53^{NS}	0.05	1.19^{00}	0.04	0.43^{0}	0.03	1.89^{NS}	0.06	2.78^{000}	0.02	3.56*	0.04
	M	0.59***	0.05	1.39**	0.06	0.60*	0.02	1.99*	0.08	2.32***	0.02	3.37^{NS}	0.07
	B	0.51^{000}	0.03	1.34^{NS}	0.03	0.55^{NS}	0.04	1.61^{0}	0.07	2.43^{000}	0.03	3.15^{00}	0.07
Fetească regală	T	0.67***	0.03	1.54***	0.02	0.53^{000}	0.07	2.07^{NS}	0.03	2.91***	0.07	3.08^{0}	0.06
	M	0.78***	0.02	1.92**	0.03	0.91***	0.05	2.83***	0.05	2.10^{000}	0.07	3.62*	0.01
	B	0.30^{000}	0.02	$0,69^{000}$	0.05	$0,30^{000}$	0.07	1.00^{000}	0.04	2.30^{0}	0.02	3.33^{NS}	0.08
Frâncușă	T	0.51^{NS}	0.03	1.27^{NS}	0.02	0.46^{0}	0.02	1.73^{00}	0.03	2.78***	0.02	3.39^{00}	0.07
	M	0.83***	0.02	2.03^{***}	0.07	0.93***	0.03	2.96***	0.05	2.19^{000}	0.03	3.56^{NS}	0.07
	B	0.35^{000}	0.07	$0,93^{000}$	0.04	$0,38^{00}$	0.02	1.31^{000}	0.03	2.42^{00}	0.06	3.74*	0.02
Grasă de Cotnari	T	0.35^{00}	0.05	1.26***	0.02	0.36^{00}	0.03	1.81***	0.02	2.66**	0.02	5.17***	0.02
	M	0.56**	0.03	1,37***	0.03	0.56**	0.07	1.89***	0.07	2.26^{00}	0.05	3.37^{00}	0.03
	B	0.50^{NS}	0.07	$0,89^{000}$	0.02	0.52^{NS}	0.06	1.24^{000}	0.05	2.46^{NS}	0.01	2.48^{000}	0.04
Tămâioasă românească	T	0.57^{NS}	0.03	1.20^{00}	0.05	0.45^{000}	0.03	2.04^{NS}	0.06	2.68***	0.02	3.57*	0.07
	M	0.59^{NS}	0.02	1.55**	0.03	0.64^{NS}	0.04	2.19***	0.02	2.41^{000}	0.03	3.71**	0.08
	B	0.55^{NS}	0.04	1.45*	0.01	0.59^{NS}	0.02	1.64^{000}	0.04	2.43^{000}	0.03	2.98^{00}	0.01
Mean		0.55	0.02	1.33	0.35	0.55	0.02	1.88	0,21	2.48	0.01	3.44	0.05
CV %		3.18	-	1.11	-	3.03	-	11,17	-	0.50	-	1.43	-

Note: Data expressed as mean values with standard deviation (n = 3). NS, *, **, *** - indicate nonsignificant and positive significant at $p \leq 0.05$, 0.01, 0.001, respectively; $^{0,\ 00,000}$ - negative significant at $p \leq 0.05$, 0.01, 0.001; T – top leaves; M – midlle leaves; B – bottom leaves.

Concentration of chlorophyll *a* in extracts presented wide variation between varieties, with a very positive statistical significance ($p<0.001$) in the case of variety Frâncușă (2.03±0.07 mg/g f.w.) middle leaves and a very negative statistical significance ($p<0.05$) in Fetească regală (0.69±0.05 mg/g f.w.) bottom leaves.

Chlorophyll *b* content of mature*V. vinifera* L. leaves varied also widely compared with the others varieties analysed, with a very positive statistical

significance ($p<0.001$) in Frâncușă and Fetească regală (0.91±0.05 mg/g f.w., respectively 0.93±0.05 mg/g f.w.) midlle leaves. Chlorophyll *a*/*b* ratio was specific to the phenophase of grape ripening, and varied within small limits between 2.10±0.07 (Fetească regală, middle leaves) and 2.78±0.02 (Frâncușă, Fetească albă, top leaves), with a mean of 2.48±0.01.

Research has shown that the leaves on the main shoots have a higher content of assimilating pigments than those of secondaryshoots and also the leaves that have a lower content of assimilating pigments are from the top and the base of the vine stock (Țâra, 1975).

Knowing that the main physiological processes, including photosynthesis that varies according to leaves age, it is necessary to determine at any time during the grapes ripining season,the concentration of photosynthetic pigments.In fact, each phenological phase corresponds to a particular floor of the photosynthetic activity when leaves reach the fully extended.

According to Gross (1991) and Wilows (2004), in mature leaves chlorophyll *a* is the major pigment and chlorophyll *b* is accessory pigment which exist in a ratio of approximately 3 to 1.Variation of chlorophyll a/b and chlorophyll/carotenoids ratio can be an indicator of senescence, stress, and damage to the photosynthetic apparatus, but can also provide distinctive informations on plant phenophase (Burzo et al., 2005).

Studies on the *V. vinifera* L. varieties growing in ecological conditions of Iași vineyard, established that at Fetească albă, Grasă de Cotnari and Tămâioasă românească genotypes, the maximum content of assimilating pigments are recorded in a more advanced stages of vegetation (50-70 days after leaf occurrence) and atthe Fetească regală and Frâncușă more later (after 90 days), fact that can be correlated with a longer vegetation period, noticing a superior production compared to the first three varieties.

Guaiacol peroxidase (E.C. 1.11.1.7) is widely distributed in plants where they catalyze the reduction of hydrogen peroxide (H_2O_2) to water, rendering it harmless (Bania and Mahanta, 2012). Peroxydase activity presented a wide variation between varieties, with a very significant ($p<0.001$) positive difference compared to the mean, in the case ofvine bottom leaves of all analysed varieties, with a maximumin Fetească regală leaves of 1.22±0.07 mg/g f.w.

Total polyphenol content of mature *V. vinifera* L. leaves harvested from the top of the plants was significantly higher compared to the midlle and bottom leaves, with a positive significance ($p<0.001$) in the case of Fetească albă (3.77±0.03 mg/g f.w.), Fetească regală (3.41±0.01 mg/g f.w.) and Frâncușă (2.84±0.01 mg/g f.w.) varieties.

Determination of moisture is essential in vegetal tissue analysis, high proportion of humidity causing a poor stability of samples, favoring microbiological and enzymatic activity (Beceanu et al., 2011; Maltini et al., 2003). In a typical grapevine leaf water content depends on the physiological condition, age and intensity of plant metabolism, ranging from 70 to 85% (Boyer et al., 1997; Mustea, 2004). In mature leaves harvested in the grape ripening stage moisture content was low, specific to this phenophase and varying from 58.59% to 72.93%,

with a mean of 66,96%. Total dry matter of leaves (%) is represented by all their constituents excluding water and is correlated with the moisture content (Tab. 2).

Table 2

Moisture dry matter (%)total polyphenol contentand peroxidase activityin mature leaves of indigenous *V. vinifera* varieties

Variety	Position	Moisture (%)	St. Dev.	Totaldry metter(%)	St. Dev.	POD (U/min/g)	St. Dev.	TPC(gGAE/ 100g f.w.)	St. Dev.
Fetească albă	T	71.09^{NS}	1.21	35.27**	1.21	0.11^{00}	0.05	3.77***	0.03
	M	72.93**	0.59	27,07^{00}	0.59	0.33^{NS}	0.01	2.15**	0.02
	B	64,73^{00}	1.50	28,91^{NS}	1.50	0.39**	0.02	1.60^{000}	0.05
Fetească regală	T	70.34^{NS}	0.90	28.21^{000}	0.90	0.24^{000}	0.03	3.41***	0.01
	M	71.79***	1.59	29.66^{NS}	1.59	1.03^{NS}	0.02	1.74**	0.01
	B	61.65^{000}	1.19	38.35***	1.19	1.22***	0.07	1.62^{000}	0.02
Frâncușă	T	66.98^{NS}	1.80	37.72**	1.80	0.56^{NS}	0.01	2.84***	0.01
	M	68.68**	1.65	31.32^{00}	1.65	0.40^{000}	0.01	2.16**	0.03
	B	62.28^{00}	0.94	33.02^{NS}	0.94	0.72***	0.02	1.70^{00}	0.01
Grasă de Cotnari	T	63.11*	1.65	41.41***	1.65	0.79^{NS}	0.01	2.50*	0.02
	M	67.45***	1.10	32.55^{000}	1.10	0.79^{NS}	0.01	2.25^{NS}	0.02
	B	58.59^{000}	1.02	36.89*	1.02	0.88**	0.01	1.90^{0}	0.01
Tămâioasă românească	T	68.54^{NS}	1.46	34.59**	1.46	0.51^{NS}	0.02	2.85*	0.01
	M	70.88***	1.35	29.12^{00}	1.35	0.34^{000}	0.02	2.33^{NS}	0.01
	B	65.41^{000}	0.89	31.46^{NS}	0.89	1.20***	0.02	1.75^{0}	0.02
Mean		66.96	1.26	33.04	1.26	0.63	0.02	2.30	0.01
CV %		1.88	-	3.80	-	2.48	-	0.64	-

Note: Data expressed as mean values with standard deviation (n = 3). NS, *, **, *** - indicate nonsignificant and positive significant at $p \leq 0.05$, 0.01, 0.001, respectively; $^{0, 00, 000}$ - negative significant at $p \leq 0.05$, 0.01, 0.001.T – top leaves; M – midlle leaves; B – bottom leaves.

Itwas found a significant correlation between low levels of moisture and a high peroxidase activity esspecialy in the leaves situated on the base of vine stock which can be related with the drought resistance of varietiesas was previously reported bySenaratna, 1985.

CONCLUSIONS

Biochemical characteristics investigated showed significant variations indicating different metabolic rates, particularly leaf age-related, which responds unitary to the cumulative action of stress factors.

Variation of chlorophyll a/b and chlorophyll/carotenoids ratio provide distinctive informations on plant phenophase, data recorded being within the range of values presented in the literature for *Vitis vinifera* L. varieties.

Carotenoid pigments were present in low concentration in grapevine leaves compared to chlorophylls, with a proper chlorophyll/carotenoids ratio ranging from 2.4 to 5.2.

The analysis of total polyphenolic compounds reported a higher content in the top leaves of vine, in the middle leaves beeing reported a higher concentration

of photosynthetic pigments. Peroxidase activity was higer in the leaves collected from the base of the vine and correlated to the lower levels of moisture phenomenon that can be related with the drought resistance of varieties.

Experimental data obtained provide further evidence that growing height of vine green parts influence their biochemical composition, in relation to plant defence activity against temperature and water stress.

REFERENCES

1. **Bania I., Mahanta R., 2012** - *Evaluation of peroxidases from various plant sources*. Int. J. of Scientific and Research Publications. Vol. 2 (5), pp. 1-5.
2. **Beceanu D., Anghel R., Filimon V. R., 2011 -***Materii prime horticole mai importante pentru industria alimentară: struguri, fructe, legume*. Cunoştinţe de bază şi aplicaţii practice. Edit. PIM Iaşi.
3. **Boyer J.S., Wong S.C., Farquhar G.D.,1997** - *CO_2 and water vapor exchange across leaf cuticle (epidermis) at various water potentials*. Plant Physiology. 114, pp. 185–191.
4. **Burzo I., Dejeu L., Şerdinescu A., Bădulescu L., 2005 –** *Fiziologi aplantelor de cultură*. Vol. III. *Fiziologia viței de vie*. Edit. Elisavaros, Bucureşti
5. **Ionescu P**.,**Condei Gh., 1971 –** *Influența îngrășămintelor chimice asupra unor procese fiziologice la vița de vie* .ICVV Analele vol. III, București.
6. **Keller M., 2010** - *The science of grapevines: anatomy and physiology*. Academic Press, Elsevier Inc.
7. **Lichtenthaler H.K., Buschmann C., 2001** - *Chlorophylls and Carotenoids: Measurement and Characterization by UV-VIS Spectroscopy*. In Current Protocols in Food Analytical Chemistry (ed. Wrolstad R.E.). John Wiley & Sons, Inc., F4.3.1–F4.3.8.
8. **Lovisolo C., Schubert A., Restagno M., 1996** -*Photosynthesis of grapevine leaves of different age at high and low light intensi*ty. ISHS Acta Hort. 427, pp. 91–93.
9. **Maltini E., Torreggiani D., Venir E., Bertolo G., 2003** -*Water activity and the preservation of plant foods*. Food Chemistry. 82, pp.79–86.
10. **Mendes-Pinto M. M., FerreiraA.C.S., Caris-Veyrat C., De Pinho G.P., 2005** - *Carotenoids, Chlorophyll, and Chlorophyll-Derived Compounds in Grapes and Port Wines*. J. Agric. Food Chem. 53, pp. 10034–10041.
11. **Mustea M.,2004 -** *Viticultură. Bazele biologice, înființarea şi întreținerea plantațiilor tinere de vii roditoare. Edit. „Ion Ionescu de la Brad", Iaşi.*
12. **Rotaru L., Țârdea C., 2002 –** *Folosirea analizei cluster în ampelografie pentru stabilirea asemănărilor fenotipce la soiurile de viță de vie*. Lucr. Ştiințifice U.S.A.M.V. Iaşi, Seria Horticultură, 45(1), pp. 189–193.
13. **Senaratna T., 1985 -** *Antioxidant levels in germinating soybean seed axes in relation to free radical and dehydration tolerance.*Plant Physiol. May 1985; 78(1), pp. 168–171.
14. **Singleton V.L., Rossi J.A., 1965** - *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid*. Am. J. Enol. Vitic. Vol. 16, pp. 144-158.
15. **Toma L.D., Jităreanu D., 2007 –** *Fiziologie vegetală*. Edit. "Ion Ionescu de la Brad", Iaşi, Romania.
16. **Țâra Gh.,1975** – A*ctivitatea fiziologică a diferitelor categorii de lăstari la vița de vie.* Analele I.C.V.V., Valea Călugărească, vol. VI, București.

USING OF CLUSTER ANALISYS FOR COARNĂ NEAGRĂ GRAPEVINE VARIETY AND ITS DESCENDENTS

ANALIZA CLUSTER LA SOIURILE DE VIȚĂ DE VIE PROVENITE DIN COARNĂ NEAGRĂ

ISTRATE A.[1], ROTARU Liliana[1], HARAS Diana Gabriela[1]
e-mail: aistrate@uaiasi.ro

Abstract. *The Coarnă neagră grapevine variety was used as maternal genitor in the creation of some new grapevine varieties. Of these varieties, the author have chosen Coarnă neagră selecționată, Azur, Milcov, Gelu, Ozana and Mara with which, on the basis of Cluster analysis will determine the phenotypical similarity among the Coarnă neagră varieties genitor and its descendants, with a view to establish the polyphyletic groupings among the biological material under analysis.*
Key words*: Cluster analysis, grapevine variety, phenotypical similarity, descendants*

Rezumat*. Soiul de viță de vie Coarnă neagră a fost folosit ca genitor matern pentru a obține creații noi românești de viță de vie. Dintre acestea autorul a ales soiurile Coarnă neagră selecționată, Azur, Milcov, Gelu, Ozana și Mara cu ajutorul cărora, pe baza analizei Cluster se va determina gradul de asemănare fenotipică dintre soiul Coarnă neagră și descendenții săi, cu scopul de a se stabili grupurile polifiletice din materialul biologic supus analizei.*
Cuvinte cheie*: analiza cluster, soi de viță de vie, asemănări fenotipice, descendeți*

INTRODUCTION

Coarnă neagră is an old grapevine variety with an oriental origin, cultivated for a long time in Romania, becoming a domestic variety. Being very well adapted to the local conditions, there were created new varieties based on it: Coarnă neagră selecționată, Azur, Milcov, Gelu, Ozana and Mara (Țârdea and Rotaru, 2003).

In order to determine the phenotypic similarities between the varieties of this group, it has been used the cluster analysis, which admits the existence of polythetic groups (similar groups of varieties /groups like varieties) and simultaneously measuring the similarity of the elements of the group and the difference between groups (Rotaru, 2000; Bosoi et al., 2011).

Based on this analysis, it was determined: the chaining / kinship levels of the varieties analyzed, the values of chaining, the histogram classification of the varieties analyzed and the dendrogram of the similarity of varieties (Rotaru, 2004).

MATERIAL AND METHOD

There was collected a total of 10 adult leaves from the Coarnă neagră variety as genitor and his descendants, considered each as monothetic groups. Based on

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

leaf architecture, there were established 51 benchmarks and has been made 68 direct ampelometric measurements. The data obtained had allowed calculation of 53 ampelometric values: amounts, ratios, product, etc. (fig. 1). For the symmetrical characters, both values were measured and calculated (Bosoi et al., 2011).

These measurements allowed to compile a statistical population made up of rows of variation of 30 values for all 121 characters analyzed on the 7 varieties under study. The genetic material analyzed is presented in table 1. (Rotaru, 2000).

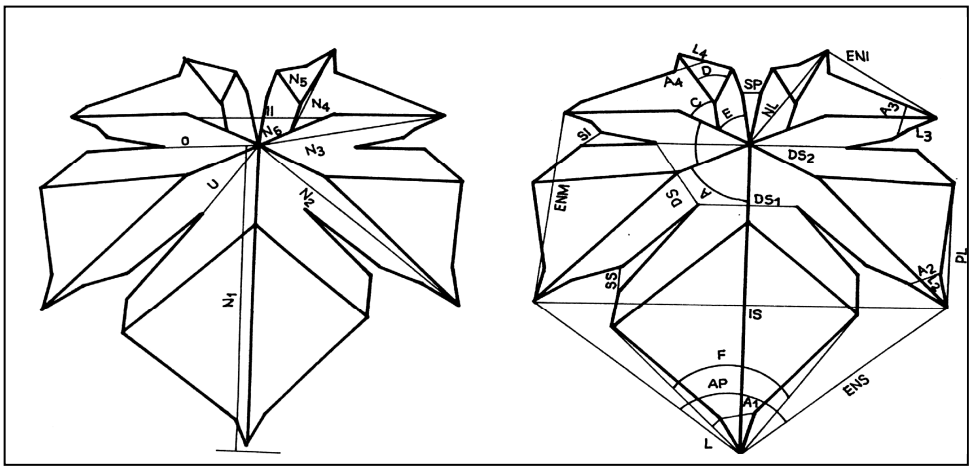


Fig. 1 – Schematic representation of characters examined at grapevine leaf

Table 1

The genetic material studied

Variety name	Genitors	Homologation year	Authors and the unity where has been approved
Coarnă neagră	Oriental origin	-	-
Coarnă neagră selecționată	Free fertilization of the Coarnă neagră variety	1970	Constantinescu Gh., Negreanu Elena, Agronomy Institute N. Balcescu, București
Azur	Coarnă neagră x Cardinal	1984	Lepădatu Victoria și Condei Gh., SCVV Drăgășani
Milcov	Coarnă neagră x Muscat de Hamburg	1988	Băditescu Margareta, Varga N., Zaharia V., Coman Gh., SCVV Odobești
Gelu	Free fertilization of the Coarnă neagră variety whose hybrid seeds were irradiated with X rays	1997	Calistru Gh., Damian Doina, SCDVV Iași
Ozana	Free fertilization of the Coarnă neagră variety	1982	Dănulescu D. și colab., SCVV Iași
Mara	SV 12303 x Ozana	2011	Damian Doina, Calistru Gh., Nechita Ancuța, Savin C., SCDVV Iași

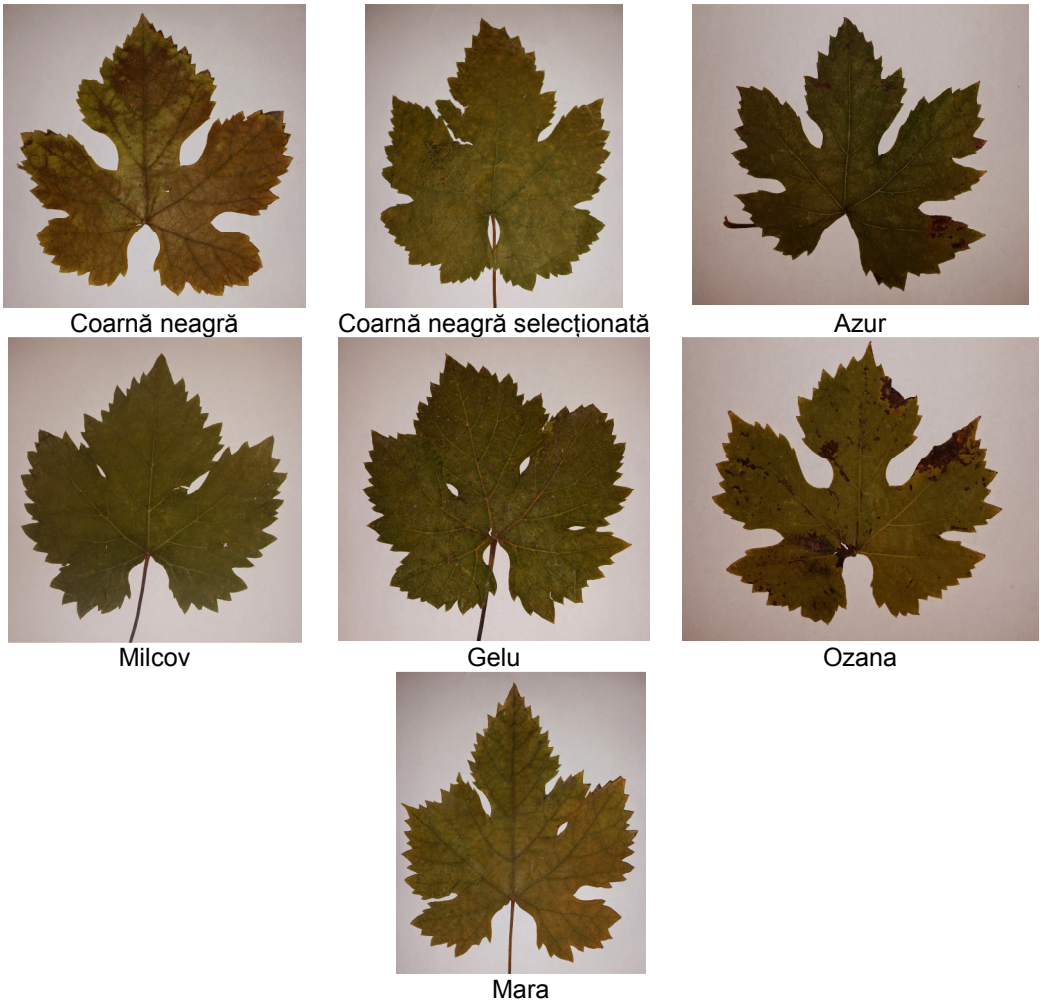


Fig. 2 – Biological material studied

RESULTS AND DISCUSSIONS

In preparing the hierarchical classification dendrogram of the genetic material, was used Pearson's correlation coefficient (generalized Ward criterion), expressed by the numerical value of the similarity index (figure 3) (Lazarevski, 1946; Rotaru, 2000).

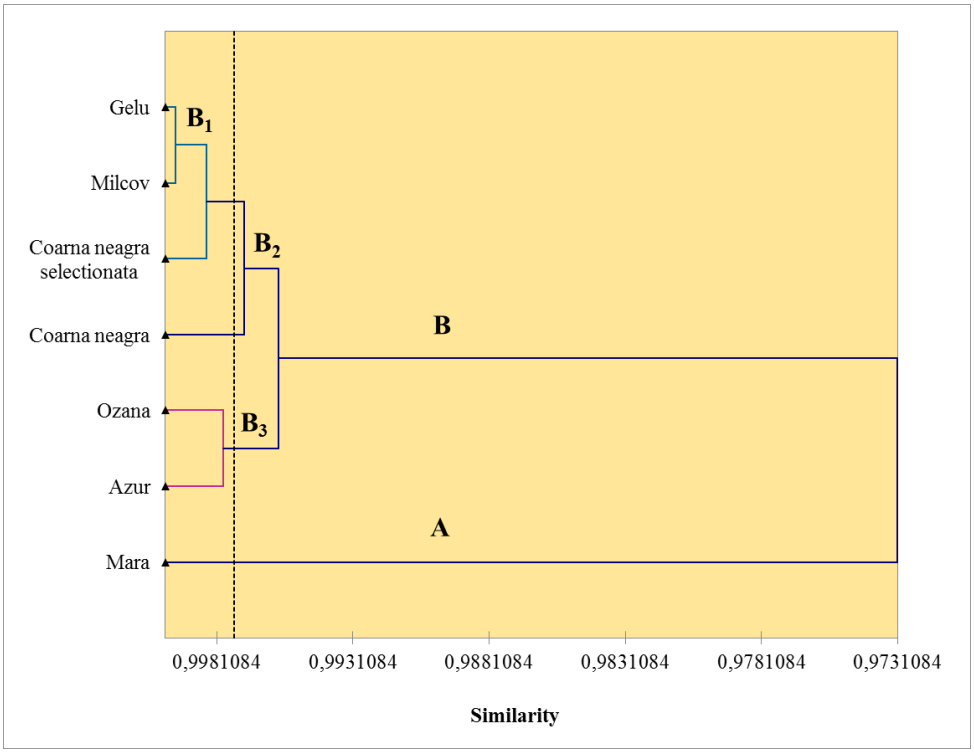


Fig. 3 – Varieties dendrogram

The higher the similarity index value is the more phenotypically similar are the varieties that make up the groups.

From analysis of the dendrogram it is found the existence of two polythetic major groups of the Coarnă neagră variety descendants.

Regarding the first group, i.e. B, it is composed of other 3 subgroups.

In the first B_1 subgroup, the first varieties that unite and have the closest phenotypic similarity are the Gelu and Milcov varieties, because they have the biggest chaining index, i.e. 0.9996. These have the biggest similarity from all 7 varieties studied. The variety that closes this subgroup is the Coarnă neagră selecționată variety, with a chaining index of 0.9984.

The second B_2 subgroup is represented by the previous subgroup plus Coarnă neagră variety, with a chaining index of 0.9970.

The third B_3 subgroup is represented by the previous subgroups plus two varieties that are very similar, Ozana and Azur varieties. To these two varieties the chaining index is 0.9978. This subgroup B_3 unites with the previous B_2 subgroup with a chaining index of 0.9958.

The second group, i.e. A, is represented by the Mara variety which unite with the rest of the dendrogram with a chaining index of 0.9731.

Table 2

Concatenation levels in developing varieties dendrogram

Concatenation levels	The number of varieties on a level	Similarity index value
Gelu ~ Milcov	2	0.9996
Gelu ~ Milcov ~ Coarnă neagră selecționată	3	0.9984
Ozana ~ Azur	2	0.9978
Gelu ~ Milcov ~ Coarnă neagră selecționată ~ Coarnă neagră	4	0.9970
Gelu ~ Milcov ~ Coarnă neagră selecționată ~ Coarnă neagră ~ Ozana ~ Azur	6	0.9958
Gelu ~ Milcov ~ Coarnă neagră selecționată ~ Coarnă neagră ~ Ozana ~ Azur ~ Mara	7	0.9731

Regarding leaf architecture, it is found that the varieties with the biggest similarity, Gelu and Milcov, have orbicular leaves, tri or pentalobed, with the lateral sinuses with a fit deep and closed ovoid, and the petiole sinus in the shape of lyre more or less open. The next variety that unite is Coarnă neagră selecționată which is different from the others through terminal elongated and sharp lobe, and the lateral sinuses less pronounced and open.

The classic Coarnă neagră variety differs in that the lateral sinuses are less deep, the upper sinuses are in the shape of lyre with sharp base, and the petiole sinus in the shape of an open V.

The two varieties which belong to the B_3 semi group, Ozana and Azur, are similar regarding the architectonics of the adult leaf, the difference being the elongated terminal lobe to Azur, short and wide to Ozana. Also, the petiole sinus is open in the shape of V to Azur, whereas to Ozana the petiole sinus is open in the shape of U.

The Mara variety which is totally different from group B, i.e. all of other varieties, differs in that having in its genetic formula a resistant variety (SV 12303), makes the cogging to be more pronounced, the leaf to be bigger, arrow-headed/cuneiform, pentalobed, sometimes with additional lobes, and the petiole sinus to be open in the shape of a lyre (Alleweldt and Dettweiller, 1986).

CONCLUSIONS

The use of the statistical-mathematical methods (the cluster analysis) in determine the phenotypic similarity of Coarnă neagră variety and its descendants revealed the following:

- The varieties which have the highest chaining index are Gelu and Milcov, which shows the high phenotypic similarity to leaf that exist between these two varieties.

- Between the 4 major polythetic groups of the descendants of the Coarnă neagră variety, the closest to this is the first group, with a chaining index of 0.9970, a group consisting of Gelu, Milcov and Coarnă neagră selecționată variety.

- Mara variety has the lowest similarity because of the low chaining index, i.e. 0.9731, which further emphasizes that this is a hybrid. Also it can be seen in its genetic formula a resistant variety (SV 12303), which makes the cogging to be more pronounced, the leaf to be bigger, arrow-headed/cuneiform, pentalobed, thus differing from the other studied varieties.

REFERENCES

1. **Alleweldt G., Dettweiller Erika, 1986** – *Ampelographic studies to characterize grapevine varietes*. Atti 4-Simp. Intern Genetica della Vite, aprilie 1985. Rev Vignevini no. 13 (suppl. No. 12), pp. 6-59.
2. **Bosoi Marioara, Mihu Gh., Bosoi Ionica, Stoian Ileana, 2011** – *Ampelometric study of native grapevine varieties by cluster analysis method*. Lucrări ştiințifice USAMV Iaşi, seria Horticultură, vol. 54(1), pp.437-443
3. **Lazarevski M. A., 1946 –** *The Methods of Grapevine Description*. Ampelography of the USSR. Vol. 1. Pishepronizdat Publ., Moscow.
4. **Rotaru Liliana, 2000 -** *Cercetări asupra caracterelor fenotipice şi însuşirilor biotehnologice la soiurile autohtone de viță de vie, prin utilizarea codurilor şi sistemelor de determinare bazate pe ampelometrie*. Teza de doctorat, Iaşi.
5. **Rotaru Liliana, 2004** – *Aplicarea analizei cluster în caracterizarea viței portaltoi din grupa Berlandieri x Riparia.* Lucrări Ştiințifice USAMV Iaşi, seria Horticultură, vol. 1 (47), pp 751-754.
6. **Țârdea C., Rotaru Liliana, 2003** - *Ampelografie, vol II (Soiuri de viță de vie pentru struguri de masă şi soiuri apirene)*. Edit. "Ion Ionescu de la Brad", Iaşi.

STUDIES ON THE COMPOSITIONAL PROFILE OF WINES OBTAINED FROM ȘARBĂ GRAPES

STUDII ASUPRA PROFILULUI COMPOZIȚIONAL AL VINURILOR DIN SOIUL ȘARBĂ

COLIBABA Cintia [1], COTEA V. V. [1], ROTARU Liliana[1], NICULAUA M. [2], NECHITA C.B. [1], ZAMFIR C.I.[3], LUCHIAN Camelia[1]
e-mail: cintia_colibaba@yahoo.co.uk

Abstract. *Wines obtained from Șarbă grapes have become more and more interesting for Romanian wine drinkers who prefer whites with semiaromatic profile. The present study underlines the physical-chemical parameters of Șarbă wines obtained from grapes harvested in two Moldavian vineyards: Iași and Cotești. The grapes were processed according to the same technological scheme, that of the Oenology laboratory within the UASVM Iași, specific for aromatic wines. The 2013 grapes were macerated for 24 hours at 15 °C and then pressed. The free fall must and the press must were fermented separately. In the end, four wine samples were obtained, two from each vineyard. After 6 months, the samples were analysed from a physical-chemical point of view, according to OIV standards.The obtained analyses accentuate the importance of the climatic conditions on the compositional profile of wines.*
Key words*: Șarbă, Cotești, Iași, compositional parameters*

Rezumat. *Vinul obtinut din soiul Șarbă este din ce în ce mai apreciat de către consumatorii de vinuri ale semi-aromate. Studiul de față se bazează pe compararea parametrilor fizico-chimici ale vinurilor obținute din soiul Șarbă, cu struguri recoltați din podgoria Cotești și Iași, vinificați însă prin același procedeu tehnologic, specific Laboratorului de Œnologie din cadrul USAMV Iași. În cursul anului 2013, strugurii au fost prelucrați folosind procedee din tehnologia vinurilor aromate. După o macerare de 24 ore la o temperatură de 15 °C, mustuiala a fost presată. Mustul ravac si mustul de presă au fost vinificate separat. În final au fost obținte patru probe, două din fiecare podgorie. Dupa 6 luni, variantele au fost analizate din punct de vedere fizico-chimic, conform cu specificațiile OIV. Analizele reliefează importanța condițiilor climatice asupra profilului compozițional al vinurilor.*
Cuvinte cheie : *Șarbă, Cotești, Iași, parametrii de compoziție*

INTRODUCTION

Șarba grape variety was considered a semi-aromatic variety, presenting, right after homologation, an intense aroma suggesting roses jam. In time, the aroma losses its power, without any scientific explanations. The cause could either be genetic, pedological-climatic, agro-technical or because of to the wine-

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
2 Oenological Research Center, Iasi Branch of the Romanian Academy, Iasi, Romania
[3] Reasearch and Development Station for Viticulture and Winemaking Iasi, Romania

making technology. Some wines present a high level of aroma, similar in intensity to that of the homologation variety, some have a very low level of aroma.

In the Romanian Official Catalogue for cultivating plants, in 2012, only one clone is registered, as Șarbă 3 Od.

This grape variety was homologated in 1972, named probably after the Șarbă Hill, the highest spot in the area, with a spectacular view of the Milcov river and the valley below.

The grape variety could initially be met only on Șarbă Hill, but soon after, it became popular in southern Moldavian vineyards, especially in Vrancea county, due to the Romanian reconversion program. Taking into the account the problem arising from the decrease in aroma, the surfaces planted with Șarbo grape variety will probably be diminished (Cotea V.D. et al. 2000).

The aromatic complexity of Șarbă wines is genetically transmitted from Tămâioasă, finely meshed together with the herbal, slightly citric hues of Riesling. Without question, Șarbă wine has its own merits. It is not a sophisticated wine, it does not have the body of a Chardonnay or the freshness of a Sauvignon. It does, however, have its advantages, or better said, its peculiarities. It is a light, semi-aromatic wine, usually demi dry, even if, in some parts, it tends to get demi-sweet. Sensorially, it reminds the wine taster of a cherry orchard in bloom and ripe peach (http://www.cramagirboiu.ro/gama-livia-sarba.php).

MATERIAL AND METHOD

Romanian grape variety Șarbă from Iasi and Cotești vineyard have been used. Șarbă is a grape variety obtained by crossing Tămâioasă românească and Riesling italian. It has been created by Gheorghe Popescu, researcher at the Station for Research and Development in the Viticultural and Winemaking field Odobești. approved for culture in 1972. The newly developed grape variety originates from a strong sensorial genitor, thus being able to produce quality aromatic or semi-aromatic wines.

The wine samples were obtained by applying the classical technology for aromatic wines (Cotea et al., 1985): harvested at full technological maturity, sorted, destemmed and crushed. Maceration was done for 24 hours at 15 ^{o}C after which the free fall must and the press must were collected and processed separately. Commercial yeasts Fermactive Muscat® (5g/hL) was used for fermentation, which was kept at 15^{o}C and lasted for 2 weeks. Fining was done with bentonite. After filtering and SO_2 addition, the sample wines were bottled. 4 variants were obtained:

V1 – Șarbă wine obtained from **free fall must** from Cotești vineyard grapes
V2 – Șarbă wine obtained from **press must** from Cotești vineyard grapes
V3 – Șarbă wine obtained from **free fall must** from Iași vineyard grapes
V4 – Șarbă wine obtained from **press must** from Iași vineyard grapes

The physical-chemical analyses were done according to the specifications from the Compendium of International Methods of Analysis of Wine and Musts, OIV, OIV-MA-AS312-01A method, in the Oenology Laboratory of USAMV Iași.

RESULTS AND DISCUSSIONS

As found in different scientific articles of specific literature (Ribereau-Gayon et al., 1972, Cotea et al., 1988), the physical-chemical parameters of wines obtained from the same batch of grapes can vary during fermentation, due to fermentation conditions, as well as because of the grapes' chemical composition.

The compositional profile of Șarbă wines is presented in table 1.

Table 1

Compositional characteristics of Șarbă wines

Sample	Total SO_2 mg/L	Free SO_2 mg/L	Volatile acidity g/L	Total acidity g/L	Density g/cm^3	Alcoholic conc.% vol.	Remanent sugars g/L	Non-reductive extract g/L	Total dry extract g/L
V1	168,91	67,75	0,34	5,87	0,99845	12	16,02	21.2	37.2
V2	165,6	70,25	0,37	5,94	0,99812	11,95	16,5	19.4	35.9
V3	71,84	14,86	0,50	6,02	0.99279	11,7	2,08	18.9	20.9
V4	73,25	17,15	0,54	6,25	0,99312	11,5	1,9		

The compositional profile of the four wines from two distinct geographical areas of Romania show without question the influence of the climatic conditions of their environmental area. The Cotești vineyard is characterized by average temperatures of 9.8 ^oC – 10 ^oC while Iași vineyard has an average temperatures of 9.5 ^oC, fact that is mirrored in the higher density, alcoholic concentration and extract of the first two samples of Șarbă wines. The alcoholic concentration is visibly higher in the Cotești wine samples (12 % vol and 11,9% vol.), while in Iași Șarbă wine samples, due to a lower concentration of glucides' accumulation, this is inferior (11,7 % vol. and 11,5 % vol.).

An essential influence on the wine's quality is represented by its acidity. The present legislation demands a minimum of 4 - 4,5 g/L tartaric acid for quality white wines. From the above analyses, it can easily be seen that the actual values are much higher, specific for the area of origin of the grapes as well as for the wine-making technology. Therefore, a difference in acidity can be observed between the wine samples obtained from free flow must and the press must. It is a fact well know that the outer layers of the grape berry contain more sugars than the area closer to the center of the berry, which is more acidic. Therefore, the total acidity of the Șarbă samples that have been obtained from press must show higher total acidity values (V2 - 5,94 g/L and V4-6,25 g/L tartaric acid) than their counterparts (V1 - 5,87 g/L and V3 - 6,02 g/L tartaric acid) obtained from free fall must. Moreover, the higher acidity of the Iași wine samples is without question the result of the Northern climatic conditions.

The experimental wine samples can be classified as quality wines, DOC, with good extracts and alcoholic concentration, giving its most intense sensorial characteristics in the case of the samples with remanent sugars.

CONCLUSIONS

The compositional quality of the wines has been strongly influenced by the climatic conditions of the harvest year as well as the natural environment conditions of the vineyard.

Șarbă wines obtained from grapes harvested from Coteşti vineyard have higher values of some of the compositional indices than Șarbă wines obtained from grapes harvested in Iași vineyard (alcoholic concentration and extract)

Total acidity is lower in Șarbă wines from grapes in Coteşti vineyard, due to higher average temperatures.

The following studies will be focused on the aroma profile of Șarbă wines, trying to determine the factors that lead to a decrease or even loss of aroma.

Acknowledgments. *The publishing of this study was made possible with the help of the USAMV internal research grant 5526/25.04.2013.*

REFERENCES

1. Cotea V.D., 1985 - *Tratat de oenologie, vol. 1*, Ed. Ceres, Bucureşti.
2. Cotea V.D., Sauciuc J., 1988 - *Tratat de Oenologie, vol. 2*, Ed. Ceres, Bucureşti.
3. Cotea V.D., Barbu N., Grigorescu C., Cotea V.V., 2000 - *Podgoriile şi vinurile României*. Ed. Academiei, Bucureşti.
4. Ribereau-Gayon J., Peynaud E., Sudraud P., Ribereau-Gayon P., 1972 - *Treatise of Oenology. Science and technology of wine, tome 1. Control and Analyse of Wine (in French).* Dunod-Paris, France.
5. OIV, 2013 - *Compendium of international methods of wine and must analysis volume 1 and 2,* Paris.
6. http://www.cramagirboiu.ro/gama-livia-sarba.php

STUDY OF THE INFLUENCE CAUSE BY SOME MATURATION PROCESS (STAVES) ON THE PHENOLIC COMPOUNDS AND THE ANTHOCYANS FROM RED WINES

STUDII PRIVIND INFLUENȚA UNOR PROCEDEE DE MATURARE (MICRODOAGE) ASUPRA COMPUȘILOR FENOLICI ȘI ANTOCIANILOR DIN VINURILE ROȘII

DUMITRIU Georgiana-Diana[1],COTEA V.V.[1], PEINADO R.A.[2], LOPEZ DE LERMA Nieves[2], ZAMFIR C.I.[1], COLIBABA Cintia[1], NICULAUA M.[1], NECHITA B.[1], VARARU F.[1]

e-mail: dumitriu.diana22@yahoo.com

Abstract. *The demand for cost-effective and simpler techniques has encouraged the development of alternative ageing systems, such as the use of oak wood pieces (oak chips, stave), that accelerate ageing, shortening the time of contact, without decreasing the quality of the wine produced. The aim of this paper is to study the influence of various stave on the red wines physical-chemical parameters and phenolic compounds, because of their positive effects on human health. Experimental material used Feteasca neagră from the Şuletea area, Fălciului hill, harvested in 2013. The wines content of phenolic compounds varied, depending on the stave variant (V0-martor wine, V1-stave fruité, V2-stave vanillé and V3-stave épicé). Following the vinification, four technologic variants were obtained and analyzed at 1.5 and 3 months. For characterizing the phenolic compounds, a spectrophotometric measurement was used in order to evaluate the total polyphenolic index and the total quantity of anthocyans from wines. Photometric measurements were made using Analytik Jena S 200 spectrometer (at 280 nm, respectively 520 nm). The analyses show a small variation of phenolic compounds which means that stave types don't influence significantly their quantity. Although, we observed a quantitative decrease of phenolic compounds registrated at 3 months in comparison with samples at 1.5 months.*

Key words*: red wines, alternative products of maturation, phenolic compounds, anthocyans;*

Rezumat. *Cererea de tehnici rentabile şi simple au încurajat dezvoltarea unor sisteme alternative de maturare, precum utilizarea unor piese de lemn de stejar (aşchii de stejar, microdoage), care accelerează procesul de maturare, scurtează timpul de contact, fără diminuarea calității vinului produs. Obiectivul acestui studiu îl reprezintă influența diferitelor microdoage asupra caracteristilor fizico-chimice şi a compușilor fenolici din vinurile roșii, datorită beneficiilor asupra sănătății omului. Materialul experimental folosit a fost preluat din zona Şuletea, Vaslui-Fetească neagră, în anul de producție 2013. Vinurile au un conținut ridicat în compuși fenolici, funcție de tipul de produs alternativ (V0-proba martor, V1-microdoage de fructe, V2-microdoage de vanilie și V3-microdoage epice). În urma*

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania
[2] University of Cordoba, Spain

vinificării s-au obţinut patru variante tehnologice, analizate la 1,5 şi 3 luni. Pentru a caracteriza compuşii fenolici, au fost utilizate măsuratori spectrofotometrice pentru a evalua indicele de polifenoli totali şi cantitatea totală de antociani. Măsurătorile fotometrice au fost realizate cu ajutorul spectrofotometrului Analitik Jena S 200 (la 280 nm, respectiv la 520 nm). În urma analizelor efectuate s-au observat mici variaţii ale compuşilor fenolici ceea ce semnifică că tipul de microdoagă nu influenţează semnificativ cantitatea acestora. Se observă însă o scădere a conţinutului de compuşi fenolici totali la 3 luni comparativ cu analizele efectuate la 1,5 luni.
***Cuvinte cheie**: vinul roşu, produse alternative de maturare, compuşi fenolici, antociani.*

INTRODUCTION

The phenolic composition of a wine is deeply influenced by grape variety, ripening, soil and climate, vinification procedures, and ageing (Zafrilla et al., 2003). The ageing process is a common technological procedure used in winemaking which seems to contribute to an increase in the antioxidant capacity of wines (Alonso et al., 2004; Canas et al., 2008; Larrauri et al., 1999). Phenolic compounds constitute one of the most important quality parameters of wines since they contribute to their organoleptic characteristics, particularly colour, astringency and bitterness (Santos-Buelga & de Freitas, 2009).

In recent years, several new techniques have been introduced in winemaking. One of these involves adding new pieces of wood (oak chips or inner staves) into inert containers (Arapitsas et al., 2004; Gomez Garcia-Carpintero et al., 2012; Alamo & Nevares, 2006). The use of stave is an alternative practice to barrels, which become widely used in the last years. Compared to traditional ageing, staves confer oak wood flavors to wines faster, easier and with a lower cost than barrels.

It is well-known that in both aging systems (alternative with oak fragments, or traditional in barrels), the characteristics of the final wines depend on the characteristics of the oak wood used (geographic origin, seasoning, toast, etc.) and on the factors that control the transfer processes of these compounds, such as contact surface between wine and wood, alcoholic degree, duration time, static or dynamic process, etc. In the traditional aging systems, the contact surface depends mainly on the size and shape of the oak barrels. However, in the "alternative systems", the contact surface depends on the size of the oak fragments (staves, chips, shavings, etc.) and on the amount used.

The aim of this paper is to evaluate the influence of stave on the physical chemical parameters, phenolic compounds and anthocyans quantity from Fetească neagră wines (local Romanian variety).

MATERIAL AND METHOD

The experiments used wines obtained from Fetească neagră grapes, from the Şuletea and Fălciului hills, Iasi region, in the vintage 2013.

The marc obtained was subjected to a maceration–fermentation process at temperature of 10-12˚C, for 7 days. In the next step, the marc was pressed and the wines obtained were transferred in fermentation tanks for the completing of alcoholic

and malolactic fermentation. At the end of the fermentation processes, we divided in four variants the wines: control wines (V0); wines with stave Medium Toast added (V1); wines with stave Medium Plus Toast added (V2) and wines with stave Heavy Toast added (V3) (Tab 1.). All the samples obtains used alternative products of maturation (4 g/L micro stave) that were immersed in 5L of wine and placed in glass vessels.

For characterizing the phenolic compounds, a spectrophotometric measurement was used in order to evaluate the total polyphenolic index and the total quantity of anthocyans from wines. The total phenolic content was determined by the enzymatic method described by Stevanato, Fabris, and Momo (2004) and total anthocyans by pH variation method. Photometric measurements were made using Analytik Jena S200 spectrometer (at 280 nm, respectively 520 nm).

The analytical methods used to characterize the above parameters are in accordance with European and OIV standards.

Wine samples, at 1.5 and 3 months, were analyzed for determining the basic physico-chemical parameters (alcohol strength (% vol.), total acidity (g/L $C_4H_6O_6$), volatile acidity (g/L $C_2H_4O_2$), relative density at 20 ˚C, reducing sugar (g/L), pH), but also the specific phenolic compounds parameters.

Analyses were done with the statistical software package Statgraphics Centurion XVI from StatPoint Technologies, Inc. (Warrenton, VI, USA). A cluster analysis, according to Ward's method, was carried out, which uses the total anthocyans as classifying variables (Fig. 3).

Table 1

Experimental design a Feteasca neagra wines

Sample code designation	Manufacturer	Produs type	Toasting level	Dose	Time
V0	-	Without products	-	-	3 months
V1	Amédée	Stave - fruité	French oak – Medium Toast	4 mini stave / 5L	3 months
V2	Amédée	Stave - vanillé	French oak – Medium plus Toast	4 mini stave / 5L	3 months
V3	Amédée	Stave - épicé	French oak – Heavy Toast	4 mini stave / 5L	3 months

RESULTS AND DISCUSSIONS

The general composition of Fetească neagră red wines (pH, alcoholic strength, total acidity, volatile acidity, relative density and reductive sugar) was analysed. We observed that the physical parameters were not affected by alternative process of maturation (Table 2).

During maturation occur many processes that modify the composition and organoleptic characteristics of the wine. Among these processes (physical, chemical and biological) we remember: the dissolution of wood components, condensation and deposition of phenolic compounds, modification of alcohol, aldehydes, acetals and esters contents and others.

Thermal process decomposes lignins in simple products and also can cause the formation of brown pigments by the effect of the Maillard reaction, favoured

by large amounts of sugars, the high temperature reached by grapes while raisining or the polymerization of phenol compounds (Moreno et al., 2007).

The pH of wine increased during the ageing process from 3.68 to 3.70 at V0 and V1, 3.64 to 3.69 at V2 and 3.65 to 3.68 at V3.

The alcohol strength doesn't present a significant variation between 1.5 and 3 months, which concludes that types of stave don't influence this parameter. Thought, we observed a decrease in alcohols during ageing caused by oxidation and esterification when ethanol is converted into other components. Acetaldehyde is the main organic by-product of ethanol metabolism, but other volatile compounds, notably acetic acid, butanediol, diacetyl, and acetoin, can also be formed.

Table 2

Physical-chemical characteristics of Fetească neagră wines

Var.	pH	Alcohol strength %	Total acidity (g/L $C_4H_6O_6$)	Volatile acidity (g/L $C_2H_4O_2$)	Relative density (g/cm^3)	Reductive sugar (g/L)
Analysis of 1.5 months						
V0	3.68±0.01[c]	14.96±0.17[c]	5.80±0.04[b]	0.56±0.01[c]	0.9675±0.02[c]	2.36±0.01[c]
V1	3.68±0.01[c]	14.94±0.24[a]	5.90±0.04[c]	0.57±0.01[d]	0.9728±0.03[d]	2.39±0.01[d]
V2	3.64±0.01[a]	14.95±0.14[b]	5.95±0.05[d]	0.55±0.02[b]	0.9624±0.03[a]	2.34±0.02[a]
V3	3.65±0.00[b]	14.95±0.09[b]	5.67±0.04[a]	0.53±0.02[a]	0.9643±0.03[b]	2.35±0.02[b]
Analysis of 3 months						
V0	3.70±0.01[c]	14.94±0.12[a]	5.78±0.00[c]	0.57±0.01[c]	0.9675±0.01[c]	2.36±0.00[c]
V1	3.70±0.00[c]	14.94±0.11[a]	5.80±0.04[d]	0.58±0.02[d]	0.9675±0.02[c]	2.36±0.01[c]
V2	3.69±0.00[b]	14.94±0.05[a]	5.63±0.00[b]	0.55±0.02[b]	0.9624±0.03[a]	2.34±0.01[a]
V3	3.68±0.01[a]	14.94±0.08[a]	5.60±0.16[a]	0.54±0.00[a]	0.9643±0.01[b]	2.35±0.02[b]

Different letters indicate significant differences at 95% confidence level. Data expressed as mean ± standard deviation.

For the total acidity, we observed that the results at 1.5 months range from 5.67 g/L tartaric acid (V3) and 5.95 g/L tartaric acid (V2) and at 3 months the values decrease and range between 5.60 g/L tartaric acid (V3) and 5.8 g/L tartaric acid (V1) in accordance with Cotea et al., 2009.

The volatile acidity values increase proportionally with ageing, due to the non-enzymatic oxidation process, that converts a part of ethyl alcohol into acetaldehyde and then into acetic acid, the main component of volatile acidity. The maximum value is 0.57 g/L acetic acid (V1) at 1.5 months and 0.58 g/L acetic acid (V1) at 3 months.

In figure 1 is presented the content of total phenolic index (D_{280}) for the two time periods chosen, where it can be observed that the maximum values are for the samples at 1.5 months and values decrease for samples at 3 months. The small variation between samples concludes that the types and toasting process of stave don't influence significantly the total polyphenolic index.

In figure 2 we can notice that the total quantity of anthocyanins (mg/L) decrease significantly at 3 months. The decreases in the anthocyanin contents during wine ageing must have resulted from the gradual conversion of monomeric compounds into more stable oligomers or polymers (Monagas et al., 2006). Taken into account that anthocyanins are the major source of colour in red wines, we observed that the anthocyanin composition of the studied wines changed considerably during ageing.

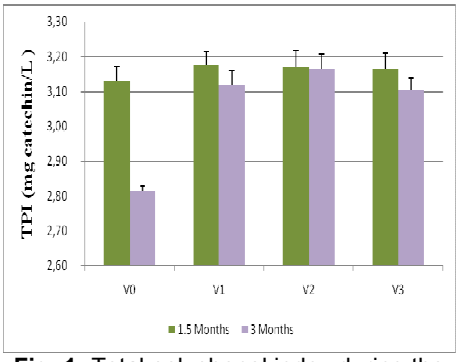


Fig. 1 -Total polyphenol index during the ageing Fetească neagră wines

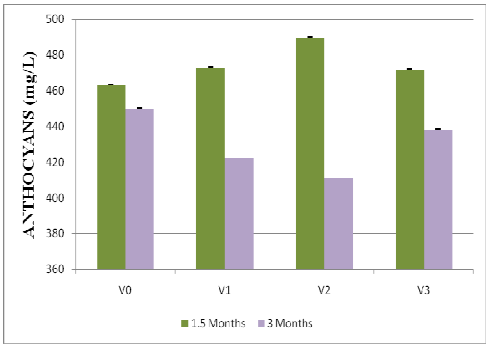


Fig. 2 - Total quantity of anthocyans during the ageing Fetească neagră wines

The maximum values recorded are 489,53 mg/L in variant V2 and 472.43 mg/L in variant V1, where V2 present the highest value for 1.5 months.

A cluster analysis according to Ward's method was carried out by using total anthocyans the as classifying variables (Fig. 3). In this work, cluster analysis was used to assess the similarity between variants analysed at 1.5 and 3 months. The distance at which the different groups were formed allowed us to differentiate sample analyzed at 1.5 and 3 months.

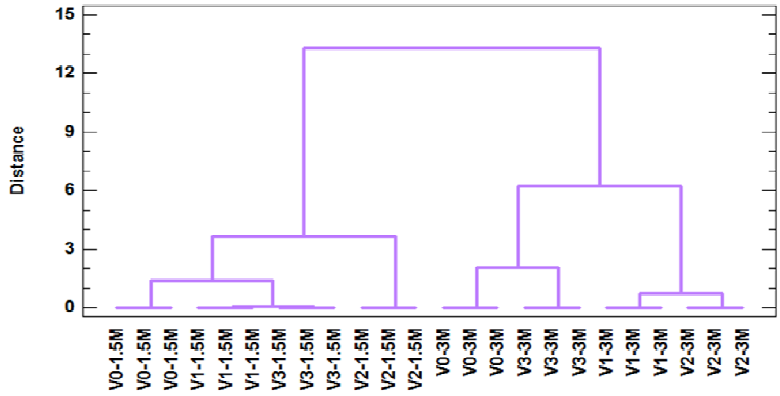


Fig. 3 - Cluster analysis performed with Ward's method and total anthocyans as classifying factors

CONCLUSIONS

1. According to the results obtained in this research, the trends of the phenolic compounds are very similar in the wines from all varieties at 1.5 months and 3 months. But the values at 3 months are slightly smaller then at 1.5 months.

2. The aging time decreased the total amount of anthocyans, as a cause of a series of mechanisms that can be related to these changes, such as adsorption by yeast, degradation and oxidation, precipitation with proteins and the progressive and irreversible formation of more complex and stable anthocyans derived pigments.

3. In conclusion the types and toasting stave don't influence significantly the physical chemical parameters, quantity of total polyphenolic index and anthocyans.

***Acknowledgments**: This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765. We are grateful to Campus de Excelencia Internacional Agroalimentario (ceiA3) and the International Doctoral Program of the University of Córdoba, in collaboration with Vitenol research Group.*

REFERENCES

1. Alonso A. M., Castro R., Rodríguez M. C., Guillén D. A., Barroso C. G., 2004 - *Study of the antioxidant power of brandies and vinegars derived from Sherry wines and correlation with their content in polyphenols*. Food Research International, 37, pp. 715–721.

2. Arapitsas P., Antonopoulos A., Stefanou E., Dourtoglou V. G., 2004 - *Artificial aging of wines using oak chips*. Food Chemistry, 86, pp. 563–570.

3. Canas S., Casanova V., Belchior A. P., 2008 - *Antioxidant activity and phenolic content of Portuguese wine aged brandies.* Journal of Food Composition and Analysis, 21, pp. 626–633.

4. Gómez García-Carpintero E., Gómez Gallego M. A., Sánchez-Palomo E., González Viñas M. A., 2012 - *Impact of alternative technique to aging using oak chips in alcoholic or in malolactic fermentation on volatile and sensory composition of red wines*. Food Chemistry, 134, pp. 851–863

5. Larrauri J. A., Sánchez-Moreno C., Rupérez P., Saura-Calixto F., 1999 - *Free radical scavenging capacity in the aging of selected red Spanish wines.* Journal of Agricultural and Food Chemistry, 47, pp. 1603–1606.

6. Santos-Buelga C., De Freitas V., 2009 - *Influence of phenolics on wine organoleptic properties*. In M. V. Moreno-Arribas, & M. C. Polo (Eds.), Wine chemistry and biochemistry (pp. 529–570). New York, NY: Springer Science and Business Media.

7. Stevanato R., Fabris S., Momo F., 2004 - *New enzymatic method for the determination of total phenolic content in tea and wine.* Journal of Agricultural and Food Chemistry, 52, pp. 6287–6293.

8. Zafrilla P., Morillas J., Mulero J., Cayuelas J. M., Martínez-Cachá A., Pardo F., 2003 - *Changes during storage in conventional and ecological wine: Phenolic content and antioxidant activity*. Journal of Agricultural and Food Chemistry, 51, pp. 4694–4700.

9. **OIV, 2012-** *International oenological codex*. Recueil des methodes internationales d'analyse des vins et des mouts. Office International de la Vigne et du vin, Paris

FLOR YEAST RESISTANCE TO ETHANOL AND ACETALDEHYDE HIGH CONTENTS

REZISTENȚA LA CONȚINUTURI RIDICATE DE ETANOL ȘI ACETALDEHIDĂ A LEVURILOR PELICULARE DE "FLOR"

MORENO-GARCÍA J.[1], VARARU F.[2], GARCÍA-MARTÍNEZ Teresa[1], MILLÁN M. Carmen[1], MAURICIO J.C.[1], MORENO J.[3]
e-mail: florinvararu@yahoo.com

Abstract. *In the elaboration of Sherry wines, flor yeast develops a biofilm on the wine surface after the alcoholic fermentation of grape which remains during the "biological aging" process. The aim of this study is to identify proteins that respond to high content of ethanol and acetaldehyde. A proteome analysis was carried for a flor yeast grown in a synthetic biological aging and in a reference fermentative condition. Further works dealing with genetics, and also utilization of different flor yeast strains could be considered and aimed to improve the quality of Sherry wines in a near future. 35 proteins were detected under the biological aging condition higher in abundance with respect to the reference condition related to ethanol resistance and 3 related to acetaldehyde resistance. This study together with genetic experiments may lead to the genetic improvement of flor yeast strains aimed to enhance the wine elaboration process.*
Key words: *Flor yeast, proteome, resistance, ethanol, acetaldehyde.*

Rezumat. *În elaborarea vinurilor de tip Sherry, levurile peliculare (Flor yeasts) formează un biofilm pe suprafața vinului după fermentația alcoolică, biofilm care rămâne în timpul procesului de "maturare biologică". Scopul acestui studiu este de a identifica proteinele care răspund la conținut ridicat de etanol și acetaldehidă. O analiză proteomică a fost efectuată pentru o levură pelliculară crescută într-un mediu sintetic de maturare biologică și în condiții fermentative de referință. Lucrări ulterioare legate de genetica levurilor și de utilizarea a diferite tulpini de levuri peliculare ar putea fi luate în considerare în scopul de a îmbunătăți calitatea vinurilor Sherry într-un viitor apropiat. 35 de proteine au înregistrat abundențe mai mari în cazul condițiilor de maturare biologică față de condițiile de referință în ceea ce privește rezistența la etanol și 3 proteine în ceea ce privește rezistența la acetaldehidă. Acest studiu împreună cu experimențe genetice poate duce la ameliorarea genetică a tulpinilor de levuri peliculare menite să îmbunătățească procesul de elaborare a vinului.*
Cuvinte cheie**:** *Levuri peliculare, proteomul, rezistența, etanol, acetaldehida*

INTRODUCTION

In some wine-producing regions around the world biological aging method is used for the elaboration of some special white types of wine known as Sherry wines. During this process, the final organoleptic properties of the

[1] Department of Microbiology, University of Córdoba, Spain
[2] University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania
[3] Department of Agricultural Chemistry, University of Córdoba, Spain

oenological product are modified principally due to the metabolism performed by peculiar yeast strains, so-called flor yeasts (Peinado et al., 2009).

Biological aging takes place after fermentation when flor yeasts become predominant (Esteve-Zaroso et al., 2001). In this moment, the medium is characterized by high ethanol content that result from a previous fermentative metabolism but also by a high acetaldehyde concentration due to a non-fermentable carbon metabolism developed by the yeasts when fermentation is over. In order to survive in the mentioned conditions, flor yeasts have the capacity to develop biological systems that ensure survival in this medium.

Protein alcohol dehydrogenase suggested by Mauricio et al. (1997) to fulfil a redox balance maintanance role in this type of medium and Hsp12p, both respond to ethanol and acetaldehyde high concentration meanwhile superoxide dismutases (Sod1p and Sod2p) are known to be implicated in the response to ethanol and oxidative stresses (Ma and Liu, 2010).

Until now, genes that codify stress response proteins have been used as targets for the genetic improvement of wine yeast strains to enhance the fermentation performance. Also, in the biological aging process, a higher quality in velum and a higher cell viability have been attained by the overexpression of response to stress genes.

In this study, we used a novel proteomic technique OFFGEL fractionator, coupled to LTQ Orbitrap XL MS to detect as much proteins as possible. All proteins related to acetaldehyde and ethanol stresses have been selected for the comparison with those in a reference fermentative condition and discussed subsequently. This knowledge alongside that provided by experiments aimed to definitively confirm the necessity of these proteins synthesis in the biological aging, can serve for the selection of target genes in order to construct flor yeast strains by biotechnological engineering and hence prolongs in time the yeast survival in the biological aging elaboration process.

MATERIAL AND METHOD

Inoculum. During 24 hours flor yeast *Saccharomyces cerevisiae* G1(ATCC: MYA-2451) was cultivated in a preinoculum medium composed by 200 mL of YPD (1% yeast extract: 2% peptone and 2% glucose). A population of 1 x 10^6 cells/mL was inoculated in the synthetic biological aging medium and in the fermentative medium.

Media. The reference fermentative medium was composed by 0,67% (w/v) YNB without amino acids (Difco), 17% (w/v) glucose and 10 mM of glutamic acid filled with distilled water until a total volume of 250 mL. The fermentations were performed at 21ºC. Cells were gathered centrifugally from broth cultures at 12 hours or middle of the exponential phase of growth when a population of 27,2 x 10^6 cells/mL was reached. This medium was used as reference trying to avoid typical biological aging stresses: high content of glucose (17%), no ethanol content and fermentable carbon metabolism through Crabtree effect. From now, the condition related to this medium will be referred as RFC (reference fermentative condition). The Biological aging medium was composed by 0.67% (w/v) YNB without amino acids (Difco), 1% w/v glycerol, 10 mM of glutamic acid

and 10% (v/v) ethanol in Erlenmeyer flasks until a volume of 250 mL cultivated at a temperature of 21 °C without shaking during 29 days when cells viability is about 90% and the velum completely formed (Mauricio et al., 1997). From now, the condition related to this medium, the biological aging condition will be referred as BAC. All media were autoclaved at 120ºC for 20 mins before the experiment. The number of yeast cells was determined in a Beckman Coulter particle counter Z2 performing previously the appropriate dilution and then mixing with a ratio 0.1:39.9 of Isoton dilution solution according to the recommendations. All experiments were carried out by triplicate in flasks closed with hydrophobic cotton.

Samples and proteome analysis. Cells from the fermentation culture were harvested by centrifugation and cells from the flor velum were collected by a suction system from the surface of each Erlenmeyer flask when the velum was fully formed. Final cell pellet obtained from each condition was resuspended in 10 mL of lysis buffer supplemented with Protease Inhibitor Cocktail tablets, and cells wall broken by vortexing in a Vibrogen Cell Mill V6 using a volume of glass beads equivalent to that of the cell pellet. Glass beads as well as cell debris were discarded by centrifugation.

Protein precipitation was carried out by overnight incubation at -20 °C after addition of 10% w/v of trichloroacetic acid and 4 volumes of ice-cold acetone to the supernatant.

After incubation, samples were centrifuged and the protein pellet was vacuum dried and then resuspended in solubilization buffer. Protein concentration was estimated by Bradford assay (1976) and samples stored at -80 °C until proteins analysis. OFFGEL High Resolution kit pH 3−10 was used for protein preparative isoelectric focusing (IEF) in solution.

Protein samples (500 µg approx. of fermentation yeast protein and 400 µg approx. of biological aging yeast) were solubilized in Protein OFFGEL fractionation buffer and aliquots were evenly distributed in a 12-well 3100 OFFGEL Fractionator tray according to the supplier instructions.

Peptides from each OFFGEL well were scanned and fragmented with the LTQ Orbitrap XL mass spectrometer equipped with a nano LC Ultimate 3000 system. The electrospray voltage was set to 1300 V and the capillary voltage to 50 V at 190 Cº. The LTQ Orbitrap was operated in the parallel mode, allowing for the accurate measurement of the precursor survey scan (400–1500 m/z) in the Orbitrap selection, a 60000 full-width at half-maximum (FWHM) resolution at m/z 400 concurrent with the acquisition of three CID Data-Dependent MS/MS scans in the LIT for peptide sequence, followed by three Data-Dependent HCD MS/MS scans (100–2000 m/z) with 7500 FWHM resolution at m/z 400 for peptide sequence and quantification. The normalized collision energies used were 40% for HCD and 35% for CID.

The maximum injection times for MS and MS/MS were set to 50 ms and 500 ms, respectively. The precursor isolation width was 3 Da and the exclusion mass width was set to 5 ppm. Monoisotopic precursor selection was allowed and singly charged species were excluded. The minimum intensity threshold for MS/MS was 500 counts for the linear ion trap and 8000 counts for the Orbitrap. Trypsin missed cleavages in peptides with a maxiumum value of 3 were detected.

Database search was performed with Proteome Discoverer 1.0 against Uniprot including fixed modification Carbamidomethylation in Cys and proteome results were statistically analyzed with the Proteome Discoverer program. The score of proteins was calculated by summing those from each peptide.

After identification, proteins related to the response to biological aging stresses (ethanol and acetaldehyde) were selected and sorted from both conditions attending to mutant phenotypes, gene ontology terms (GO Terms) or references using YeastMine tool from SGD and Uniprot databases.

In order to compare quantity of proteins detected in within and among conditions, a relative quantification has been carried by using the emPAI index or exponentially modified protein abundance index. The emPAI is calculated as follows: emPAI = 10^{PAI}–1. The PAI index is obtained by dividing the observed peptides of a specific protein in the analysis by the observable peptides.

The observable peptides were determined by using the "MS Digest" software. Fragmentation spectra matching the same peptide sequence but with different charge, modification state or containing missed cleavage (max. 3) sites were counted separately. The protein contents in each condition were calculated using the next equation: Protein content (% weigh)= [(emPAI x Mr)/(Σ(emPAI x Mr))] x 100.

RESULTS AND DISCUSSIONS

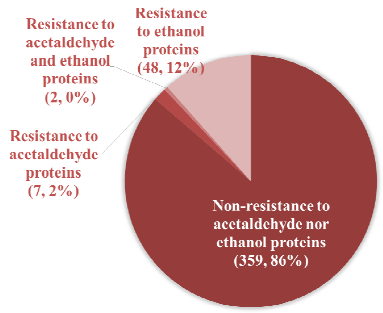
416 proteins were detected under the BAC condition and 611 under RFC.

Similar percentages in proteins related to acetaldehyde and ethanol resistances have been reported in both conditions (2,2% of acetaldehyde resistance proteins in BAC and 2,4% in RFC; 12,0% of ethanol resistance proteins in BAC and 12,9% in RFC) although proteins in each of them are different (Fig. 1).

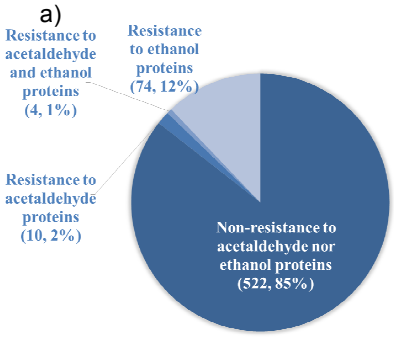
With regard to acetaldehyde, in a condition such as BAC, this molecule is produced throguh the ethanol degradation while it can also be produced as a sub-product of the glucose fermentation in RFC.

In this experiment, it reached a higher concentration under BAC than under RFC (3,7 mM and 0,3 mM, respectively) (Moreno-García et al., 2014). 3 proteins were detected with a higher acetaldehyde content under BAC and are known to be implicated in the following biological processes: cellular amino acid metabolic process (Arc1, Hom6p), pentose-phosphate shunt (Tkl1p) and tRNA aminoacylation for protein translation (Arc1p).

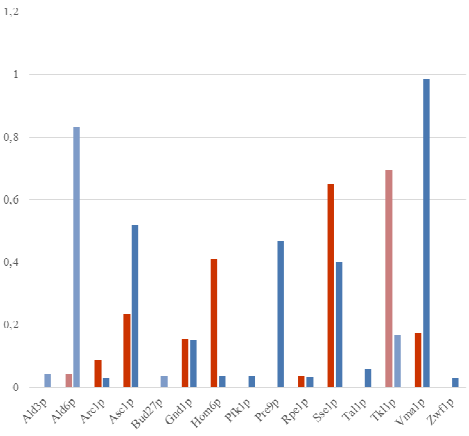
It has been reported that, acetaldehyde provokes mutations in the yeast chromosome DNA having a more deleterious effect than ethanol (Ristow et al., 1995) however much more proteins were related to ethanol resistance rather than acetaldehyde in the present study.



a)



b) Resistance to acetaldehyde



c) Resistance to ethanol

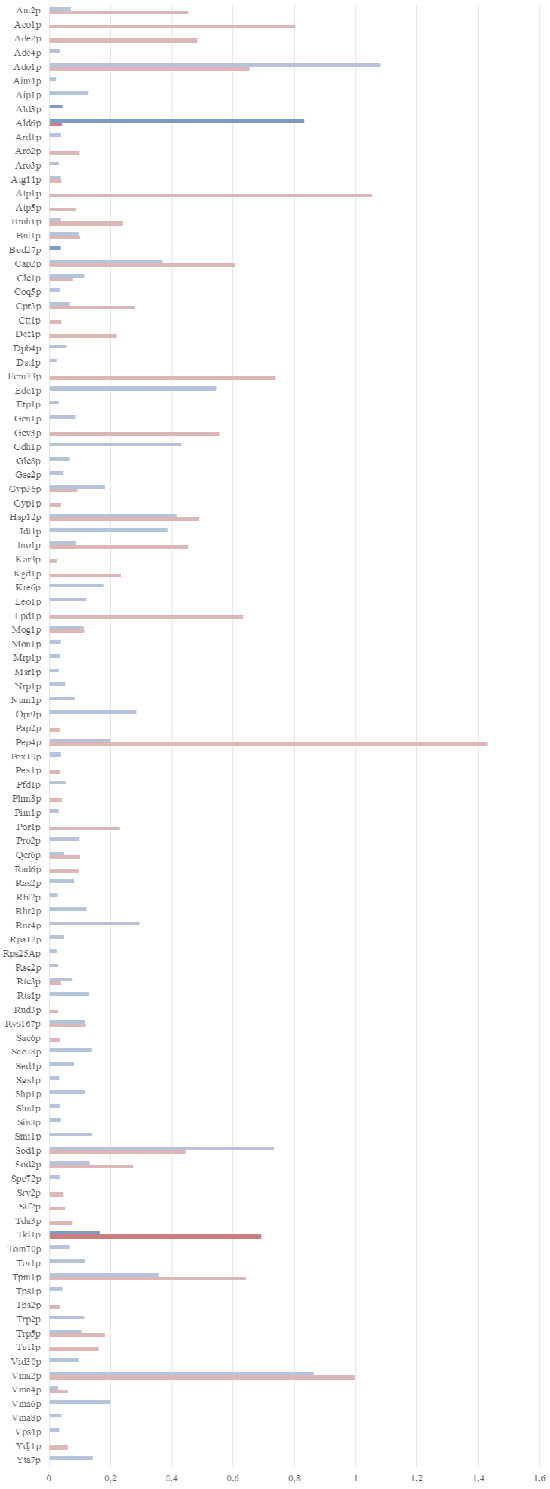


Fig. 1 - Cake diagrams showing the number and percentage of BAC (in red) and RFC (in blue) proteins related to acetaldehyde and ethanol stresses (a). Proteins related to the acetaldehyde resistance (b) and ethanol resistance (c).

Ethanol concentration reached a value of 1282 mM (7,8% v/v) at the time of sampling in BAC and 28 mM in RFC as result of the fermentative metabolism (equivalent to 0,16 % v/v) (Moreno-García et al., 2014). Although more proteins were identified under RFC, the protein content sum in BAC is higher than in RFC, this is 14,6 compared to 11,7, respectively. Etp1p or ethanol tolerance protein surprisingly was only found in RFC. It is known that this is needed to adapt efficiently to ethanol, either as sole carbon source or as cell stressor its gene expression is higher during the first stage of the stationary phase (Gasch et al., 2000). More abundant resistance to ethanol proteins in BAC were found to be involved mainly in biological processes like: nucleobase-containing small molecule metabolic process (Ade2p, Atp1p, Atp5p, Phm8p, Qcr6p and Tkl1p), cellular response to DNA damage stimulus (Bmh1p, Def1p, Pap2p and Rad6p) and carbohydrate metabolic process (Bmh1p, Ino1p, Tkl1p, Tps2p).

CONCLUSIONS

1. Although having acetaldehyde a more deleterious effect than ethanol, more ethanol resistance proteins were detected in the proteome analysis.

2. A similar percentage of proteins either related to the acetaldehyde and ethanol resistance were reported in BAC and in RFC.

3. The ethanol tolerance protein, Etp1p, was detected under RFC and not under BAC.

***Acknowledgments**: Spain's Ministry of Economy and Competitiveness and the European Community (FEDER), Grant RTA2011-00020-C02-02, MINECO-INIA-CCAA.*

REFERENCES

1. Esteve-Zaroso B., Peris-Toran M. J., García-Maiquez E., Uruburu F., Querol A., 2001 - *Yeast population dynamics during the fermentation and biological ageing of sherry wines*. Appl. Environ. Microbiol. 67, pp. 2056-2061.

2. Gasch A. P., Spellman P. T., Kao C. M., Carmel-Harel O., Eisen M. B., Storz G., Botstein D., Brown, P. O., 2000 - *Genomic expression programs in the response of yeast cells to environmental changes*. Mol. Biol. Cell., 12, pp. 4241-4257.

3. Ma M., Liu Z. L., 2010 - *Mechanisms of ethanol tolerance in Saccharomyces cerevisiae*. Appl. Microbiol. Biotechnol. 87, pp. 829-845.

4. Mauricio J. C., Moreno J. J., Ortega J. M., 1997 - *In vitro specific activities of alcohol and aldehyde dehydrogenases from two flor yeasts during controlled wine ageing*. J. Agric. Food Chem. 45, pp. 1967-1971.

5. Moreno-García J., García-Martínez T., Moreno J., Millán M. C., Mauricio J. C., 2014 - *A Proteomic and Metabolomic Approach for Understanding the Role of the Flor Yeast Mitochondria in the Velum Formation.* Int J Food Microbiol, 172, pp. 21-29.

6. Peinado R.A., Mauricio J.C., 2009 - *Biologically aged wines.* In: Moreno-Arribas, M.V., Polo, M.C. (Eds.), Wine Chemistry and Biochemistry. Springer, pp. 81-103.

7. Ristow H., Seyfarth A., Lochmann E. R., 1995 - *Chromosomal damages by ethanol and acetaldehyde in Saccharomyces cerevisiae as studied by pulsed field gel electrophoresis*. Mutat. Res. 326, pp. 165–170.

ISOLATION OF NEW TYPES OF YEASTS STRAINS FROM INDIGENOUS FLORA OF IAŞI VINEYARDS

NOI SUŞE DE LEVURI CU CARACTER ALCOOLIGEN RIDICAT IZOLATE DIN FLORA INDIGENĂ A PODGORIEI IAŞI

***NECHITA Ancuta*[1], *SAVIN* C.[1], *PAŞA Rodica*[1], *ZAMFIR C.I.*[2], *CODREANU Maria*[1]**
e-mail: ancuta.vasile@yahoo.com

Abstract. *The alcoholic fermentation of grapes must is highly desired when the yeasts are tolerant to high osmotic pressure due to high concentrated sugar and resistant to the toxic effects of the high alcohol concentration. In this context, the research led to the selections made from Iasi vineyard – Copou vine centre of new yeast strains with strong alcooligen character, Sacchoramyces genus. New strains of isolated and selected yeasts have the ability to achieve full alcoholic fermentation and has a good tolerance to high alcohol concentrations (15.0 to 16.0 vol % alcohol). They may also restart the alcoholic fermentation when the process was stopped at a concentration of 8.5 to 11.5% alcohol by volume and still have a residual sugar content of 60 - 100 g/L.*
Key words: *yeast, fermentation, tolerant*

Rezumat. *În procesul fermentaţiei alcoolice a mustului din struguri sunt foarte apreciate suşele de levuri tolerante la presiune osmotică ridicată, cauzată de concentraţi mari de zaharuri, şi rezistenţă la efectul toxic al concentraţiei mari de alcool. În acest context, cercetările întreprinse au dus la selecţia din plantaţiile centrului viticol Copou Iaşi a unor noi suşe de levuri cu puternic caracter alcooligen, aparţinând genului Sacchoramyces. Suşele noi de levuri izolate şi selectate au capacitatea de a realiza fermentaţia alcoolică integral şi posedă o bună toleranţă la alcool (15,0- 16,0 vol. % alcool). De asemenea, pot redeclanşa şi finaliza fermentaţiile alcoolice în cazul proceselor fermentative care s-au oprit la concentraţii de 8,5 – 11,5 vol. % alcool şi care au încă un conţinut în zaharuri rezidual de 60 – 100 g/L.*
Cuvinte cheie: *levuri, fermentaţie, toleranţă*

INTRODUCTION

In the alcoholic fermentation of grape musts are very popular yeast strains that are tolerant to osmotic pressure, caused by high concentrations of sugars and resistance to the toxic effects of high concentrations of alcohol. Through their use prevents stagnation of alcoholic fermentation, which can lead to the production of wines with a lower alcohol content and unfermented sugar residue. In recent years, stopping alcoholic fermentation is a phenomenon encountered in both large units and wine from small producers (Ekunsanmi and Odunfa S.A., 1990). For this reason wine research were discussed studies on the isolation of yeasts strains tolerant to high

[1] Reasearch and Development Station for Viticulture and Winemaking Iasi, Romania
[2] Oenological Research Center, Iasi Branch of the Romanian Academy, Iasi, Romania

concentrations of sugars and alcohol (Buescher W.A. et. Al., 2001; Oshoa, 2005; Tikka C. et.al., 2013).

In this paper we proposed the selection of yeast strains from Iasi vineyard – Copou vine, properties with high concentrations of alcohol tolerance, with the ability to restart, and to complete the alcoholic fermentation where it was stopped at a concentration of 8.5 to 11.5 % vol . alcohol and the residual sugar 60-100 g/L.

MATERIAL AND METHOD

New strains isolated in pure culture yeasts were preliminary tested in view of the technological (foaming, initiation and completion of alcoholic fermentation, clarity wine, yeast deposit type etc). Of the yeast strains tested were selected seven strains noted coded RF1, RF2 , RF3 , RF4 , RF5 , RF6 and RF7. The selected yeast strains, the next step was tested for tolerance to alcohol. Was used YPG culture medium (yeast extract, peptone, dextrose), after sterilization and cooling, alcohol was aseptically added in concentrations of 10%, 12%, 13%, 14% and 15%. Assessment of alcohol tolerance was performed by determining the dynamics (24, 48 and 72 hours), the optical density at 615 nm , and viability of yeast cells by staining smears with methylene blue. Yeast strains selected on the basis of tolerance to alcohol, the following experiment was verified using the sugar concentration must be 230 g/L and the wort was added to the rectified must in order to increase the sugar concentration 276 g/L. For each yeast strain to prepare a set of recipients with the capacity of 1000 mL were distributed in 750 mL of musts those inoculated with 4 x 10^6 cells/mL. Recipients were thermostated at 20°C. The wines were characterized physico - chemical OIV standards. The verification experiment refermentare capacity wines with sugar residue to use a wine with 11.5 % vol alcohol and 70 g/L sugars fermented. The amount of yeast inoculum was 8 x 10^6 cells/mL. The wines produced at the end of the experiment were characterized physico - chemical and organoleptic.

RESULTS AND DISCUSSIONS

With a view to the selection of new strains of yeast tolerant performance properties at high concentrations of alcohol have been studied 18 yeast strains have been isolated in pure culture. In the preliminary selection of all yeast strains isolated 11 yeast strains were eliminated by failure of technological characteristics: foaming, triggering delayed or stalled fermentation, producing quality wines with unsatisfactory organoleptic point of view. The selected yeast strains (RF1, RF2, RF3, RF4, RF5, RF6 and RF7) alcoholic fermentations were initiated by 18 to 20 hours, very little foam, and have completed the first 24 hours after 10 days the alcoholic fermentation, the wine to give dry, valued for organoleptic quality. Capacity development of yeast strains with alcohol concentrations of 10% vol., 12% vol., 13 % vol., 14 % vol., and 15 % vol. appreciated by determining the dynamics of the optical density at 615 nm of non inoculated culture medium. Increase in optical density indicates the ability of strains of yeast propagation under the conditions established in experiment (fig. 1 – 7).

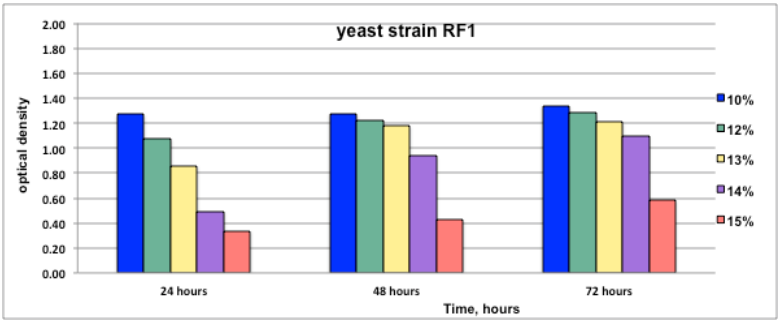


Fig. 1 - Determination of dynamic optical density, yeast strain RF1

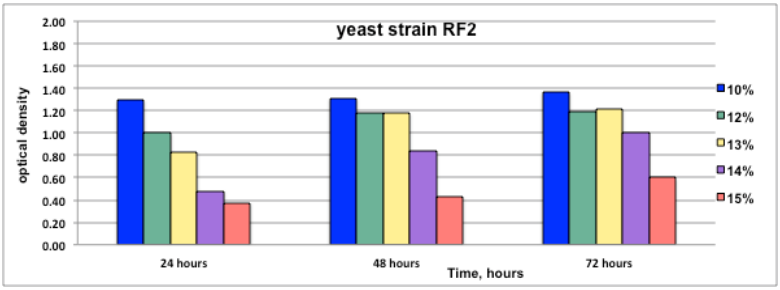


Fig. 2 - Determination of dynamic optical density, yeast strain RF2

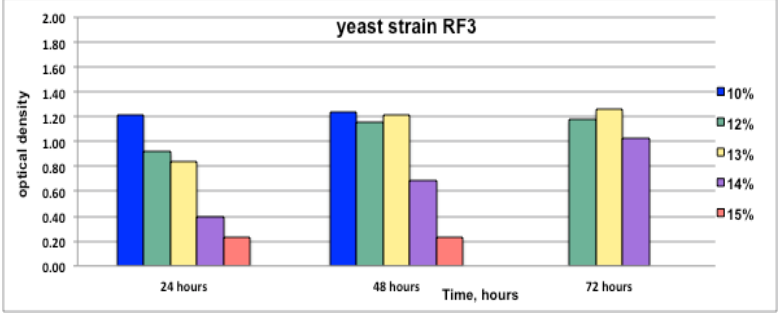


Fig. 3 - Determination of dynamic optical density, yeast strain RF3

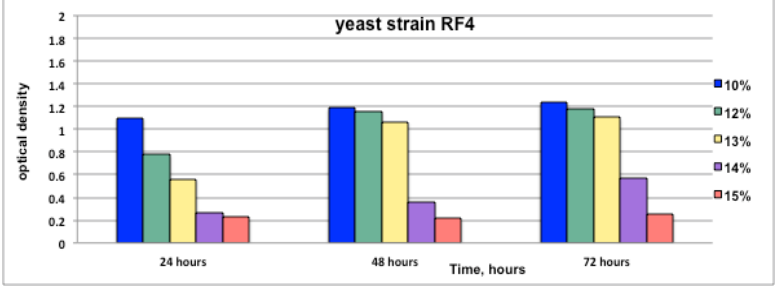


Fig. 4 - Determination of dynamic optical density, yeast strain RF4

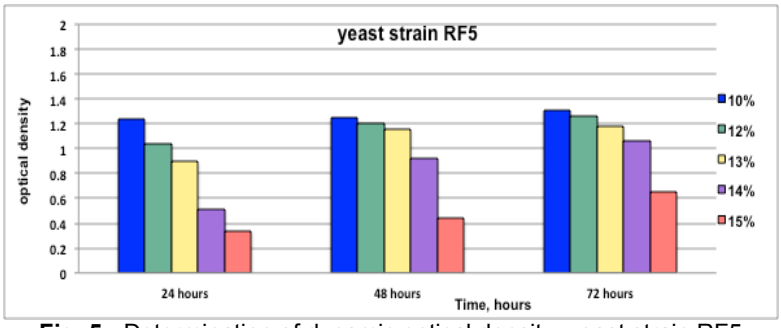


Fig. 5 - Determination of dynamic optical density, yeast strain RF5

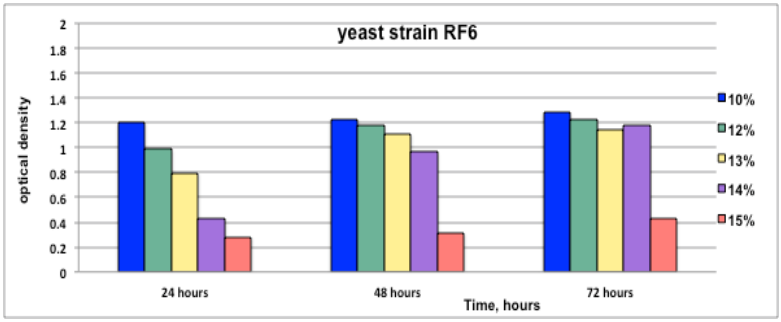


Fig. 6 - Determination of dynamic optical density, yeast strain RF6

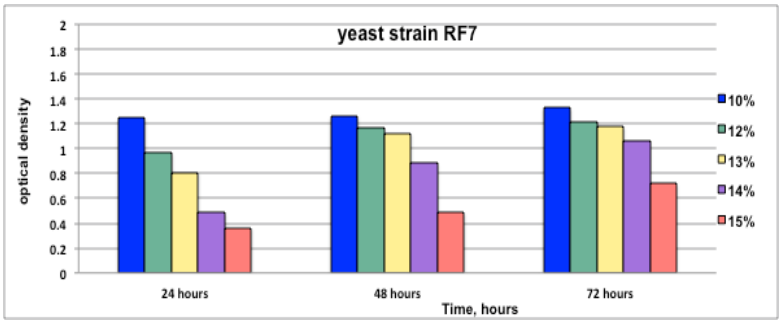


Fig. 7 - Determination of dynamic optical density, yeast strain RF7

After 24 hours of the onset of the experiment all strains tested reached the highest optical density at levels of 10% vol. alcohol. Along with increasing alcohol concentrations optical density values were becoming smaller. The results confirmed that the time lag in the concentration of alcohol 10% vol. is low and increases with increasing concentration.

In the following period of 48 hours the optical density increased to values close to those reached after 24 hours and yeast strains for the development of alcohol concentrations of 12-13 % vol. It was also an increase in the optical density of 42 % and 47 % alcohol concentrations of 14 % vol. Yeast strains for RF1, RF2, RF3 , RF5, RF6 and RF7 except strain RF4, whose optical density increased compared to the value at 24 hours only 26%.

Regarding the development of yeast strains for alcohol concentrations

of 15 % vol., optical density increased only by 10 % when RF6 strain, 20 % for RF1 and RF2 strains and 25 % for strain RF7. It will be appreciated that the alcohol concentration of 14-15 % vol. lag period of adaptation of yeast strains requires a longer time, namely 48 hours. Examination of methylene blue stained smears revealed that the number of viable cells as a percentage increase with increasing alcohol concentration.

Within 72 hours there was a progressive increase in the number of cells at concentrations of 14 % vol. alcohol, registering an increase of optical density by 13 % for yeast strain RF5, by 14% for yeast strain RF1, with 13 % for yeast strain RF2 and 17% pentru RF6 and RF7. Also, an increase in the optical density values found and for the development of yeast strains at a concentration that is 15 % alcohol and 27 % in the case of the yeast strains RF1, RF2 and RF6 and 30 % for yeast strains RF5 and RF7.

The data obtained by evaluating the dynamic optical density, it is clear that only five strains of yeast can be considered tolerant to alcohol concentrations of 15%. Yeast strains RF3 and RF4 are tolerant to high concentrations of alcohol, by means of the high number of viable cells after 24 hours, about 80 %.

Recovery capacity fermentation alcohol tolerant yeast strains was performed on must sugars concentrations of 230 g/L (table 1) and rectified concentrated grape must adjusted to the sugar concentration 276 g/L (tab. 2).

The amount of inoculum used was 4 x 10^6 cells/mL. Fermentations were conducted at 20°C. The wines were characterized physico - chemical and alcoholigenic results confirmed the ability of yeast strains tested in both alcoholic fermentation yielding dry wines with high alcoholic degrees depending on the sugar concentration musts used.

Table 1

Compositional characteristics of wines produced using yeast strains tolerant to alcohol the must with 230 g/L sugars

Physical parameters - chemical	Yeast strains tolerant the alcohol				
	RF1	RF2	RF5	RF6	RF7
Alcohol % vol.	13,1	13,1	13,2	13,6	13,6
Total acidity, g/L $C_4H_6O_6$	4,2	4,2	4,1	4,3	4,3
Volatile acidity, g/L CH_3COOH	0,32	0,36	0,32	0,36	0,36
Reducer extract, g/L	21,6	21,0	21,8	21,06	21,6
Non-fermented sugars, g/L	-	-	-	-	-

Table 2

Compositional characteristics of wines produced using yeast strains tolerant to alcohol the must with 276 g/L sugars

Physical parameters - chemical	Yeast strains tolerant the alcohol				
	RF1	RF2	RF5	RF6	RF7
Alcohol % vol.	15,9	15,7	15,9	15,8	16,0
Total acidity, g/L $C_4H_6O_6$	3,6	4,0	3,8	4,0	4,1
Volatile acidity, g/L CH_3COOH	0,48	0,51	0,48	0,36	0,45
Reducer extract, g/L	21,2	21,1	21,6	21,2	21,4
Non-fermented sugars, g/L	-	-	-	-	-

Ability alcohol tolerant strains in the refermented process was tested on a wine with an alcoholic strength of 11.5 % vol. and non fermented sugar residue of 70 g/L. Because during alcoholic fermentation which yielded this wine sold out a series of compounds essential for the development of yeasts (nitrogen, phosphorus), the wine used in the experiment was added 0.1 g diammonium phosphate and increased the amount of inoculum from the 8 x 10^6 cells / mL .

If the musts completion of the fermentation time was 10 days in this experiment , the length of the completion of fermentation was high, reaching 18 days. The wines were characterized physico - chemical (table 3), the data obtained showing that all strains tolerant to alcohol sugars are fermented entirely existing affording dry wine .

Table 3

Compositional characteristics refermented wine yeast strains using alcohol tolerance

Physical parameters - chemical	Yeast strains tolerant the alcohol				
	RF1	RF2	RF5	RF6	RF7
Alcohol % vol.	14,9	15,7	15,9	15,8	16,0
Total acidity, g/L $C_4H_6O_6$	3,9	4,1	4,0	4,0	4,1
Volatile acidity, g/L CH_3COOH	0,48	0,51	0,48	0,36	0,45
Reducer extract, g/L	21,8	20,9	21,6	21,8	21,4
Non-fermented sugars, g/L	-	-	-	-	-

CONCLUSIONS

1. Following isolation of new activity alcooligene yeast strains were isolated in pure culture 18 strains from Iasi vineyard – Copou vine and the preliminary test selection based on technological characteristics were selected seven strains of yeast.

2. Yeast strains selected checked for tolerance to alcohol concentration in the range of 10 to 15 % shows that five are tolerant to high concentrations of alcohol (14-15 %) namely RF1, RF2, RF5, RF6 and RF7.

3. In the alcoholic fermentation restart of stagnated at 11.5 % vol. alcohol and 70 g/L sugars, alcohol - tolerant yeast strains have restart and completed fermentations leading to the production of dry wines.

REFERENCES

1. **Buescher W. A., Siler C. E., Morris J. R., Threlfall R. T., Main G. L., Cone G. C., 2001 -** *High Alcohol Wine Production from Grape Juice Concentrates*. Am. J. Enol. Vitic 2001 vol. 52 no. 4, pp. 345 - 351
2. **Osho A., 2005 -** *Ethanol and sugar tolerance of wine yeasts isolated from fermenting cashew apple juice*. African J Biotechnol. no. 4, pp. 660–662.
3. **Tikka C, Osuru HP, Atluri N, Raghavulu PC, Yellapu NK, Mannur IS, Prasad UV, Aluru S, K NV, Bhaskar M, 2013 -** *Isolation and characterization of ethanol tolerant yeast strains.* Bioinformation vol. 9(8), pp. 421-425
4. **Ekunsanmi T. J., Odunfa S.A., 1990 -** *Ethanol tolerance, sugar tolerance and invertase activities of some yeast strains isolated from steep water of fermenting cassava tubers*. Journal of Applied Bacteriology vol. 69, pp. 672–675.

MAJOR AROMA COMPOSITION AND COLOR OF ALIGOTE WINES DEPENDING ON THE YEAST STRAINS

COMPUȘII MAJORITARI DE AROMĂ ȘI CULOAREA VINURILOR ALIGOTÉ ÎN FUNCȚIE DE SUȘELE DE LEVURI UTILIZATE

VARARU F.[1], MORENO-GARCIA J.[2], MORENO J.[2], NICULAUA M.[1], NECHITA C.B.[1], ZAMFIR C.I.[1], COLIBABA Cintia[1], DUMITRIU Georgiana-Diana[1], COTEA V.V.[1]
e-mail: florinvararu@yahoo.com

Abstract: *Must from Aligote grapes was homogenized and divided into nine batches to produce dry wine. The first eight batches were inoculated with different yeast strains in order to study the influence of the yeast strains to the major aroma compounds and color of wines. The ninth batch was left without inoculums for spontaneous fermentation, as control sample. 12 major volatile compounds were quantified by gas chromatograph – flame ionization detector (GC-FID) technique. Color measurements (CIELab parameters: L*, a*, b*, C^*_{ab}, and h_{ab}) were made in a Perkin Elmer Lambda 25 spectrophotometer (PerkinElmer, CA, USA), using 1 mm path length quartz cells, following the recommendations of the Commission Internationale de L'Eclariage (CIE, 2004). Significant differences in composition of volatile compounds and significant color differences were found depending on the yeast strain applied. The sensorial analysis of the wine samples by a tasting panel performed confirms the analytical results.*
Key words*: aroma compounds, wine color, yeast, sensorial analysis, Aligote*

Rezumat. *Mustul din soiul Aligote a fost omogenizat și împărțit în nouă loturi pentru producerea de vinuri seci. Primele opt loturi au fost inoculate cu diferite sușe de levuri cu scopul de a studia influența sușelor de levuri asupra compușilor de levuri majoritari și a culorii vinurilor. Al noulea lot a fost lăsat fără inoculum pentru fermentația spontană, ca proba martor. 12 compuși volatili majoritari au fost cuantificați cu ajutorul tehnicii gaz-cromatograf cu detector de ionizare prin flacara (GC-FID). Parametrii de culoare (parametrii CIELab: L*, a*, b*, C^*_{ab}, și h_{ab}) au fost măsurați cu ajutorul unui spectrofotometru Perkin Elmer Lambda 25(PerkinElmer, CA, USA),folosind celule de cuarț de 1mm grosime, urmând recomandările Commission Internationale de L'Eclariage (CIE, 2004). Au fost înregistrate diferențe semnificative de culoare și de compoziție în substanțe de aromă, în funcție de sușa de levuri utilizată. Analiza senzorială a probelor de vin realizată de 15 degustători confirmă rezultatele analitice.*
Cuvinte cheie*: compuși de aromă, culoarea vinurilor, levuri, analiza senzoriala, Aligote*

[1] University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania
[2] Department of Agricultural Chemistry, University of Córdoba, Spain

INTRODUCTION

Aligoté is one of the grape varieties that experienced a large appreciation in last years in Romania, being used for high quality dry wines. Unfortunately there are not many studies done for this variety.

The quality of any wine is based specially on its color and flavor. Both, the color and the flavor characteristics are the result of complex interactions among different factors, the most important being: grape variety, yeast strain and technical conditions of wine-making (Lilly et al., 2000). Also, the effect of yeast strain on volatile compounds differs according to the original grape must, as the same yeast strain could produce different results (Romano et al., 2003).

The aroma of wine can be classified accordingly to its origin, in varietal aroma, pre-fermentative aroma, fermentative aroma and post-fermentative aroma (Cotea et al., 2009, Moreno and Peinado, 2010). Although a number of flavor components are found in the original grape, the dominant and major compounds contributing to white wine aroma are formed during yeast fermentation (Patel and Shibamoto, 2003; Estévez et al., 2004) and are mainly higher alcohols, fatty acids, acetates, ethyl esters, aldehydes and ketones (Lilly et al., 2000; Estévez et al., 2004). It was demonstrated that not only the yeast species but also the particular strain of the individual species can influence the aroma of wines (Patel and Shibamoto, 2003; Romano et al., 2003).

The aim of this study is to determine the influence of some commercial yeast strains to the color and aroma composition of Aligoté wines from Odobesti vineyard, 2013 vintage.

MATERIAL AND METHOD

2.1. Wine samples

Wines obtained from Aligote grape variety from Vrancea region, Romania were studied. The wines were produced in the micro winery belonging to the Oenology department of the University of Agricultural Sciences and Veterinary Medicine, Iasi. Healthy ripe grapes of *Vitis vinifera* cv. Aligoté collected at full maturity were destemmed, crushed, and the must was homogenized and transferred to 9 glass containers in equal quantities, for the alcoholic fermentation. Eight different pure cultures of selected yeasts were added to unsterilized must, the 9-th must being left without inoculums, as a control (AV0). The pure yeast cultures were commercial *S. cerevisiae* sold under the name of: Fermol aromatic (AV1), Cross Evolution (AV2), Zymaflore X16 (AV3), Fermol Cryoarome (AV4), Fermactiv Thyol (AV5), Fermactiv AP (AV6), Fermactiv Muscat (AV7), and one *S. cerevisiae* yeast selected from Iasi vineyard by the Research and Development Station for Viticulture and Wine, Iaşi (AV8). The yeast strains selected are the most frequent yeasts used by the wine makers in this region for dry white wines.

2.2. General characteristics analyses

The analysis of pH, reducing sugars, titratable acidity and volatile acidity was performed by the official European Union methods (1990). The ethanol content was quantified by oxidation with dichromate according to Crowell and Ough (1979) and measuring the absorbance at 600nm. The absorbance at 280, 420, 520 and 620 nm

was measured in a spectrophotometer Perkin Elmer Lambda 25 using 10 mm path length glass cells, after filtering the samples through a HA-0.45 µm paper (Millipore, Bedford, MA, USA).

2.3. Gas-Chromatographic Quantification of Major Volatile Compounds and Polyols

Considering the most abundant alcohols (methanol, 1-propanol, isobutanol, isoamylic alcohol and 2-phenylethanol), two carbonyl compounds (acetaldehyde, and acetoin), three ethyl esters (ethyl acetate, ethyl lactate and ethyl succinate) and two polyols (glycerol and 2,3-butanediol), twelve wine aroma compounds were quantified by Gas-Chromatographic Analysis (GCA). An Agilent 6890 series plus gas chromatograph (Palo Alto, CA) with a fused silica 60 m long, 0.25 mm diameter and 0.40-µm film thickness CPWAX-57 CB column from Varian (Palo Alto, CA) was used. A quantity of 0.5 µL aliquots from 10 mL of wine or standard samples previously supplied with 0,5 mL of 2 g/L 4-methyl-2-pentanol as internal standard solution were injected into the injector of the GC instrument. The temperature program was as follows: 50 °C for 15 min and then raised to 190 °C at 4 °C/min and held for 35 min. The flow rate of the carrier gas (helium) was held at 0.7 mL/min for 16 min and then raised at 0.2 mL/min^2 to 1.1 mL/min for 52 min. The injector was equipped with an open tubular liner type in borosilicate glass, 4 mm of i.d., using glass wool at the center to ensure repeatability in the injection volume and no tapers for consistent split injection. A 1:30 split ratio and an injector temperature of 275 °C were used. The flame ionization detector temperature was 300 °C, and the hydrogen and air flow rates were 40 and 400 mL/min, respectively. A post run purge program at 200 °C for 25 min and a helium flow rate of 1.3 mL/min were used after the chromatographic peaks of interest were eluted. Each compound was quantified from the response factor provided by standard solutions analyzed as the wine samples. The chemical compounds used, preparation of standards and method validation was detailed by Peinado et al. (2004). Also, each compound was confirmed by using the Willey 7 N spectral library and a Mass Spectrometric detector model HP-5972-A from Agilent Technologies, coupled to the same GC equipment used for the quantification of standards and wine samples.

2.4. Colour measurements

Color measurements were made in a Perkin Elmer Lambda 25 spectrophotometer (PerkinElmer, CA, USA), using 1 mm path length quartz cells. The wine samples were filtered through Millipore- HA-0.45 µm paper filters (Bedford, MA, USA), prior to the spectrophotometric analysis.

The whole visible spectrum (380–770 nm) was recorded ($\Delta\lambda$ = 2 nm) and Illuminant D_{65} and 10º Observer were used in the calculations as standard conditions. The CIE-Lab parameters (L*, a*, b*, C^*_{ab}, h_{ab}) were determined by using the software COLVIN (PerkinElmer, CA, USA), following the recommendations of the Comission Internationale de L'Eclairage (CIE, 1986).

2.5. Sensory analysis

The wines were assessed for color, aroma and flavor acceptability by 15 tasters in a panel in accordance with ISO 8586-1:1993. The tasting room was kept at 20 ºC and wines served in tasting glasses certified and coded. Evaluation of the quality of the wines was made using the method according to ISO 4121:2003, with options of desirable (7–9), acceptable (4–6) and undesirable (1–3). The final punctuations were calculated as the mean, taking into account the evaluation of each taster.

RESULTS AND DISCUSSIONS

The general characteristics of wines are presented in tab.1. As it can be seen, the volatile acidity differs between 0,3 and 0,66 g acetic acid/L; ethanol between 10,82 and 12,38 %v/v, all the wines being semidry to semisweet wines.

Table 1.

Physical-chemical parameters (mean and standard deviation, n=3) of wines made with different yeast strains.

	pH	Volatile acidity (g/L Acetic acid)	Total acidity (g/L Tartaric acid)	Ethanol (%v/v)	Density	Reducing sugars (g/L)
AV0	3,09±0,02	0,47±0,02	6,2±0,1	11,05±0,1	0,996±0,005	20,6±0,1
AV1	3,24±0,01	0,49±0,02	6,7±0,09	10,89±0,07	0,987±0,001	24,99±0,01
AV2	3,22±0,005	0,45±6,8	7,1±0,04	10,82±0,02	1,004±0,0001	25,2±0,2
AV3	3,19±0,005	0,35±0,02	7,4±0,04	11,35±0,02	0,995±0,0004	19,7±0,01
AV4	3,28±0,005	0,66±0,01	6,8±0,09	12,38±0,3	0,975±0,003	6,5±0,05
AV5	3,25±0,005	0,52±0,02	7,1±0,01	12,33±0,08	0,973±0,001	8,98±0,01
AV6	3,18±0,005	0,37±0,02	7,5±0,04	10,94±0,08	0,9867±0,0006	23,56±0,01
AV7	3,2±0,01	0,3±0,01	6,9±0,04	11,73±0,31	0,9803±0,0008	14,15±0,01
AV8	3,23±0,01	0,35±0,02	6,7±0,04	11,33±0,03	0,992±0,001	14,4±0,01

Fig. 1 shows the major volatile compounds quantified in our samples. Regarding the content in alcohols, the highest values were registered at AV3 followed by AV6 and AV0, the lowest values being registered at the variant coded AV4. The highest concentration in polyols was registered at AV4 and AV2, AV2 and AV3 having the highest concentration in ethyl esters. AV1 is the sample that registered the highest concentration in carbonyl compounds which means that the yeast strain sold under the name of Fermol aromatic is the most suitable for obtaining wines rich in carbonyl compounds under the given experimental conditions. The most pleasant odor given by the ethyl esters was found in the wines fermented with the yeast strains sold under the name of Cross Evolution (AV2) and Zymaflore X16 (AV3). Also, high quantities of ethyl esters were registered at the blank sample, which means that the yeasts that are present in the must can be used to obtain wines with floral and fruity characteristics.

The chromatic parameters analyzed by CIELab method are presented in table 2. The lightness (***L****) parameter has values that varies between 92.8 and 99.5, with higher values registered at AV1 and AV5, indicating that the wines tend towards transparency. Four samples (AV1, AV3, AV5 and AV7) have negative values for coordinate a*, meaning that these wines have a greenish color; the other four samples registered positive values. It has to be mentioned that the differences between these values are small, all values being close to 0.

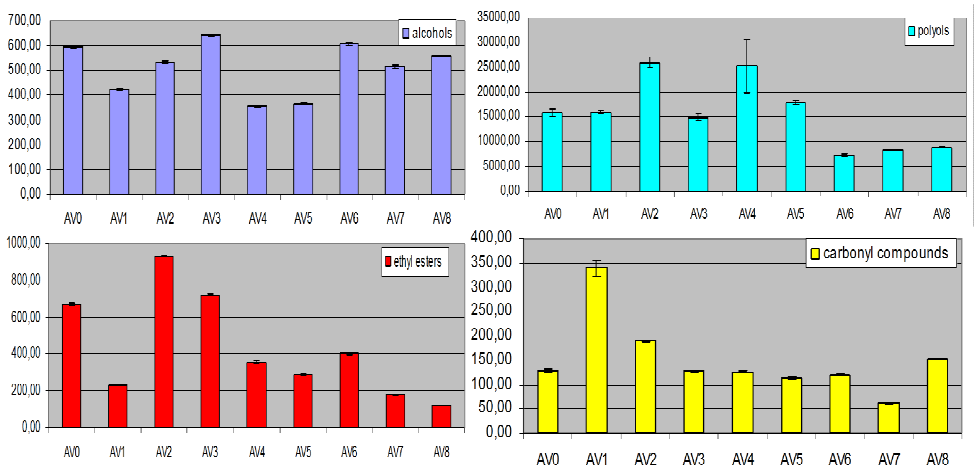


Fig. 1 - Major volatile compounds (mean of n=3 (mg/L) and standard deviation) quantified in wines made with different yeast strains

Regarding the chromaticity +b*/-b*, all the samples have positive values (yelow nuances prevail against the blue ones) with higher values for AV2 and AV4. Also, these two samples registered the highest color intensity out of all the 9 samples studied (Tab. 2).

Table 2

Chromatic parameters of wines made with different yeast strains

Sample	Luminosity L*	Chromaticity +a*/-a*	Chromaticity +b*/-b*	Saturation C*	Tonality H*	Intensity	Color hue
AV0	96,07	0,05	6,57	6,58	91,09	0,20	2,93
AV1	99,13	-0,25	2,77	2,78	95,06	0,06	3,82
AV2	92,80	0,43	10,65	10,66	87,68	0,36	2,18
AV3	97,45	-0,21	3,91	3,92	93,14	0,13	2,34
AV4	93,73	0,43	10,32	10,33	87,61	0,32	2,27
AV5	99,48	-0,32	3,31	3,32	95,47	0,05	5,63
AV6	94,51	0,13	9,73	9,73	89,26	0,29	2,44
AV7	98,57	-0,19	3,48	3,49	93,17	0,08	2,85
AV8	92,88	0,39	8,38	8,39	87,31	0,33	1,95

The sensorial analysis carried out by 15 tasters confirms the analytical results. Samples AV2 and AV7 were mostly appreciated by the majority of the tasters (fig. 2).

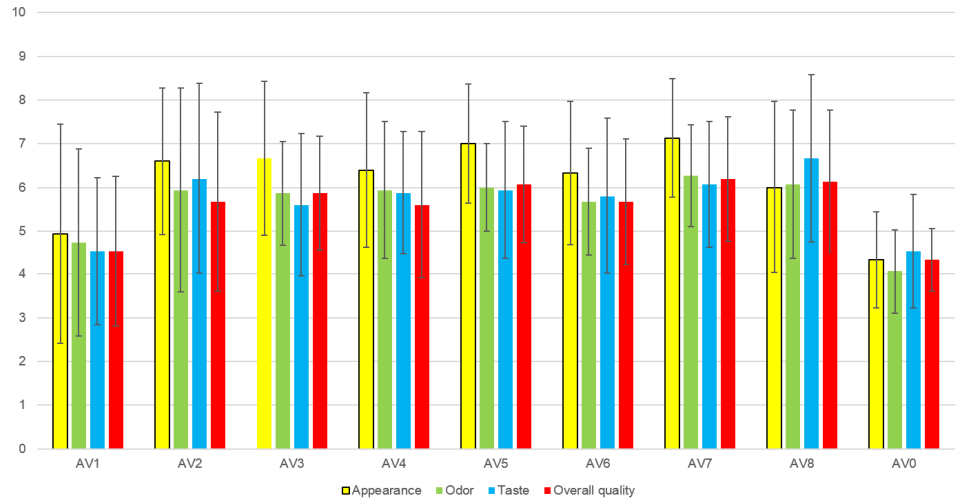


Fig. 2 - Sensorial analyses of wines made with different yeast strains

CONCLUSIONS

The yeast strains used influence the general characteristics of wines.

1. 12 major volatile compounds have been quantified, grouped in four chemical groups (alcohols, polyols, ethyl esters and carbonyl compounds). The quantity of each compound varied depending on the yeast strain used.

2. There are color differences between the studied wines.

3. The sensorial analysis carried out confirms the analytical results.

Acknowledgment: *This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765. We are grateful to Campus de Excelencia Internacional Agroalimentario (ceiA3) and the International Doctoral Program of the University of Córdoba, in collaboration with Vitenol research Group.*

REFERENCES

1. Cotea V. D., Zănoagă C. V., Cotea V. V., **2009** - *Tratat de Oenochimie, vol. 1.,* Editura Academiei Române, București.

2. Estévez P., Gil M.L., Falqué E., **2004** - *Effects of seven yeast strains on the volatile composition of Palomino wines*. International Journal of Food Science and Technology 39, pp. 61–69.

3. Lilly M., Lambrechts M.G., Pretorius L.S., **2000** - *Effect of increased yeast alcohol acetyltransferase activity on flavour profiles of wine and distillates*. Applied and Environmental Microbiology 66, pp. 744–753.

4. Moreno J., Peinado R., **2012** - *Enological Chemistry*, Academic Press.

5. Patel S., Shibamoto T., 2003 - *Effect of different yeast strains on the production of volatile components in Symphony wine*. Journal of Food Composition and Analysis 16, pp. 469–476.

6. Romano P., Fiore C., Paraggio M., Caruso M., Capece A., 2003 - *Function of yeast species and strains in wine flavour.* International Journal of Food Microbiology 86, pp. 169–180.

EVALUATION OF CHLOROGENIC ACID AND TOTAL PHENOLIC CONTENT OF GREEN COFFEE (*COFFEA CANEPHORA*) DRIED BEANS

EVALUAREA CONȚINUTULUI DE ACID CLOROGENIC ŞI TOTAL FENOLIC DIN BOABELE USCATE DE CAFEA VERDE (*COFFEA CANEPHORA*)

***FILIMON V.R.*[1], *ROTARU Liliana*[1], *PATRAŞ Antoanela*[1], *FILIMON Roxana*[1]**
e-mail: razvan_f80@yahoo.com

Abstract. *Chlorogenic acids are a group of cinnamic acid derivatives with biological effects mostly related to their antioxidant and antiinflammatory activities. This paper aims to determine the chlorogenic acid (CGA) and total phenolic (TP) concentration in various solvent extracts of green coffee beans (Coffea canephora syn. Coffea robusta). Also, the influence of brewing time (water at 90 °C) on the extraction of CGA was analyzed. 70% methanol and 70% 2-propanol were the most effective solvents in extraction of CGA from dried grinded green coffee beans of C. canephora (34.80±0.21 mg CQAE/g d.w. and 31.20±0.32 mg CQAE/g d.w., respectively). The most important CGA concentration was registered after 10 to 15 minute of coffee brewing (38.20±0.24 mg CQAE/g d.w.), when CGA represents approximately 45% of total phenolic compounds. Data obtained are useful both for experts from the food (functional food) and pharmaceutical industry, and also for traders and consumers.*
Key words: *chlorogenic acid, phenolic compounds, solvent extraction, time of brewing, Coffea canephora*

Rezumat. *Acizii clorogenici reprezintă un grup de derivaţi ai acidului cinamic cu proprietăţi biologic active datorate în principal activităţii lor antioxidante şi antiinflamatoare. Lucrarea are ca scop determinarea concentraţiei de acid clorogenic (ACG) şi a totalului de compuşi fenolici (TF) a extractelor de cafea verde (Coffea canephora sin. Coffea robusta) obţinute utilizând diferiţi solvenţi. De asemenea, a fost analizată influenţa timpului de infuzie (apă, 90 °C) asupra extracţiei ACG. Metanol 70% şi 2-propanol 70% au fost solvenţii cei mai eficienţi în extracţia ACG din boabele uscate şi măcinate de cafea verde C. canephora (34,80±0,21 mg EACQ/g m.u., respectiv 31,20±0,32 mg EACQ/g m.u.). Cea mai importantă concentraţie de ACG a fost înregistrată între 10 şi 15 minute de infuzare a materialului vegetal (38,20±0,24 mg EACQ/g m.u.), când ACG a reprezentat circa 45% din totalul compuşilor fenolici. Datele obţinute sunt utile atât de specialiştilor din industria alimentară (alimente funcţionale) şi farmaceutică, cât şi comercianţilor şi consumatorilor.*
Cuvinte cheie: *acid clorogenic, compuşi fenolici, extracţie cu solvenţi, timp de infuzare, Coffea canephora*

[1]University of Agricultural Sciences and Veterinary Medicine Iasi, Romania

INTRODUCTION

Chlorogenic acids (CGA) are phenolic compounds, secondary metabolites in plants derived from phenylalanine, formed through the esterification of cinnamic acids, such as caffeic, ferulic, and *p*-coumaric acids, with quinic acid (Clifford, 1999; Vermerris and Nicholson, 2006).

A series of health benefits have been associated with the consumption of CGA in the last decade, such as reduction of the risk of cardiovascular disease, type II diabetes, Alzheimer, and also antibacterial and antiinflammatory activities (Almeida et al., 2006; Santos et al., 2006; Farah et al., 2008). Moreover, it was demonstrated that CGA have protective effects against liver, colon and tongue carcinogenesis (Glezer, 2003), and reducing of DNA damages (Bakuradze et al., 2011). On the other hand, the reducing of the absorption of different types of sugar from the gastrointestinal tract due to consumption of CGA was recently reported (Bakuradze et al., 2011; Vinson et al., 2012).

Coffee plant is an evergreen shrub belonging to the *Rubiaceae* family, genus *Coffea*. Among the 85 species of this genus only two are of commercial importance: *Coffea arabica* and *Coffea canephora*, commonly known as „*arabicas*" and „*robustas*" (Flament, 1995).

Coffee is one of the most frequently consumed beverage worldwide. Since the 1960s and 1970s, epidemiological studies have shown that consumption of three or more cups of coffee per day may reduce several risk factors for obesity. Phenolic compounds other than caffeine that are found in coffee may contribute to these effects (Pimentel et al., 2014). The intake of green coffee (unroasted) has been associated with a lower risk of diseases of oxidative etiology apparently related to its high phenolic content (Baeza et al., 2014). At least five major groups of chlorogenic acid isomers were identified in green coffee beans: caffeoylquinic acids, dicaffeoylquinic acids, feruoylquinic acids, coumaroylquinic acids and caffeoylferuloylquinic acids (Shahidi and Naczk, 2004). Literature data indicate that „robustas" coffee has a substantially higher content of chlorogenic acid than „arabicas", varying from 7 to 10% of dry weight (d.w.) (Shahidi and Naczk, 2004).

In recent years, clinical studies conducted on humans and animals regarding the efficacy of green coffee beans and extracts in weight loss, suggest that CGA intake is an effective method in reducing weight, and might be considered as a less expensive means of preventing obesity (Vinson et al., 2012).

MATERIAL AND METHOD

Coffea canephora (syn. *Coffea robusta*) green beans were imported from India (under the name of „Indian Cherry") by local romanian traders. Coffee „dry processing" (also known as „unwashed" or „natural coffee") was used. The entire coffee cherry after harvest was first cleaned and then placed in the sun to dry in thin layers. The dried cherries were stored in bulk until they were sent to hulling, sorting and grading. Green coffee was transported in jute bags and milled 2 days before extraction.

Weight of 100 beans, moisture content (drying oven, 4 hours at 105 °C; ISO 1446:2001) and total mineral concentration (calcination oven, 4 hours at 525 °C; SR EN 1135:1997) of dried beans were determined.

The milled coffee beans were passed through a sieve with a 0.7 mm aperture. The ground material was extracted by shaking a 1 g of sample in a screw-capped tube with the following solvents: pure water, acetone (99.98%), 70% 2-propanol, 70% methanol and 70% ethanol, for a minimum of 30 minutes. The suspension was allowed to settle and decanted onto a Whatman No. 1 filter paper. The residue was re-extracted five times, the filtered extracts were bulked and diluted to 100 mL with solvent (Ohiokpehai, 1982).

Chlorogenic acid content was quantified using the molybdate assay proposed by Clifford and Wight, 1976 and presented by Chan et al., 2011. Molybdate reagent was prepared by dissolving 16.5 g sodium molybdate, 8.0 g dipotassium hydrogen phosphate, and 7.9 g potassium dihydrogen phosphate in 1 L distilled water (pH 6.5). An aliquot of each sample (0.2 mL) was added to 10 mL of molybdate reagent and mixed. This solution was examined spectrophotometrically (UV-vis Shimadzu 1700 Pharmaspec) against a blank (sample and buffer without sodium molybdate) at 370 nm. Different concentrations of caffeoylquinic acid solutions was used for expressing the results as caffeoylquinic acid equivalent (CQAE). The calibration equation used for CGA estimation was that proposed by Ohiokpehai (1982): $y = 0.003x + 0.0296$ ($R^2 = 0.9971$), where y represents absorbance while x is concentration of caffeoylquinic acid in mg/250 mL.

Total phenolic content was determined by Folin-Ciocâlteu colorimetric method, measuring the absorbance at 750 nm (Singleton and Rossi, 1965). A calibration curve of different concentrations of gallic acid solutions was used for expressing the results as gallic acid equivalent (GAE), with the equation $y = 0.8757x + 0.0438$ ($R^2 = 0.991$), where y represents concentration of gallic acid in mg/mL, while x is the absorbance. For obtaining the CGA percentage of total phenolic compounds, the results were also expressed as mg CQAE by equation mentioned.

All samples were examined in triplicate and standard deviation was mentioned.

The method used to discriminate among the means was Fischer's least significant difference procedure at 95% confidence level. P values lower than 0.05 ($p \leq 0.05$) were considered to be significant.

RESULTS AND DISCUSSIONS

Weight of 100 beans of *Coffea canephora* samples varied from 22 to 28 g, with a mean of 24.67±3.06 g, the values being within the range previously reported by Sureshkumar et al. (2013).

Moisture content of green beans influences the stability during storage and alters sensorial quality of end product, thus, a moisture content ranging between 8.0% and 12.5% is considered to be adequate (Reh et al., 2006). Moisture content of coffee beans analysed was about 11.42±0.94%, with a total mineral content, represented by ash, of 4.08±0.81%, in accordance to Bicho et al. (2012), which presented a mineral content in "robusta" green beans of about 4% (3.6%–4.8%).

The extraction of CGA into the coffee beverage depends on a variety of factors like: the proportion of grinded coffee to water, coffee freshness, grinding size, method of coffee brewing (temperature and time) (Shahidi and Naczk, 2004).

Concentration of chlorogenic acids in dry green coffee infusions (water at 90 °C) has increased during first 5 to 15 minutes of brewing, with a maximum of 38.20±0.24 mg CQAE/g d.w. (after 15 minutes), followed by a decrease in concentration, the trend line forming a plateau after 20 to 30 minutes of infusion (fig. 1). According to Shahidi and Naczk (2004), holding the coffee brew at elevated temperatures results in a loss of CGA, depending on the temperature and time; about 15% of chlorogenic acid was lost after 24 h at 83°C.

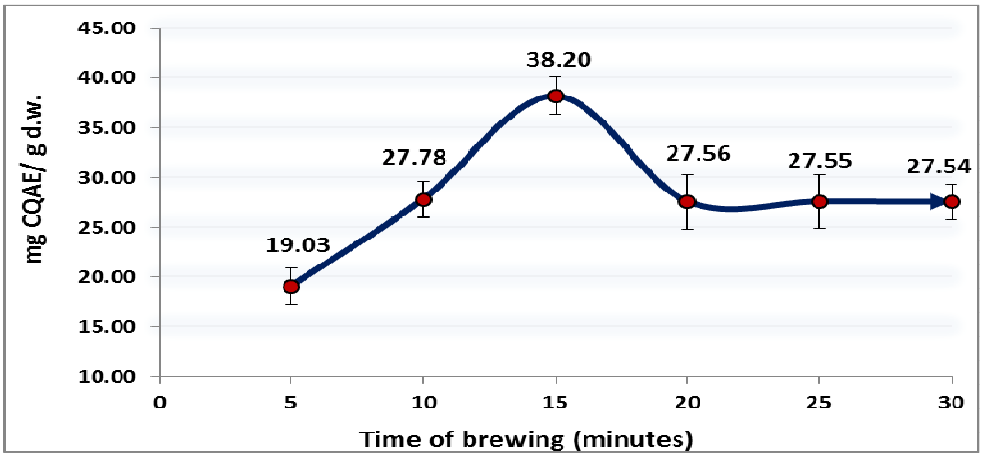


Fig. 1 - Concentration of chlorogenic acid (mg CQAE/g d.w.) in green coffee infusions (90°C) during brewing

Acetone does not seems to be specific for CGA extraction as long as the its concentration in acetone extract was only 4.48±1.02 mg CQAE/g d.w. 70% methanol was 7.76 times more effective than acetone, 1.33 times than water and only 1.12 times more effective than 70% 2-propanol (fig. 2).

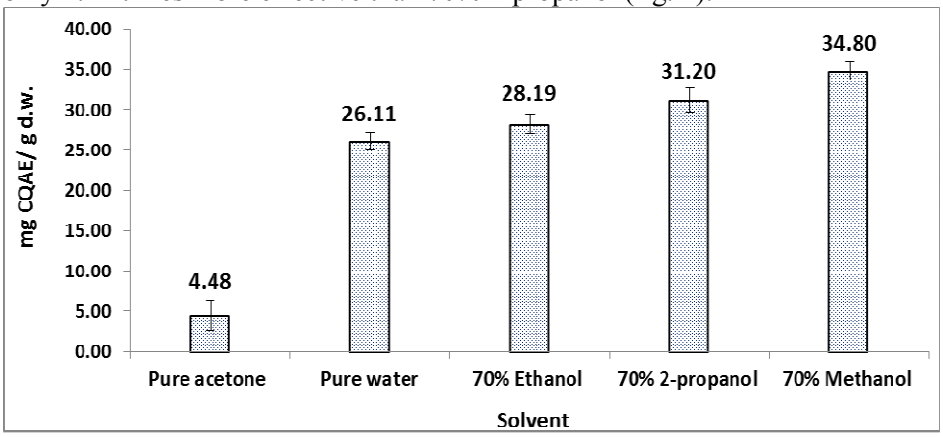


Fig. 2 - Concentration of chlorogenic acid (mg CQAE /g d.w.) in green coffee extracts obtained with different solvents

Regarding total phenolic content (TP) of green coffee infusions, positive statistically significant differences to the mean, were determined only for the values recorded after 10 and 15 minute of infusion, when the percentage of CGA from TP increased to a maximum of 45.18±0.68% (table 1).

Total phenolic content expresed as mg GAE, is useful in order to allow comparison with other species and to compare data reported for similar species. Thus, total phenolic content of analysed beans (23 mg GAE/g d.w.) was higher than that reported for *C. arabica* (21.00 mg GAE/g d.w.), but lower than that for *C. canephora* green beans (26.08 mg GAE/g d.w.) (Komes and Voivodic, 2014).

Table 1

Total phenolic content (TP) of green coffee infusions (water at 90 °C) during brewing and the percentage of chlorogenic acid (CGA) from TP

Time (min.)	TP (mg GAE/g d.w.)	St. error.	TP (mg CQAE/g d.w.)	St. error.	% of CGA from TP	St. error.
5	15.81^{000}	1.02	80.31^{000}	5.18	23.70^{000}	1.20
10	16.61^{***}	0.84	85.02^{***}	4.27	32.67^{000}	0.98
15	16.53^{***}	0.65	84.56^{***}	3.30	45.18^{***}	0.68
20	15.83^{000}	0.87	80.46^{000}	4.42	34.26^{***}	1.04
25	15.82^{000}	1.10	80.41^{000}	5.59	34.26^{***}	1.02
30	15.83^{000}	0.55	80.43^{000}	2.79	34.24^{***}	0.43
Mean	16.07	0.39	81.87	2.27	34.05	6.83
CV%	2.41	-	2.77	-	20.06	-

Note: Data expressed as mean values with standard error (n = 3). $^{NS, *, **, ***}$ - indicate nonsignificant and positive significant (at $p \leq 0.05$, 0.01, 0.001) differences to the mean; $^{o, oo, ooo}$ - indicate negative significant (at $p \leq 0.05$, 0.01, 0.001) differences to the mean. CV% - coefficient of variability (ratio of standard error to the mean (%)).

In the case of extraction with various solvents, total phenolic content of green coffee extracts followed the same trend as for CGA, the percentage of CGA from TP being maximum (24.30±1.19%) in 70% 2-propanol extracts (table 2).

Table 2

Total phenolic content (TP) of green coffee extracts obtained with different solvents and the percentage of chlorogenic acid (CGA) from TP

Solvent	TP (mg GAE/g d.w.)	St. error.	TP (mg CQAE/g d.w.)	St. error.	% of CGA from TP	St. error.
Pure acetone	9.07^{000}	1.02	37.22^{000}	4.18	12.05^{000}	1.12
Pure water	18.60^{**}	0.98	109.74^{***}	5.82	25.69^{***}	1.01
70% ethanol	19.06^{***}	1.23	113.20^{***}	7.26	23.06^{***}	1.64
70% 2-propanol	21.05^{***}	1.11	128.40^{***}	6.77	24.30^{***}	1.19
70% methanol	23.26^{***}	0.65	145.20^{***}	4.06	23.97^{***}	1.01
Mean	18.21	5.43	106.75	41.33	21.81	5.54
CV%	29.82	-	38.71	-	25.39	-

Note: Data expressed as mean values with standard error (n = 3). $^{NS, *, **, ***}$ - indicate nonsignificant and positive significant (at $p \leq 0.05$, 0.01, 0.001) differences to the mean; $^{o, oo, ooo}$ - indicate negative significant (at $p \leq 0.05$, 0.01, 0.001) differences to the mean. CV% - coefficient of variability (ratio of standard error to the mean (%)).

Statistical analysis of the data revealed a negative significant difference to the mean only for acetone extraction of phenolic compounds.

CONCLUSIONS

1. The most important concentration of chlorogenic acids from dried green coffee beans of *C. canephora* was registered after 15 minute of coffee brewing (38.20±0.24 mg CQAE/g d.w.), when CGA represents approximately 45% of total phenolic compounds ($p<0.05$).

2. 70% methanol and 70% 2-propanol were more effective in extraction of chlorogenic acids from green coffee beans in comparison to acetone, pure water and 70% ethanol; total phenolic content of extracts following a similar trend.

3. Temperature appears to be a decisive factor in the extraction of CGA from green coffee; hot water (90-93 °C) extracted, at the maximum point (15 min), an additional CGA percentage of 32% comparing to cold water (25 °C).

4. High amounts of chlorogenic acid in green coffee *C. canephora* beans, justify their consumption as *functional food* in preventing diseases and obesity.

***Acknowledgments**: This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. **Almeida A.A., Farah A., Silva D.A.M., Nunam E.A., Gloria M.B.A., 2006** - *Antibacterial activity of coffee extracts and selected coffee chemical compounds against enterobacteria*. J. Agric. Food Chem. Vol. 54, pp. 8738–8743.
2. **Baeza G., Amigo-Benavent M., Sarriá B., Goya L., Mateos R., Bravo L., 2014** - *Green coffee hydroxycinnamic acids but not caffeine protect human $HepG_2$ cells against oxidative stress.* Food Research Int. Vol. 62, pp. 1038–1046.
3. **Bakuradze T., Boehm N., Janzowski C., Lang R., ..., Baum M., 2011** - *Antioxidant-rich coffee reduces DNA damage, elevates glutathione status and contributes to weight control: results from the intervention study*. Mol. Nu. Food Res. Vol. 55, pp. 793–797.
4. **Bicho N.C., Leitão A.E., Ramalho J.C., Lidon F.C., 2012** - *Use of colour parameters for roasted coffee assessment.* Ciênc. Tecnol. Aliment. Vol. 32, no.3, pp. 436-442.
5. **Chan W.C., Lim Y.Y., Shiau P.T., 2011** - *Standardised herbal extract of chlorogenic acid from leaves of Etlingera elatior Eric.* Pharmacog. Res. Vol. 3 (3), pp. 178–184.
6. **Clifford M.N., 1999** - *Chlorogenic acids and other cinnamates - nature, occurrence, and dietary burden*. J. Sci. Food Agric. Vol. 79, pp. 362–372.
7. **Clifford M.N., Wight J., 1976** - *The measurement of feruloylquinic acids and caffeoylquinic acids in coffee beans. Development of the technique and its preliminary application to green coffee beans*. J. Sci. Food Agr. Vol. 27(1), p. 73-84.
8. **Farah A., Monteiro M., Donangelo C.M., Lafay S., 2008** - *Chlorogenic acids from green coffee extract are highly bioavailable in humans*. The J. of Nutrition. Vol. 138, no. 12, pp. 2309-2315.
9. **Flament I., 1995** - *Coffee, cacao, and tea*. In Volatile Compounds in Foods and Beverages (Ed. Maarse H.). Marcel Dekker, New York, pp. 617–669.
10. **Glezer V., 2003** - *Environmental effects of substituted phenols*. In The Chemistry of Phenols (Ed. Rappoport Z.). John Wiley & Sons, Ltd. p. 1347–1368.
11. **International Organization for Standardization, 2001** - *Green coffee - Determination of water content*. Basic reference method: ISO 1446:2001.
12. **Komes D., Voivodic A., 2014** - *Effects of varieties and growing conditions on antioxidant capacity of coffee*. In Processing and Impact on Antioxidants in Beverages (Ed. Preedy V.). Academic Press, USA. pp. 77–87.
13. **Ohiokpehai O., 1982** - *Chlorogenic acid content of green coffee beans*. Doctoral thesis, University of Surrey.
14. **Pimentel G.D., Micheletti T.O., Nehlig A., 2014** - *Coffee Intake and Obesity*. In Nutrition in the Prevention and Treatment of Abdominal Obesity (Ed. Watson R.R.). Academic Press, Elsevier. pp. 245–259.
15. **Reh C.T., Gerber A., Prodolliet J., Vuataz G., 2006** - *Water content determination in green coffee – Method comparison to study specificity and accuracy.* Food Chemistry. Vol. 96 (3), pp. 423–430.
16. **Santos M.D., Almeida M.C., Lopes N.P., Souza G.P., 2006** - *Evaluation of the antiinflamatory, analgesic and antypiretic activity of the natural polyphenol chlorogenic acid.* Biol. Pharm. Bull. Vol. 29, pp. 2236–2240.
17. **Shahidi F., Naczk M., 2004** - *Phenolics in Food and Nutraceuticals.* CRC Press, USA.
18. **Singleton V.L., Rossi J.A., 1965** - *Colorimetry of total phenolics with phosphomolybdic-phosphotungstic acid*. Am. J. Enol. Vitic. Vol. 16,p p. 144-158.
19. **Sureshkumar V.B., Nikhila K.R., Prakash N.S., Mohanan K.V., 2013** - *Interrelationship and association of characters in Robusta coffee (Coffea canephora var. robusta)*. Agriculture, Forestry and Fisheries. Vol. 2, No. 2, pp. 98–104.
20. **Vermerris W. and Nicholson R., 2006** - *Phenolic compound biochemistry*. Springer, Dordrecht, The Netherlands.
21. **Vinson J. A., Burnham B. R., Nagendran M. V., 2012** - *Randomized, double-blind, placebo-controlled, linear dose, crossover study to evaluate the efficacy and safety of a green coffee bean extract in overweight subjects.* Diabetes Metab. Syndr. Obes. Vol. 5, pp. 21–27.

RESEARCH ON THE APPLES QUALITY MARKETED IN THE MOLDOVA AREA FROM THE PHYSICO – CHEMICAL AND SENSORIAL POINT OF VIEW

CERCETĂRI PRIVIND CALITATEA MERELOR COMERCIALIZATE ÎN ZONA MOLDOVEI DIN PUNCT DE VEDERE FIZICO CHIMIC ŞI SENZORIAL

MURARIU Otilia Cristina[1], IRIMIA L.M., ANGHEL Roxana[1], MURARIU F.[1]
e-mail: otiliamurariu@yahoo.com

Abstract. *The main purpose of this study was to identify the assortment of apple varieties traded in Moldova and an radiography performing on the products quality in terms of physico-chemichal and sensorial properties. The biological material is represented by 8 varieties of apple (Generos, Golden Delicious, Jonagold, Granny Smith, Starkimson, Florina, Jonathan şi Idared) purchased from different supermarkets or markets. The physico-chemichal analysis consisted on determination of dry powder, water, sugar and vitamin C content, titrable acidity, maturation degree by starch hydrolisis and diameter, weight and firmness of apples. Research findings indicated that apples marketed in autumn come mainly (65%) from internal production while the imported apples representing 35%. Fruits folding is mostly in bulk (62,5%), the difference being found in the retail packed in bags or polythene bags, cardboard boxes and crates alveolar plates of wood or cardboard. The results obtained for apples firmness reveals that Starkimson (17,45 UP/5 sec), Granny Smith (20,45 UP/5 sec),Jonathan (20,45 UP/5 sec) şi Generos varieties (25,85 UP/5 sec) are distinguished by a low consistency and a degree of maturation advanced, results that are consisistent with the degree of starch hydrolysis where the values range between 9R to 10R projecting over the state of supramaturation for this varieties. Therefore, it is believed that the produsts of this varieties are not suitable for long term storage being indicated the trade on markets or on processing units of apple in short term.*
Key words: *apples, Moldova, physico-chemichal and sensorial properties*

Rezumat. *Scopul principal al cestui studiu a vizat identificarea varietăţilor sortimentale de mere comercializate în zona Moldovei precum şi efectuare unei radiografii asupra calităţii acestui produs sub aspect fizico-chimic, fitosanitar şi senzorial. Materialul biologic este reprezentat de 8 soiuri de mere (Generos, Golden Delicious, Jonagold, Granny Smith, Starkimson, Florina, Jonathan şi Idared) prelevate din diferite supermarketuri şi pieţe. Analizele fizico-chimice efectuate au constat în determinarea substanţei uscate solubile, a conţinutului de apă, glucide şi vitamina C, a acidităţii titrabile, a gradului de maturare prin hidroliza amidonului precum şi a diametrului, greutăţii şi fermităţii merelor luate în studiu. Rezultatele cercetărilor au indicat că merele comercializate toamna (septembrie-noiembrie), provin cu preponderenţă din producţia internă (65%), cele importate reprezentând 35%. Desfacerea fructelor se face cu precădere în vrac în proporţie de 62,5%, diferenţa fiind regăsită în domeniul retail ambalată sau preambalată în pungi sau de*

[1] University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

polietilenă, cutii din carton cu platouri alveolare şi lăzi din lemn sau carton. Rezultatele obținute pentru fermitate relevă faptul că soiurile Starkimson (17,45 UP/5 sec), Granny Smith (20,25 UP/5 sec), Jonathan (20,45 UP/5 sec) și Generos (25,85 UP/5 sec) se evidențiază printr-o consistență a pulpei scăzută și un grad de maturare avansat, rezultate ce sunt în concordanță cu gradul de hidroliză a amidonului unde valorile au variat între 9R și 10R, reliefându-se starea de supramaturare pentru aceste soiuri. Prin urmare, se consideră că produsele ce aparțin acestor soiuri nu se pretează pentru un termen lung de păstrare fiind indicată comercializarea spre piata sau către unitățile de prelucrare a merelor în termen scurt.
Cuvinte cheie: mere, Moldova, proprietăți fizico-chimice si senzoriale

INTRODUCTION

In Romania the apple culture have pedoclimatic conditions very favorable for the obtaining of high quality productions. Once with Romania's accession to the European Union, the requirements related to the product quality have been harmonized with the European standards to facilitate commerce, on one hand and to increase the life standard at the same standards. To obtain a superior quality, besides the technological processes applied in the orchard, the fruit must be valorized according to an adequate technology that might allow the maintaining of quality at high levels from harvesting until the delivery to the consumer (Irimia, 2013; Beceanu and Chira, 2003).

MATERIAL AND METHOD

Biological material is represented by eight varieties of apples: Generos, Golden Delicious, Jonagold, Granny Smith, Starkrimson, Jonathan, Florina and Idared. These were taken from different areas specialized trade (hypermarket, supermarket, market) from Iasi. Analyzes and determinations were performed on a total of 10 samples, denoted by H1 - H3 - varieties from the hypermarket (Auchan) S1 - S3 - varieties of supermarket (Kaufland); and P1 - P4 - varieties from fruit and vegetable markets in Iasi. Research method consisted of: market diversity research; physico-chemical analyses (titratable acidity, soluble solids, the degree of starch hydrolysis, the content of L-ascorbic acid); organoleptic analysis method and analysis points of the product with marketing standard (Regulation (EC) NO. 1221/2008). Total titratable acidity (TA) was determined by colorimetric method; soluble dry matter (SUS%) was determined by refractometry, the degree of hydrolysis of starch (HA) was determined by the colorimetric method with iodine in potassium iodine and the L-ascorbic acid content (vit. C) was achieved by titrimetric method.

RESULTS AND DISCUSSIONS

Results regarding the diversity study of commercial offer: The research results show that in the autumn (September to November), apples predominate (Figure 1) are from internal production (62.5%) as opposed to imports (37.5%). The varieties were well represented: General, Golden Delicious, Jonagold, Granny Smith, Strakrimson, Jonathan, Florina and Idared. Variety Jonagold and Golden Delicious, were among the best represented species as found both in hypermarket, supermarket and fruit and vegetable markets.

In the market regime, the predominant packaging method was packing in bulk (62.5% of species). The supermarket and hypermarket, varied types of packaging from cardboard boxes with a specific number of apples (Granny Smith), simple cardboard boxes (Golden Delicious) to packaging in cardboard boxes with alveolar plates. In the supermarket and hypermarket products, packaging, specify variety, country of origin, quantity, quality, price, and size sometimes (Hui et al., 2006).

The results of organoleptic analysis

Quality evaluation of apples by organoleptic examination and points method revealed the following aspect: the varieties sampled in hypermarket, supermarket and the market falls into class I, with scores between 69.48 pts. (Jonagold) or 84.87 points. (Idared). The most appreciated varieties by oganoleptic points were Golden Delicious (S2 - 77.11 pts.), Florina (79.21 pts.) Starkrimson (80.61 pts.) and Idared (84.87 pts). The Jonagold variety (H3) didn't meet the requirements of apple shape and appearance (scab presence of Venturia inaequalis), earning the lowest score (69.48 pts.) (Fig. 1).

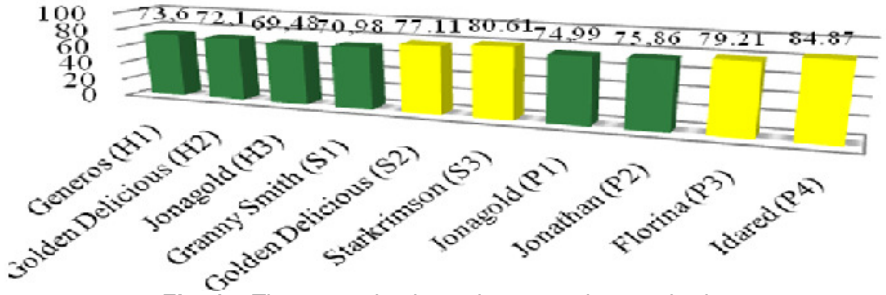


Fig. 1 – The organoleptic analyses – points method

The results of physico-chemical analyses

The titratable acidity (g/l malic acid) is an maturation indicator, of freshness, health status and an important indicator of fruit taste. The values of acidity are hight before firstfruits level is high and deficient on supramaturation. Malic acid is an organic acid with the largest share in the fruits of apple, its content being correlated with titratable acidity (Nour et al., 2010).

Titratable acidity values showed a hight variability for the 8 varieties of analyzed apple, oscillating in the range of 0.14 (mg malic acid/100 g) lower bounded for Starkrimson variety . and upper of 0.73 (mg malic acid/100 g) for Granny Smith, values that fall into those mentioned in the literature (0.2-0.9) from other authors except Starkrimson variety that show inferior values. The higher values of acidity are responsible for the astringent taste characteristic of the variety Granny Smith.

The starch content of fruits and structo-textural firmness is in perfect correlation, meaning that the extent of starch hydrolysis determin the fruit firmness decreases. Thus, are highlights the Jonagold and Florina varieties with a higher of starch percentage (5R - Jonagold and 7R - Florina) which shows higher values for firmness (39.77UP/5 sec. and 36 UP/5 sec.) showen that these varieties are suitable for storage and their sale in winter season. The apples that present reached full maturity belong on Idared varieties (10R and 16,25 UP/ 5 sec) and Starkrimson with (17.25 UP/ 5 sec. and 9R) indicated that they must be sent on processing.

Dry powder content of varieties studied, varies between 8.52% (Idared) and 15.06% (Golden Delicious). High levels of soluble solids were recorded and Granny Smith (13.96%) and Jonathan (14.86%).

Mean of soluble solids content was 12.65%, which means that apples were marketed for fresh consumption only. Although the variety Idared obtained the lowest soluble dry matter (8.52%), according to organoleptic analysis results, it was most appreciated variety in terms of taste (16.75 pts.). Unlike the variety Golden Delicious (15.06%) with a score of only 11.87 points. varieties Florina (13.86%) and Jonathan (14.86%) were noted as sweet varieties.

Soluble solids (SUS%) is an indicator of apples sugar content and sweet taste. The amount of soluble solids expresses the degree of maturation, freshness and fruits health. Being a climacteric fruit, apple maturation continues after his storage placing by starch hydrolysis, with the decreasing of dry matter after subtracting the respiration process.

The S.U.S. content of investigated varieties, ranging from 8.52% (Idared) to 15.06% (Golden Delicious). High levels of soluble solids were recorded on Granny Smith varieties (13.96%) and Jonathan (14.86%).

The vitamin C content of apple giving rise to the antioxidant properties of the fruit, the values ranges from 1.67 mg / 100 g for Golden Delicious apple varieties (H2) and 42.15 mg / 100 g on Granny Smith. The high levels of Granny Smith apples variety may be due to treatment with L-ascorbic acid by different methods, before the placed of commercial distribution. Similar proportions of vitamin C were noted in the Strakrimson (3.08 mg vit.C / 100 g apple) and Generic varieties (3.43 mg vit.C / 100 g apple). High levels of L-ascorbic acid were obtained on Idared varieties, the vit. C content range from 8.36 mg / 100 g to 11.44 mg / 100 g for Florina apple). Small quantities of vitamin C may be due to the metabolic processes carried out in storage.

CONCLUSIONS

Conclusions showed the need for this type of studies in the context of research activity at national level and mainly world level as well as the trend to promote a healthy diet.

The research releaved that the six analysed varieties (Generos, Golden Delicious, Granny Smith, Starkrimson, Jonathan and Idared) are not suitable for long term storage being indicated the trade on markets or on processing units of apple in short term. The Jonagold and Florina varieties may be comercilized later.

The high levels obtained for Granny Smith apples variety may be due to treatment with L-ascorbic acid by different methods, before the placed of commercial distribution.

REFERENCES

1. **Beceanu D., Chira A., 2003** - *Tehnologia produselor horticole. Valorificarea in stare proaspata si industrializare*. Editura Economica, Bucuresti.
2. **Hui Y.H et al., 2006** - *Handbook of fruits and fruit processing*. Editura Blackwell, USA, UK, Australia.
3. **Irimia L.M., 2013** - *Controlul și expertiza produselor calității legumelor, fructelor și produselor derivate*. Editura Ion Ionescu de la Brad, Iași.
4. **Nour Violeta et. al., 2010** - *Compositional Characteristics of fruit of several (Malus Domestica Borkh.) cultivars*, Not. Bot. Agrobot., Cluj 38(3), pp.228 - 233.

PHENOLOGICAL STAGES OF *SPIRAEA X VANHOUTTEI* ACCORDING TO BBCH CODE

STADIILE FENOLOGICE ALE SPECIEI *SPIRAEA X VANHOUTTEI* CONFORM CODULUI BBCH

***TOMA Raluca*[1], *ZAHARIA D.*[1]**
e-mail: ralucatoma17@yahoo.com

Abstract. *Phenological observations of deciduous shrubs with ornamental role in urban spaces could provide a better understanding of stress condition that affect plant growth. Researchers, especially in Europe, are suggesting that using a common language as BBCH code can be helpful when describing, collecting and sharing phenological data. Using BBCH code to describe the seasonal development of ornamental plants can be an instrument in understanding the way that plants can be affected by the environment .It also could provide information that can be employed to improve the management and maintenance of urban green spaces due to the extensive data that can be collected around the world, especially in Europe, using the same way to codify phenological information. An application of the BBCH code is the description of phenological stages of Spiraea x vanhouttei.*
Key words: *phenology, BBCH code, Spiraea x vanhouttei*

Rezumat. *Observaţiile fenologice efectuate asupra arbuştilor foioşi cu rol decorativ în spaţiile urbane pot furniza informaţii care să ajute la o mai bună înţelegere a condiţiilor de stres care afectează creşterea plantelor. Cercetătorii, mai ales în Europa, propun utilizarea unui sistem comun de codificare a informaţiilor fenologice cum este codul BBCH. Utilizarea acestui cod poate fi un instrument util în înţelegerea modului în care mediul afectează dezvoltarea plantelor şi poate furniza informaţii car pot fi utilizate la întreţinerea şi managementul spaţiilor verzi datorită numeroaselor date care pot fi adunate din întreaga lume utilizând acest limbaj comun. Stabilirea stadiilor fenologice ale speciei Spiraea x vanhouttei constituie o exemplificare a modului de aplicare a acestui cod.*
Cuvinte cheie: *fenologie, codul BBCH, Spiraea x vanhouttei*

INTRODUCTION

Data about plant development cycles and the relations between vegetation and the environment has been recorded since the Antiquity even though there was no systematic data collection (Schwartz, 2003). Once the data collection of phenological observations expanded on global scale and recording became continuous, by the mid of XX century, phenology has transformed from a discipline which had applications almost exclusively in the agricultural field to one which has an interdisciplinary character, the book „*Phenology. An Integrative environmental science*" edited by M.D. Schwartz supporting this.

[1] University of Agricultural Sciences and Veterinary Medicine of Cluj-Napoca, Romania

During the last decades, even if there is a strong evidence of agricultural application research predominance (Chmielewski, 2003), due to the obvious relationship between plant development and environment the observation of plant species which have certain peculiarity which makes them appropriate for use as environmental markers (spontaneous flora plants that can become guide species in phenological development for other more important agricultural plants) which are cultivated and observed in the so called Phenological Gardens (Mandrioli, 1993). Meanwhile the observations of vegetation in the urban environment have been focused more on the effects of urbanization over phenological development stages such as flowering, because it is generally considered that climate conditions of urban environment are similar to changing global climate conditions (Kaesha and Jianguo, 2006).

Phenological applications on the field of environmental sciences become more important as time goes by and national and international phenological networks have appeared and developed. The aim of these networks is to provide, by analyzing phenological data, information about global climate change that are ongoing in the last fifty years (Dal Monte, 2007). The creation of phenological databases and their development has generated the need of a common language, a method for coding phenological observations that allows data collection, archiving and analysis can be applied to any plant by anyone. Scientists developed a uniform coding system of similar phenological growth stages, named BBCH - **B**iologishe **B**undesanstalt, **B**undessortenamt and **Ch**emical industry. Following other methods of codifying phenological data such as Flekinger's code for pome fruit or Zadoks's code for cereals, BBCH code was created on the purpose of describing any mono- and dicotyledonous plant development during the crop year (Meier et. al., 2009).

MATERIAL AND METHODS

Spiraea x vanhouttei is a common species very much used on green spaces design in Cluj-Napoca city (Constantinescu and Szilagyi, 2002) especially in the form of hedges and less as isolated shrubs. Observations were conducted during four years especially in spring and summer. The phenological development of ornamental shrubs located on green urban spaces, placed along the east-west city axis, situated in urban climate, more precisely the microclimate of west, central and east quarters (Moldovan and Fodorean, 2002) was monitored.

BBCH is a decimal code which divides the plant development in principal growing stages (using numbers from 0 to 9) that indicate clearly recognizable, long lasting development phases like leaf development, flowering or fruit development and secondary development stages (also codified using numbers from 0 to 9) which indicate a precise moment during the plant evolution within the main development stage. The numbers in the secondary development stage correspond to the respective ordinal numbers or percentage values (e.g. 2 can represent 2nd true leaf or 2nd node or 20% of the flowers open or 20% of the final size typical of the species) (Hack et al., 1992).

The adaptability of this code to various species is exemplified by the many papers in the scientific literature like those that can be found in the "*Growth stages of mono- and dicotyledonous plants – BBCH Monograph"* (ed. Meier, 2001). One of the most important example for this research is the adaptation of BBCH code for the woody vegetation (Finn et

al., 2007). The codification proposed by Finn *et al*. emphasises those growing stages that are typical for the morphology and biology of woody plants like shoot development and fruits and discards stages like formation of side shoots or development of harvestable vegetative plant parts or vegetatively propagated organs.

This study proposes an adaption of the method used by Saskia and Kuzovina for the species of *Salix* genus (Saska and Kuzovina, 2010) which describes more accurately and detailed the phase of flower development and renounce to the description of stages referring of fruit development and ripening because *Spiraea x vanhouttei* fructification isn't important for ornamental purposes.

RESULTS AND DISCUSSION

The paper aims to present the phenological development of a very common species used in landscape design in Romania based on the BBCH code. Following other examples found in the scientific literature, this study tries to adapt the code to the peculiarities of *Spiraea x vanhouttei*.

Phenological stages of *Spiraea x vanhouttei* according to the BBCH code are presented as follows. Usually they are accompanied by images which illustrates the most important stages (figure 2).

Principal growth stage 0: Bud development
00 Dormancy: buds covered with scales
01 Buds begin to swell and change colour
05 Buds break, scales are open
09 Green tips can be observed

Principal growth stage 1: Leaf development
10 Leaves are completely green, still attached one to another
11 First leaves are completely separated
13 More leaves are separated
15 Majority of leaves are completely separated without reaching their final size, shoot emergence
19 Leaves get the typical variety size and shape

Principal growth stage 3: Stem elongation
30 Stem elongation visible
31 Stem about 10% of final length
35 Stem about 50% of final length
39 Stem about 90% of final length. Stem growth ends

Principal growth stage 5: Inflorescence emergence
50 Inflorescence buds can be observed
51 Inflorescence take shape
53 Inflorescence growth
55 Floral cluster growth
57 Floral buds become white
59 Floral buds are white and still closed

Principal growth stage 6: Flowering
60 First flowers open
61 Beginning of flowering: 10% of flowers open
65 Full flowering: at least 50% of flowers are opened, first flowers begin to fade
67 Majority of flowers have faded
69 End of flowering: All flowers have faded or have fallen
Principal growth stage 9: Senescence
90 Stem growth completed; foliage still green and buds developed
91 Beginning of leaf discoloration
93 Beginning of leaf fall
99 End of leaf fall

Because the aspects regarding flowering are considered very important for *Spiraea x vanhouttei* species, detailing the stages of inflorescence formation and development was considered more important than a percentage evaluation of inflorescence growth. Because fruits are insignificant describing principal growth, stages 7 and 8 (fruit development and ripening) was dropped out. The 2nd (formation of side shoots) and 4th principal growth stages (booting / development of harvestable vegetative plant parts or vegetatively propagated organs) have been discarded also.

Difficulties in evaluation of principal growth stage number 3 appeared because observations conducted *in situ* were affected by the frequent and inopportune cuttings on hedges.

During summer time, more exactly at the beginning of summer, buds were emerging at the leaves axil. At the end of the season they measured 1-1,5 mm. Branch development took place while inflorescence was emerging and continued during the mid summer.

Describing with accuracy the phenological development of *Spiraea x vanhouttei* is useful when the management plan for intervention on hedges is made. An association between 3rd and 5th principal growth stages and aphids apparition was observed. That drives people to wrongfully take action by cutting hedges before flowering which deprives the users of one of the most aesthetic characteristic of the species - the arching branches full of white flowers (fig. 1).

Fig. 1 - *Spiraea x vanhouttei* hedges (photo by Raluca Toma, 2014)

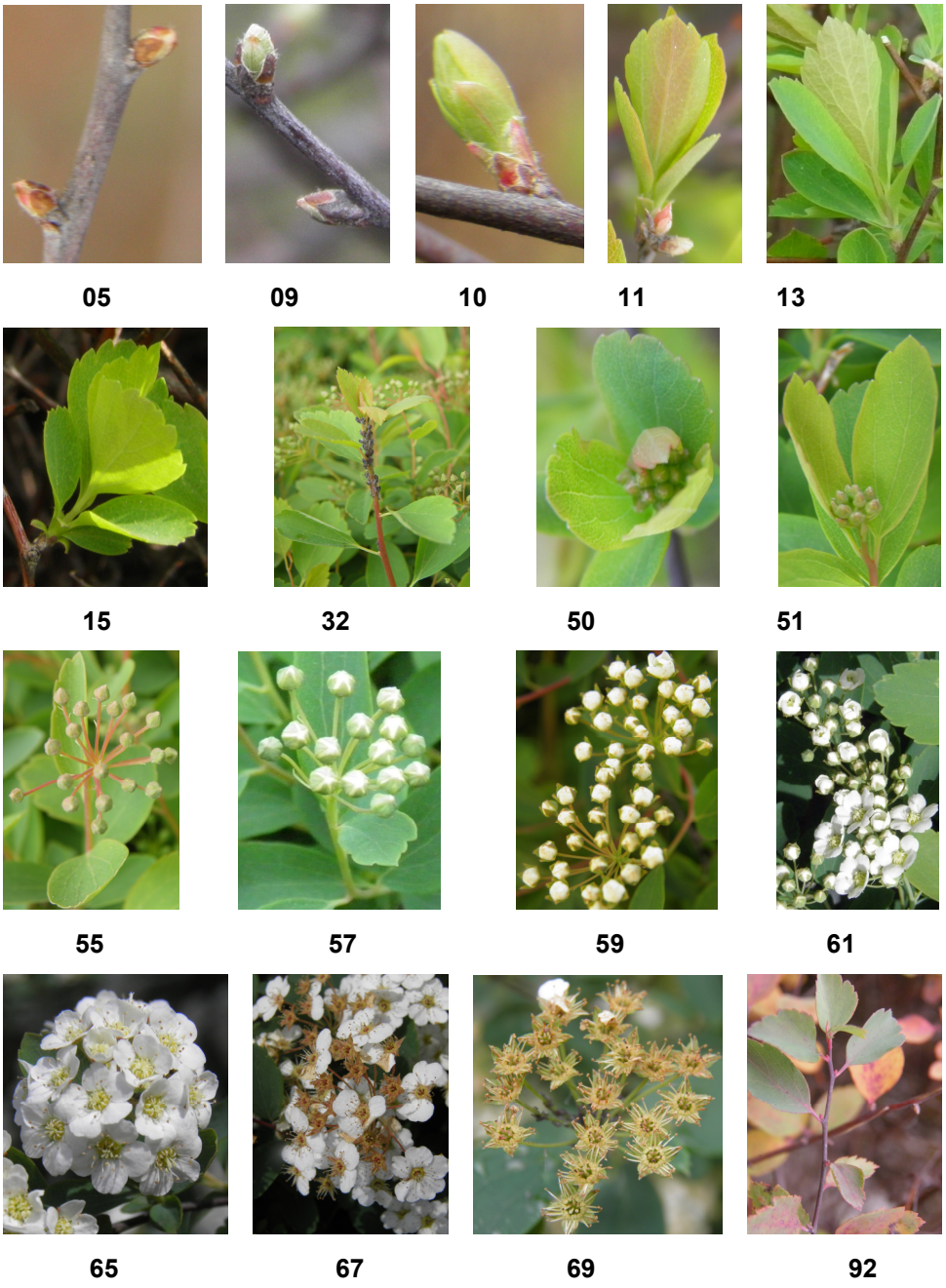


Fig. 2 - Phenological stages of *Spiraea x vanhouttei* (photo by Raluca Toma, 2014)

CONCLUSIONS

Studying the phenology of ornamental vegetation in urban environment is necessary because the interventions for maintaining a certain aesthetic level of green spaces implies substantial costs for the community due to the fact that landscape and economic value of ornamental vegetation is the result of aesthetic characteristics.

1. Emphasizing the aesthetic qualities of deciduous ornamental woody plants cannot be done without an attentive study of their development during the seasons.

2. Establishing the key moments in phonological development of ornamental plants can improve the efficacy of works necessary for maintaining a high level of aesthetic qualities necessary for the vegetation to be perceived as an ornamental element in the urban landscape.

3. Observing phenological development can help to identify and understand the relations between urban environment and vegetation that affect plant development.

REFERRENCES

1. **Dal Monte G., 2007 –** *An international outlook on phenology.* Journal of Agromereorology, xx xxx (3), pp. 52-58.
2. **Chmielewski F.M., - 2003** – *Phenology and agriculture. Phenology. An integrative environmental science,* Kluwer Academic Publishers, Dordrecht, pp.505-522
3. **Constantinescu M., Szilagyi Camelia, 2002 –** *Spațiile verzi.* Municipiul Cluj-Napoca şi zona periurbană. Studii ambientale. Ed. Accent, Cluj-Napoca pp. 157-166.
4. **Finn G.A., Straszewski A.E., Peterson V., 2007 –** *A general growth stage key for describing trees and woody plants.* Annals of Applied Biology, 151, pp. 127-131.
5. **Hack H. et al. - 1992 –** *The extended BBCH - scale.* În Growth stages of mono- and dicotyledonous plants – BBCH Monograph, pp. 6–12
6. **Kaesha N., Jianguo Wu, 2006 –** *Effects of urbanization on plant flowering phenology. A review.* Urban Ecosystems, 9 (3), pp. 243–257
7. **Mandrioli P., 1993 –** *Una Rete di Giardini Fenologici in Italia: Finalità e criteri*. În Guida al rilevamento dei Giardini Fenologici Italiani. M.I.P.A. Progetto finalizzato "Phenagri: Fenologia per l'Agricoltura", sottoprogetto 2: "Fenologia delle piante arboree, ricerca 2.3: Studio dello sviluppo fenologico di specie guida nei giardini fenologici. Coordinatore generale dott. A. Brunetti, pp. 1-5.
8. **Meier U., Bleiholder H., Buhr Liselotte, Feller Carmen, Hack H., Heß M., Lancashire P.D., Schnock Uta, Stauß R., van den Boom T., Weber Elfriede, Zwerger P., 2009 –** *The BBCH system to coding the phenological growth stages of plants – history and publications.* Journal für Kulturpflanzen, 61(2), pp. 41-52.
9. **Moldovan F., Fodorean I., 2002 –** *Caracterizare climatică.* Municipiul Cluj-Napoca şi zona periurbană. Studii ambientale. Ed. Accent, Cluj-Napoca pp. 49-66.
10. **Saska Margaret. M., Kuzovina Yulia A., 2010 –** *Phenological stages of willow (Salix).* Annals of Applied Biology, 156, pp. 431-437.
11. *****, Schwarz M.D. (ed.), 2003** – *Phenology. An integrative environmental science.* Kluwer Academic Publishers, Dordrecht.
12. *****, Meier U. (ed.), 2001** – *Growth stages of mono- and dicotyledonous plants – BBCH Monograph.* http://www.bba.de/veroeff/bbch/bbcheng.pdf.

STUDIES ON THE PHENOLOGY OF *COTONEASTER HORIZONTALIS* SPECIE, IN THE CONDITIONS OF "TUDOR NECULAI" NURSERY, IASI REGION

STUDII FENOLOGICE ASUPRA SPECIEI *COTONEASTER HORIZONTALIS* ÎN CONDIȚIILE PEPINIEREI „TUDOR NECULAI" IAŞI

***BERNARDIS R.R.*[1]*, SANDU Tatiana*[1]**
e-mail: roberto041069@yahoo.com

Abstract. *From the multitude of dendrological species, Cotoneaster genus, with all the species included, is particularly important in garden design, especially that the conditions of our country are generally favorable and very favorable for the growth of these species. The purpose of the paper is to highlight the ornamental potential of the most popular specie of Cotoneaster genus, respectively Cotoneaster horizontalis that was studied in the conditions of "Tudor Neculai" nursery, Iasi region. During the growing season there were made observations and determinations concerning shoots annual growth rate, the growth rhythm of leaves and the number of flowers on the stems.*
Key words: *shoots, leaves, flowers.*

Rezumat. *În cadrul multitudinii de specii dendrologice, speciile genului Cotoneaster au importanță deosebită în amenajarea spațiilor verzi şi mai ales în condițiile de la noi din țară care sunt în general favorabile şi foarte favorabile pentru aceaste specii. Scopul lucrării este de a pune în evidență potențialul ornamental al celei mai cunoscute specii, Cotoneaster horizontalis, care se întâlneşte în cadrul pepinierei „Tudor Neculai" Iaşi. Pe parcursul perioadei de vegetație s-au efectuat observații şi determinări privind ritmul de creştere a lujerilor anuali, ritmul de creştere a frunzelor şi numărul de flori pe lujeri.*
Cuvinte cheie: *lujeri, frunze, flori.*

INTRODUCTION

One of the basic components of the green spaces which provide the aesthetic aspect of the cities, is represented by the landscape arrangements (Zaharia and Dumitras, 2003).

Within the numberless dendrological species of the ornamental shrubs, the species of the *Cotoneaster* class are given a special importance when arranging the green spaces, while the environmental conditions of our county are generally favourable and very favorable for this species (Iliescu, 2002).

The need to diversify the class becomes a priority, taking into consideration the diversity of the biological material, especially, the results obtained at international level (Donita et al., 2004).

[1] University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania

The literature in the field reveals that, within this type, various species and varieties with unknown ornamental value have been discovered. As the ornamental importance diversity is known, it was suggested that, for the *Cotoneaster* class, phenological studies should be carried out, for the whole shrub, meaning: stalk, leaves and flowers (Bernardis, 2011; Mihail, 2005).

MATERIAL AND METHOD

The investigations regarding the *Cotoneaster horizontalis* species, were carried out within the "Tudor Neculai" nursery of Iași.

The nursery is located in the unincorporated area of Iași, on an area pertaining to Miroslava, being designated to produce the dendrological and floricultural seeding material, in order to decorate and beautify the green spaces from the city of Iași.

The nursery possesses a rich type of *Cotoneaster* species, among which we mention:

1. Procumbent species with persistent leaves:

-*Cotoneaster dammeri* Schneid.,
-*Cotoneaster microphyllus* Wall. Ex Lindl.

2. Prostrate plants with caducous leaves:

-*Cotoneaster horizontalis* Decne.

3. Medium-size erected species, with caducous leaves:

-*Cotoneaster dielsianus* Pritz.,
-*Cotoneaster divaricatus* Rehd. et Wils.

4. High-size species with caducous leaves:

-*Cotoneaster bullata* Bois.,
-*Cotoneaster multiflorus* Bge.

5. High-size species, with semi-persistent leaves

-*Cotoneaster salicifolius* Franch.,
-*Cotoneaster franchetii* Bois.

The analyses have been carried out at *Cotoneaster horizontalis* Decne, with the purpose of highlighting the ornamental potential of the most popular species, *Cotoneaster horizontalis,* which can be found in the dendrological nursery "Tudor Neculai" of Iași.

These analyses were performed during 2012-2013 and there have been carried out determinations which regarded: the stalks' growing rhythm, leaves' growing rhythm and the number of flowers in the stalks.

RESULTS AND DISCUSSIONS

Following the determinations carried out at the annual stalks in 2012, it can be stated that the minimum value of the stalks' length was 0,7 mm, while the maximum one was 13,3 cm.

In 2013, the minimum value was 1cm, while the maximum one was 12,5 cm. (table 1, fig. 1).

Regarding the results that obtained for the leaves, we could say that, in 2012, the minimum value was 0,2 mm, while the maximum value was 2,2 cm; in 2012, the minimum value was 0,5 mm, while the maximum value was 2,5 mm (table 2, fig. 2).

Table 1

Annual stalks growing rhythm in 2012-2013

Date on which the analyses was carried out	Growths (cm)	
	2012	2013
25 march	0,7	-
30 march	2	1
10 april	3,7	2,5
15 april	4,4	3
20 april	5,6	3,9
25 april	6,7	5,5
30 april	7,7	7
10 may	10	8,2
15 may	11,8	10,4
20 may	13,3	12,5

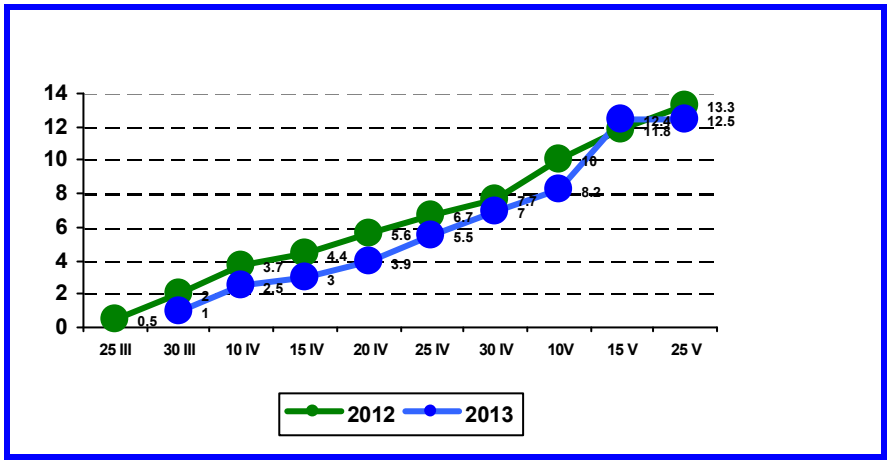


Fig. 1 - The annual stalks growing rhythm in 2012-2013

Table 2

The leaves growing rhythm in 2012-2013

Date on which the analyses was carried out	Growths (cm)	
	2012	2013
25 march	-	0,5
30 march	0,2	1
10 april	0,8	1,2
15 april	1,1	1,3
20 april	1,1	1,3
25 aprili	1,3	1,3
30 aprili	1,3	1,3
10 may	2	1,4
15 may	2	2
20 mai	2,2	2,5

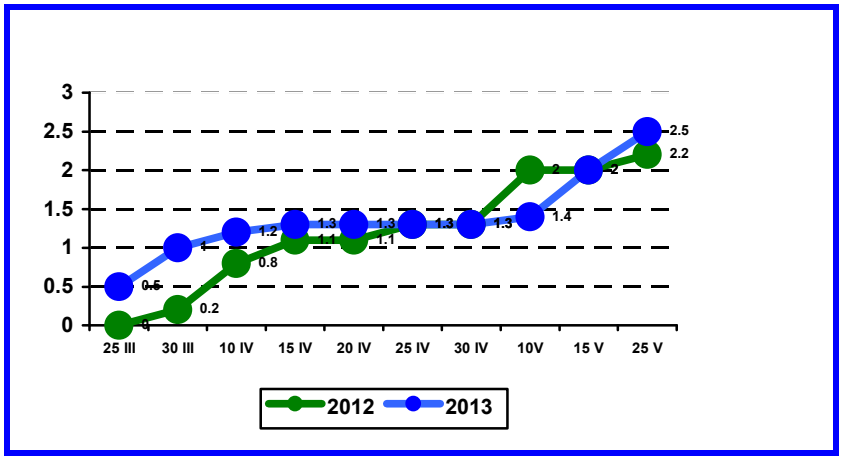


Fig. 2 - The leaves growing rhythm in 2012-2013

Following the researches carried out for the *Cotoneaster horizontalis* species, regarding the number of flowers (fig. 3) performed on 10 stalks, it was revealed that in 2012, on the stalk 1, a minimum number of 10 flowers was registered, while on the stalk 10, a maximum number of 42 flowers was registered.

In 2013, the species had a lower number of flowers, compared to 2012, and registered a number of 6 flowers on the stalk 1 and 36 flowers on the stalk 10 (table 4, fig. 4):

Table 4

Number of flowers on 10 stalks, in 2012-2013

Number of stalks	Number of flowers	
	2012	2013
1	10	6
2	13	8
3	14	14
4	15	15
5	20	22
6	28	26
7	32	28
8	36	31
9	38	34
10	42	36

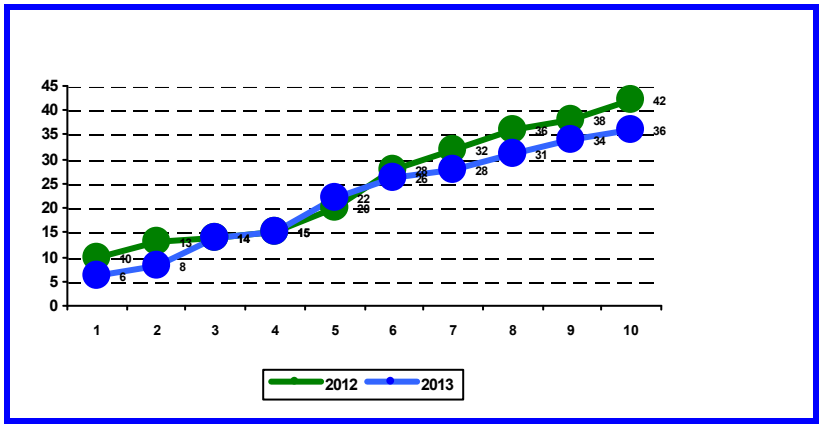


Fig. 4 - Number of flowers on 10 stalks, in 2012-2013

The analyses of the leaves

Following the researches carried out in 2012, the *Cotoneaster horizontalis* species started to come into leaf in the 3rd decade of the March. At the first measurement performed on 25th March, the leaves were 0,2 mm. Due to the cold temperature and precipitations, the same value was registered also on 30th March.

At the measurement performed on 10th April, the leaves had the value of 0,8 mm, after which, on 15th April, their size was to 1,1 cm, period in which stagnation was registered, until 20th April. From 20th April until 25th April, a growth was registered, up to 1,3 cm, which was maintained until 30th April. At the measurement performed on 10th May, it was noticed that the leaves' size was 2 cm, value which was maintained until 15th May, after which, at the last measurement the leaves were 2,2 cm.

In 2013, the species started to come into leaf 10 days later. This way, at the first measurement, performed on 25th March, the leaves were 0,5 cm, after which, on 30th March, their size was 1 cm. At the measurement performed on 10th April, the leaves were 1,2 cm, after which, on 15th April until 30th April, no growth was registered.

Starting from 30th April, until 20th May, the leaves grew as follows: on 10th May, they were 1,4 cm, on 15th May they were 2 cm, while on 20th May, and they were 2,2 cm.

The analysis of the annual stalks

In 2012, the *Cotoneaster horizontalis* species entered into vegetation in the 2nd decade of March.

From their first apparition, until the second measurement, performed on 30th March, the stalks have grown up to 0,7 mm.

From 25th March, until the second measurement performed on 30th March, the stalks have grown up to 2 cm.

At the following measurement performed on 10th April, it was revealed that the stalks have grown up to 3,7 cm. In April, the measurements were performed every ten days: on 10th April-4,4 cm, 20th April-6,7 cm, 30th April-7,7 cm.

At the performance of the last measurements, in May, it was revealed a reduced growth, as a consequence of the first inflorescence.

This way, on 10th May, the stalks reached the size of 10 cm, on 15th May – at 11,8 cm, and at the final measurement, the stalks were 13,3 cm.

Following the analyses performed in 2013, it was revealed that the species came into vegetation in the 2nd decade of March, but 12 days later.

At the first measurement, performed on 30th April, the stalks were 1 cm. But at the last measurement, performed on 10th April, the stalks have grown up to 1,5 cm. In that month, the measurements were performed more often and this way it was revealed that the stalks had the following values: 15th April- 3cm; 20 april-3,9 cm.

From 20th April until 25th May, the stalks had a more punctuated growth, meaning: from 25th April- 5,5 cm, at 30th April-7cm, 10th May- 8,2 cm, on 15th May- 10,4 cm, while at the last measurement, the stalks had a growth of 12,5 cm.

CONCLUSIONS

1. The pedoclimaic conditions provided by the dendrological nursery, are adequate for a positive growth and development of the biological material which was studied, and may allow to highlight the ornamental value of the species.

2. Comparing the apparition and the dynamics of the stalks' development, it was observed that there are no major differences during the two vegetation periods, in which the analyses were performed.

3. After the phenological analysis, performed on the leaves of *Cotoneaster horizontalis* species, it resulted that there were no major modifications of the initial sizes of the leaves, the average was preserved between 2,2 cm for 2012, and 2,5 cm for 2013.

4. Regarding the number of flowers, due to the favourable environmental conditions and to the maintenance works which were applied; a relatively good number was observed, situated within the maximum biological values of this plant, the number of lowers providing a special ornamental value.

REFERENCES

1. **Bernardis R., 2011**- *Ornamental Arboriculture 2nd* volume. Ion Ionescu de la Brad, Publishing House of Iași.
2. **Doniță N., Geambaşu T., Brad R., 2004** – *Dendrologie*. Vasile Goldiş University Press, Arad.
3. **Iliescu Ana Felicia, 2002** – *Cultivation of ornamental arbors and shrubs*. Ceres Publishing House of Bucharest
4. **Mihail M., 2005** – *Dendrology*. "Bioterra" Publishing House of Bucharest.
5. **Zaharia D., Dumitraş Adelina, 2003** – *Ornamental Arboriculture.* „Risoprint" Publishing House of Cluj-Napoca.

THE STUDY OF SOME SPECIES AND CULTIVARS OF *HEUCHERA* GROWING IN IAŞI CONDITIONS

STUDIUL UNOR SPECII ŞI SOIURI DE *HEUCHERA* CULTIVATE ÎN CONDIŢIILE DE LA IAŞI

DRAGHIA Lucia[1], BAHRIM C.[1],
CHELARIU Elena-Liliana[1], MUNTEANU Gianina[1]
e-mail: lucia@uaiasi.ro

Abstract. *The genus Heuchera (fam. Saxifragaceae) includes over 53 species of perennial herbaceous plants with persistent leaves. Plants of this genus form globular bushes with heights between 15 - 25 cm and the diameter between 15-50 cm. Although the main ornamental part of the species and cultivars of Heuchera is represented by leaves, because of their shape and color, the decorative value of the flowers can not be neglected. In this paper were studied some morphological characters that give ornamental value to Heuchera plants (bush diameter, leaf size, number and size of flowers and inflorescences). Observations were made on five Heuchera cultivars grown in the field, in conditions of Iaşi (H. sanguinea 'Coral Forest', H. hybrida 'Mini Monster', H. hybrida 'Venus', H. hybrida 'Marmalade' and H. hybrida 'Pewter Purple'). The highest number of leaves and most compact bushes stands out 'Mini Monster', and the best flowering capacity had 'Mini Monster' and 'Venus', while 'Marmelade' has formed fewest inflorescences.*
Key words: *Heuchera,* ornamental value, caractere morfologice

Rezumat. *Genul Heuchera (fam. Saxifragaceae) cuprinde peste 53 de specii de plante erbacee perene, cu frunze persistente. Plantele acestui gen formează tufe globuloase cu înălţimi de 15 - 25 cm şi diametrul de 15 - 50 cm. Deşi principala parte ornamentală a speciilor şi cultivarelor de Heuchera o constituie frunzele, prin forma şi coloritul acestora, nu poate fi neglijată nici valoarea decorativă a florilor. În lucrare sunt studiate o serie de caractere morfologice care dau valoare ornamentală plantelor de Heuchera (diametrul tufei, dimensiunile frunzelor, numărul şi dimensiunea florilor şi inflorescenţelor), observaţiile fiind efectuate la cinci cultivare de Heuchera cultivate în câmp, în condiţiile de la Iaşi (H. sanguinea 'Coral Forest', H. hybrida 'Mini Monster', H. hybrida 'Venus', H. hybrida 'Marmalade' şi H. hybrida 'Pewter Purple'). Cu numărul cel mai mare de frunze şi cu tufele cele mai compacte se remarcă 'Mini Monster', iar cea mai bună capacitate de înflorire au avut 'Mini Monster' şi 'Venus', în timp ce 'Marmelade' a format cele mai puţine inflorescenţe.*
Cuvinte cheie: *Heuchera,* valoare ornamentală, morphological characters

INTRODUCTION

The genus *Heuchera* contains at least 53 species of herbaceous perennial plants with persistent leaves, originating in North America and belonging to the family *Saxifragaceae*. The most popular name used is "coral bells" which refers

[1] University of Agriculture Sciences and Veterinary Medicine of Iaşi, Romania

to campanulate flowers, red as coral, of *Heuchera sanguinea* species. It is also used as "alum root", because the roots are rich in tannin and were used as alum pickling process or for medicinal purposes, to stop nose bleeding, to treat ulcers etc. (http://www.plantdelights.com/Article/Heuchera-Heucherella-Coral-Bells).

Heuchera plants form the globular clumps with heights between 15 and 25 cm and the diameter of the bush with values between 15 and 50 cm. The appearance and color of the leaves and flowers varies depending on the species and cultivar. *Heuchera* species and cultivars are decorative by both leaves and flowers. In order to obtain new forms, crosses were made between different species. The first were made between *Heuchera sanguinea* and *Heuchera americana*; to create hybrids for cut flowers there were used *Heuchera micrantha*, *H. americana* and *H. sanguinea;* to obtain hybrids with red flowers and resistance to low temperatures there were used *Heuchera sanguinea* and *Heuchera richardsonii*.

Due to the origin of climatic zones plant resistance to cold (4-9), *Heuchera* species and cultivars have resistance to low temperatures and it is recommended for growing in colder areas where they can overwinter in the field (Iles and Agnew, 1993; Dimke *et al*., 2008; Collicutt and Davidson, 1992) Multiplication of *Heuchera* species can be done by traditional methods but also through cell cultures and "in vitro" tissues (Hosoki and Kajino, 2003; Rout *et al.,* 2006).

Species of the genus *Heuchera* are also recommended to be grown on contaminated soils. Studies conducted for *H. parvifolia* showed a very good tolerance of plants to strongly acidic pH and to high content of heavy metals (King *et al*., 2009; Marty L., 2000). From *Heuchera sanguinea* seeds were isolated "plant defensins" type HsAFP1 (antimicrobial peptides), which inhibits a wide range of fungi (*Candida albicans, Candida krusei, Aspergillus flavus* etc.). They are not toxic to human cells, thus providing a possible source for fungal infections treatment (Aerts *et al.,* 2011).

In this paper were studied some morphological characters that give ornamental value to *Heuchera* plants growing in Iaşi conditions.

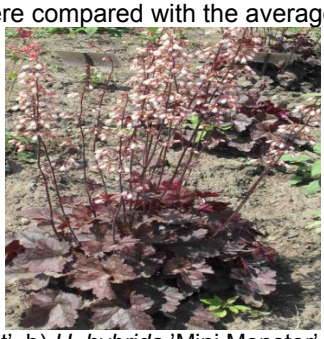
MATERIAL AND METHOD

Experimental cultures were established in October 2013 with *Heuchera* plants purchased from nurseries in Hungary. Each variant was represented by one cultivar, resulting five variants: V_1 - *H. sanguinea* 'Coral Forest'; V_2 - *H. hybrida* 'Mini Monster'; V_3 - *H. hybrida* 'Venus'; V_4 - *H. hybrida* 'Marmelade'; V_5 - *H. hybrida* 'Pewter Purple'. 'Coral Forest' has lobed leaves, rounded, bright green (fig. 1a); 'Mini Monster' has mottled pattern leaves and pale pink flowers (fig. 1b); 'Venus' shows large silver leaves with dark green ribs and yellowish-white flowers (fig. 1c); 'Marmelade' has the shape of maple leaves, bright colors varying from amber to bronze- peach and copper (fig. 1d); 'Pewter Purple' has silvery-gray leaves, mottled with gray and brown ribs (fig. 1e).

The experiment planning was made by the randomized blocks method with three replications. Biometric determinations were made: number and size of leaves, stems height, number of flowers or inflorescences per plant, number of flowers in inflorescence, flower size etc. The data was interpreted statistically by analysis of

variance and the results were compared with the average of the variants.


a) *H. sanguinea* 'Coral Forest'


b) *H. hybrida* 'Mini Monster'


c) *H. hybrida* 'Venus'


d) *H. hybrida* 'Marmelade'

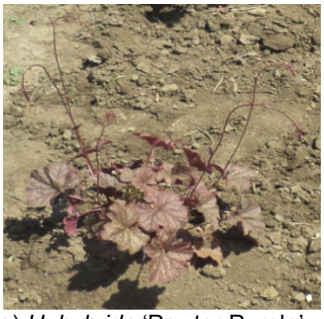

e) *H. hybrida* 'Pewter Purple'

Fig. 1 (a-e) - Experimental *Heuchera* species and cultivars

RESULTS AND DISCUSSIONS

Considering that *Heuchera* plants are appreciated especially due to the decorative appearance of the leaves, there were analyzed and compared a number of leaf characteristics of studied cultivars.

Table 1

Biometric characteristics of the leaves

Var.	Species / Cultivar	Total length (cm)	From which:		Rapport petiole/ lamina
			petiole	lamina	
V_1	*H. sanguinea* 'Coral Forest'	11,0	6,7	4,3	1,56
V_2	*H. hybrida* 'Mini Monster'	16,8	9,8	7,0	1,40
V_3	*H. hybrida* 'Venus'	17,6	9,2	8,4	1,10
V_4	*H. hybrida* 'Marmelade'	19,3	10,7	8,6	1,24
V_5	*H. hybrida* 'Pewter Purple'	14,2	8,1	6,1	1,33

In table 1 there are presented the results of biometric measurements performed at *Heuchera* cultivars leaves, regarding length of petiole and lamina (fig. 2). The ratio between the length of the petiole and lamina indicates that all plant variants were characterized by leaves with long petiole (the ratio was over unity) and the highest values were recorded in V_1 and V_2 ('Coral Forest' and 'Mini Monster').

a) *H. sanguinea* 'Coral Forest' **b)** *H. hybrida* 'Mini Monster' **c)** *H. hybrida* 'Venus'

d) *H. hybrida* 'Marmelade' **e)** *H. hybrida* 'Pewter Purple'

Fig. 2 (a-e) - Aspects regarding leaves and inflorescences

Plants with the richest foliage were those of V_2 ('Mini Monster'), with an average of 106.5 leaf/plant, registering very significant positive differences (tab. 2).

Table 2

Number of leaves and bush diameter

Variants	Species / Cultivar	Nr. leaves/ plant		Bush diameter (cm)	
		Absolute	±D	Absolute	±D
V_1	*H. sanguinea* 'Coral Forest'	63,5	$-4,2^{000}$	22,0	$-9,9^{000}$
V_2	*H. hybrida* 'Mini Monster'	106,5	$38,8^{xxx}$	32,5	0,6
V_3	*H. hybrida* 'Venus'	68,0	0,3	35,3	$3,4^{xx}$
V_4	*H. hybrida* 'Marmelade'	53,0	$-14,7^{000}$	39,7	$7,8^{xxx}$
V_5	*H. hybrida* 'Pewter Purple'	47,6	$-20,1^{000}$	30,0	$-1,9^{o}$
Average (control)		67,7	0	31,9	0
LSD 5%			1,0		1,8
LSD 1%			1,4		2,6
LSD 0,1%			2,1		3,9

Significant negative differences compared to average had variants V_1, V_4 and V_5 ('Coral Forest', 'Marmalade' and 'Pewter Purple'). At cv. 'Venus' (V_3), the differences were not statistically assured. Bush diameter (tab. 2) is one of morphological characters that can give indications about the ability of ground cover plant. Bushes with the largest diameter and very significant positive differences from the average were those of cv. 'Marmalade', following with distinct positive significant differences cv. 'Venus'. The smallest diameter it was at cv. 'Coral Forest' plants (22 cm), from the average differences are negative, very significant. At the cv. 'Mini Monster' differences were not statistically assured.

At the studied cultivars there were analyzed aspects regarding the number of floriferous stems / plant and their length, inflorescence length and number of flowers / inflorescence (tab. 3). Data regarding the average number of floriferous stems/plant indicates variations between 3 inflorescences / plant at V_4 ('Marmelade') and 23.5 inflorescences / plant at V_2 ('Mini Monster'). The differences compared to average were very significant positive to the V_2 ('Mini Monster') and V_3 ('Venus'), and negative to variants V_1 ('Coral Forest'), V_4 ('Marmelade') and V_5 ('Pewter Purple'). Quite large variations were noted regarding the height of floriferous stems (tab. 3), from 34.0 cm ('Marmelade') to 79.2 cm ('Venus'). To variants V_3 and V_5 ('Venus' and 'Pewter Purple'), positive differences compared to average were very significant, while on the other three variants there were recorded values below average and highly negative significant differences to V_1 and V_4 ('Coral Forest' and 'Marmelade') and significantly distinct to V_2 ('Mini Monster').

Table 3

Characteristics of flowers and inflorescences

Var.	Nr. flowers/plant		Height of flowers (cm)		Length of inflorescence (cm)		Nr. flowers / inflorescence	
	Absolute	±D	Absolute	±D	Absolute	±D	Absolute	±D
V_1	5,7	$-5,6^{000}$	41,3	$-11,4^{000}$	12,4	$-10,3^{000}$	72,5	$-28,0^{000}$
V_2	23,5	$12,2^{xxx}$	50,4	$-2,3^{00}$	19,7	$-3,0^{000}$	93,8	$-6,7^{000}$
V_3	18,7	$7,4^{xxx}$	79,2	$26,5^{xxx}$	41,3	$18,6^{xxx}$	178,1	$77,6^{xxx}$
V_4	3,0	$-8,3^{000}$	34,0	$-18,7^{000}$	19,0	$-3,7^{000}$	74,0	$-26,5^{000}$
V_5	5,7	$-5,6^{000}$	58,7	$6,0^{xxx}$	20,9	$-1,8^{000}$	84,3	$-16,2^{000}$
Average control)	11,3	0	52,7	0	22,7	0	100,5	0
LSD 5%		0,2		1,4		0,7		1,6
LSD 1%		0,3		2,0		1,0		2,3
LSD 0,1%		0,5		3,0		1,6		3,4

Of the total length of floriferous stems, inflorescences represented 30.0% for *H. sanguinea* 'Coral Forest', 39.1% for *H. hybrida* 'Mini Monster', 52.1% for *H. hybrida* 'Venus' and 55, 9% for *H. hybrida* 'Marmelade'. Inflorescence length was between 12 and 20 cm, except for *H. hybrida* 'Venus', to which the length of inflorescence was 41,3cm, the medium difference being highly significant positive. For the other 3 cultivars, significant differences were very negative. The average number of flowers/ inflorescence on the 5 cultivars was 100.5 (tab. 3).

Detached however with 178.1 flowers / inflorescence, was *H. hybrida* 'Venus' (V_3), where medium differences were highly significant positive. At the remaining cultivars, differences from average were very significant negative.

CONCLUSIONS

1. Morphological characters analyzed at five cultivars of *Heuchera* plants indicate a profile with special decorative features that can be valued both for the appearance of leaves and flowers.

2. The highest number of leaves (106 leaves / plant) and most compact bushes stands out 'Mini Monster'. Plants with long leaves formed large bushes ('Venus' and 'Marmelade'), while plants with small leaves formed bushes with reduced diameter ('Coral Forest').

3. The best flowering capacity had 'Mini Monster' and 'Venus', while 'Marmelade' has formed fewest inflorescences.

REFERENCES

1. **Aerts An M., Bammens L., Govaert G., Carmona-Gutierrez D., Frank Madeo F., Cammue B.P.A., Thevissen K., 2011** - *The antifungal plant defensin HsAFP1 from Heuchera sanguinea induces apoptosis in Candida albicans.* Frontiers in microbiology, vol. 2, pp: 1-9, www.frontiersin.org
2. **Collicutt L.M., Davidson C.G., 1992** - *Landscape Plant Improvement for Cold Climates*. Acta Horticulturae 320
3. **Dimke K.C., Still S.K., Gardner D.S., 2008 -** *Effect of Overwintering Environment on the Survival of 30 Species of Herbaceous Perennials.* J. Environ. Hort. 26(4), pp:222–228
4. **Hosoki T., Kajino E., 2003** - *Shoot regeneration from petioles of coral bells (Heuchera sanguinea Engelm.) cultured in vitro, and subsequent planting and flowering ex vitro*. In Vitro Cellular Developmental Biology—Plant 39, pp:135–138.
5. **Iles J. K., Agnew N.H., 1993** - *Determining Cold Hardiness of Heuchera sanguinea Engelm. 'Chatterbox' Using Dormant Crowns.* Hortscience, vol. 28(11), pp:1087–1088
6. **King Sandra, King M., Jennings S., Neuman D., 2009** - *Using Plant Tissue Culture to Develop Plants with Acid Soil, Heavy Metal Tolerance (AHMT), Potentially Useful for Hard-Rock Mine Land Reclamation*. pp: 673-692. In: Proceedings of the 2009 National Meeting of the American Society of Mining and Reclamation, Billings, MT. Published by ASMR, 3134 Montavesta Rd., Lexington, KY 40502.
7. **Marty L., 2000 -** *The Use of Local Ecotypes for the Revegetation of Acid/Heavy Metal Contaminated Lands in Western Montana*. pp. 216-229. *In*: 2000 Billings Land Reclamation Symposium. Billings, MT. March 20-24, 2000. Reclamation Research Unit Publication No. 00-01. Montana State University, Bozeman.
8. **Rout G.R., Mohapatra A., Mohan Jain S., 2006** - *Tissue culture of ornamental pot plant: A critical review on present scenario and future prospects*. Biotechnology Advances, vol 24, pp: 531-560.
9. http://www.plantdelights.com/Article/Heuchera-Heucherella-Coral-Bells

THE INFLUENCE OF SOME CULTURE SYSTEMS ON THE ORNAMENTAL VALUE OF *SEDUM SPURIUM* 'FULDAGLUT' AND *SEMPERVIVUM TECTORUM* SPECIES

INFLUENȚA UNOR SISTEME DE CULTURĂ ASUPRA VALORII ORNAMENTALE A SPECIILOR *SEDUM SPURIUM* 'FULDAGLUT' ȘI *SEMPERVIVUM TECTORUM*

NEGREA Roxana [1], DRAGHIA Lucia[1], CIOBOTARI Gh. [1]
e-mail: roxana.acfrance@gmail.com

Abstract. *The purpose of the paper is to analyze the behavior of some ornamental flowering plants cultivated on roofs, on three types of substrate. The used biological material was comprised of mature Sedum spurium 'Fuldaglut' and Sempervivum tectorum samples, purchased from specialized nurseries and cultivated in 80/480 cm containers. The experimental scheme includes three variants, with three repetitions each in which various types of culture substrates were used: V_1- forest soil; V_2 -mixture of peat Novobalt (43%), coconut fiber (30%), composted bark (23%), alginate (4%); V_3-blonde peat (40%), brown peat (30%), sand 10% and forest soil (20%). It was found that the substrate type has influenced the growth and development of the Sedum spurium 'Fuldaglut' and Sempervivum tectorum plants. Comparing the types of substrate we concluded that the substrate a_3, a mixture of* blonde peat (40%), brown peat (30%), sand (10%) and forest soil (20%), *had the most positive influence, while the substrate a_2, a mixture of* Novobalt peat (43%), coconut fiber (30%), composted bark (23%), alginate (4%), *had the least influence.*
Key words: *substrates, green roofs, Sedum, Sempervivum.*

Rezumat. *Lucrarea are ca scop analiza comportării unor plante floricole ornamentale cultivate pe acoperiș, în trei tipuri de substrat. Materialul biologic utilizat a fost format din exemplare mature de Sedum spurium 'Fuldaglut' și Sempervivum tectorum, achiziționate din pepiniere de profil și cultivate în 12 containere de 80/480 cm. Schema experiementală cuprinde trei variante, cu câte trei repetiții în care s-au utilizat diferite tipuri de substraturi de cultură: V_1- pământ de pădure; V_2 – amestec de turbă Novobalt (43%), fibră de cocos (30%), scoarță compostată (23%), alginit (4%); V_3 - turbă blondă 40%, turbă brună 30%, nisip 10% și pământ de pădure (20%). S-a constatat faptul că tipul substratului a influențat creșterea și dezvoltarea plantelor de Sedum spurium 'Fuldaglut' și Sempervivum tectorum. Comparând tipurile de substrat, s-a constatat, la ambele specii, influența pozitivă a substratului a_3, alcătuit din - turbă blondă (40%), turbă brună (30%), nisip (10%) și pământ de pădure (20%); mai puțin favorabil a fost substratul a_2, format din amestec de turbă Novobalt (43%), fibră de cocos (30%), scoarță compostată (23 %), alginit (4%).*
Cuvinte cheie: *substraturi, acoperișuri verzi, Sedum, Sempervivum.*

[1] University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania

INTRODUCTION

Green roofs are a landscaping branch that combines esthetics with the ecologic functions of these types of setups. The esthetics and especially the functionality of such an area are primordial aspects, anchored in the selection of the most suited flower species to enter its composition.

Among the species recommended to be used in such setups we find *Sedum spurium* L., a specie native to the Caucasian area, (*Sedum spurium* (L.) M.Beib.) which has been introduced and naturalized in almost all of Europe, including in Romania (Sîrbu and Oprea, 2011). At the beginning of the last century, it was considered a „boyar" plant, as it was mainly found in the boyars' gardens, rarely in those of wealthy peasants; until the middle of the last century when it is mentioned as sub-spontaneous plan in some areas of Transylvania and Banat (Borza, 1947). In many studies, it is treated as an invasive plant (Zheng, 2013; Bruce, 2011; Higgins, 2005) with the ability to build dense populations in a short time (Zheng, 2013).

Also originating in the mounting, but this time from the mountains in central Western and Southern Europe, *Sempervivum tectorum* L. is cultivated in our country as an ornamental plant on roofs, walls and in cliff arrangements, especially in Transylvania, since 1816 (Borza, 1925; Sîrbu and Oprea, 2011).

Suggestively called „mother hen and chicken" name referring to the tendency of this plant to expand (Șelaru, 2007), *Sempervivum tectorum* L. is one of the species with superficial root system frequently met in cliffy gardens or even on walls. It flowers starting with May until the end of August. The vigor, rusticity and abundance of the flowering make this species be almost always present in the ornamental assortment of alpine gardens. It is suited for the setup of green fences and cliffs (Draghia and Chelariu, 2011; Cantor, 2008).

MATERIAL AND METHODS

The biological material used was 108 mature *Sedum spurium* 'Fuldaglut' specimens and 108 *Sempervivum tectorum* specimens, purchased in 12 cm flower pots from specialized nursery gardens. The uniform vegetal material has been transplanted in the spring of 2014 in the field of the Floriculture department and on the roof of a building belonging to University of Agricultural Sciences and Veterinary Medicine of Iasi, by mounting 18 containers of 80/480 cm, in order to also make a comparative analysis between their development on the ground and on roofs. The experimental scheme for each place of culture (roof and field), includes three variants, of three repetitions each, with 9 repetition plants, in which various type of culture substrates were used: a_1 (control) - forest soil a_2– mixture of Novobalt peat (43%), coconut fiber (30%), composted bark (23%), alginate (4%); a_3– blonde peat (40%), brown peat (30%), sand (10%) and forest soil (20%). This results in a total of 6 variants: rooftop - variants V_1 (substrate a_1), V_2 (substrate a_2) and V_3 (substrate a_3); and field (ground level) - variants V_4 (substrate a_1), V_5 (substrate a_2) and V_6 (substrate a_3).

Thus, for the mounting of the containers innovative materials were used, meant to protect the insulation of the roof where they were mounted, and the plants involved in the experiment as well. For a proper insulation o the containers, a special

membrane called MacTex BN40.1 200 gr was used and in order to retain rainwater we used Maxistud, which is a membrane with troconical protuberations of HDPR with a thickness lager than 20mm and with exceptional mechanical characteristics, which can retain up to 6L of water per $1m^2$. Over this membrane, in order to distribute the weight of the substrate and for a better water drainage we used a Q-Drain ZM 8 membrane (fig. 1).

a) b) c) d)

Fig. 1 - Alveoli mounting: on the roof (a-b); on the field (c-d)

The study has focused on phenological observations made depending on the planting date, the date the sprouts and flowers appeared. During the experiments we have ascertained the morphologic characteristics of the species: the number of flowers and leaves, the length and width of bushes and flowers and the number of sprouts, floriferous stems length. The data was interpreted statistically by analysis of variance and the results were compared with the average of the variants.

RESULTS AND DISCUSSIONS

Following the investigations made for *Sedum spurium* 'Fuldaglut', planted on the experimental field on 25th May 2014 we have ascertained that buds started appearing approximately 20 days since planting, on the 14th of June (fig. 2) and only 5 days later at the *Sempervivum tectorum* samples, without any difference based on substrates variants.

The flowering start for la *Sedum spurium* 'Fuldaglut', happened 6 days later, continuing until 20th September (tab. 1).

Table 1

Phenological and technological data for the *Sedum spurium* 'Fuldaglut' and *Sempervivum tectorum* plants

Species	Planted	Occurance of buds	Flowering		
			Start	Full	End
Sedum spurium 'Fuldaglut'	25.05.2014	14.06.2014	20.06.2014	1.07.2014	20.08.2014
Sempervivum tectorum	20.05.2014	25.05.2013	30.05.2013	28.06.2013	3.08.2013

The two species assured blossoming for approx. 3 months (fig. 2), starting with *Sempervivum tectorum* (late May - early June) and ending with *Sedum spurium* 'Fuldaglut' (until almost the end of August).

Species/month	May			June			July			August		
Sedum spurium 'Fuldaglut'												
Sempervivum tectorum												

Fig. 2 - Flowering period of *Sedum spurium* 'Fuldaglut' and *Sempervivum tectorum*

The biometric measurements (fig. 3) performed during the vegetation period (from late May to early September), to the specimens belonging to the species under study highlighted the fact that the plants had a normal development specific to each gender.

a)

b)

Fig. 3 - Flowering plants: a) *Sedum spurium* 'Fuldaglut'; b) *Sempervivum tectorum*

The *Sedum spurium* 'Fuldaglut' results concerning the bush diameter recorded values between 18,0 to 19,7 cm, and the differences from the average of the variants was not statistical relevant.

Table 2 presents the results on the number of tillers per plant, length of the floriferous stems, number of flowers per plant and number of flowers in an inflorescence at *Sedum spurium* 'Fuldaglut'. In regard to the number of tillers/plant, by comparing the values obtained for each variant to the average of the variants, we found that the highest values and significant positive differences from the average were recorded for V_4 and V_6 versions; above average values were recorded for V_1 and V_3 variants, but the differences were not significant. Weaker and fewer tiller growth was recorded for V_2 and V_5 (grown on a substrate made of a mixture of Novobalt peat, coconut fiber, composted bark and alginate), where the difference from the average was distinctly significant negative. A similar trend was registered also for the

other characters (number and length floriferous stems, number of flowers/inflorescence), meaning that the worse results, below average, have been the variants V_2 and V_5. It stands out to be more obvious (very significant) differences in the number of formed floriferous rods. Regarding the number of flowers/inflorescence, the richest flowering plants have grown on the substrate made from blonde format peat, brown peat, sand and forest soil placed in the field (V_6).

Table 2

Results regarding plant morphological characteristics of *Sedum spurium* 'Fuldaglut'

Var.	No. tillers/plant		Floriferous stems length (cm)		No. floriferous stems / plant		No. flowers/ inflorescence	
	Abs.	±d	Abs.	±d	Abs.	±d	Abs.	±d
V_1	28.0	+0.9	6.2	+0.2	2.7	0	11.0	+0.1
V_2	24.5	-2.6 ^{00}	4.7	-1.3 ^{00}	1.8	-0.9 ^{000}	9.0	-1.9 ^{0}
V_3	28.0	+0.9	7.0	+1.0 ^{x}	3.5	+0.8 ^{xxx}	12.0	+1.1
V_4	29.0	+1.9 ^{x}	6.0	0	3.0	+0.3 ^{x}	11.0	+0.1
V_5	24.7	-2.4 ^{00}	5.0	-1.0 ^{0}	1.5	-1.2 ^{000}	8.3	-2.6 ^{00}
V_6	28.7	+1.6 ^{x}	7.3	+1.3 ^{xx}	3.4	+0.7 ^{xxx}	14.0	+3.1 ^{xxx}
Average	27.1	-	6.0	-	2.7	-	10.9	-
LSD 5%		1,4		0.9		0.3		1.4
LSD 1%		1,9		1.2		0.4		2.0
LSD 0,1%		2,8		1.8		0.6		2.9

For *Sempervivum tectorum* plants the results obtained from measurements done on the bush diameter were similar, and the differences were insignificant from the average, with values between 9.7 and 11.7 cm. Similar results, with insignificant differences from the average, have been obtained for floriferous stems height (where the values were between 8.0 and 9.7 cm), and the number of flowers in inflorescence (values between 9.7 and 12 cm).

Table 3 presents the results of measurements performed at *Sempervivum tectorum* just for the characters that yielded statistically differences (no. rosette/plants also no. leaves/rosette).

Table 3

Results regarding the morphological character of *Sempervivum tectorum* plants

Var.	No. rosette/plants		No. leaves/rosette	
	Abs.	±d	Abs.	±d
V_1	7.3	+0.2	30.7	-0.2
V_2	6.3	-0.8 ^{0}	26.7	-4.2 ^{000}
V_3	7.3	+0.2	33.8	+2.9 ^{xxx}
V_4	7.6	+0.5	31.3	+0.4
V_5	6.5	-0.6	29.0	-1.9 ^{0}
V_6	7.8	+0.7	34.0	+3.1 ^{xxx}
Average	7.1	-	30.9	-
LSD 5%		0.7		1.4
LSD 1%		1.0		2.0
LSD 0,1%		1.5		2.9

Greater differences between variants occurred in the number of leaves in rosette. Both crops, from the roof and at the field, the substrate a_3, in variants V_3 and V_6, had a positive influence, leading to the formation of a larger number of leaves, with significant differences from the average. On the contrary, the substrate a_2 led to a poorer development of the plants, especially on crops located on the roof (V_2).

CONCLUSIONS

1. Composition of substrate used to grow *Sedum spurium* 'Fuldaglut' and *Sempervivum tectorum* placed both on the roof or in the field, influences plant growth and development.

2. Both species recorded positive influence from substrate a_3, made up of blond peat (40%), brown peat (30%), sand (10%) and forest soil (20%); less favorable was a_2 substrate, consisting of peat Novobalt (43%), coconut fiber (30%), composted bark (23%), alginates (4%).

3. Plant response to the type of substrate was more obvious at *Sedum spurium* 'Fuldaglut'

4. Regardless the type of substrate, the cultivated plants on the roof had weaker growth and lower flowering capacity than those situated at ground level.

Acknowledgements: *This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765*

REFERENCES

1. Borza Al., 1925 – *Flora grădinilor țărănești române II.* Ed. Grăd. Bot., Cluj, p. 49-72.
2. Borza Al., 1947 – *Conspectus Florae Romaniae, Regionumque affinium*. Ed. Cartea Românească, Cluj, p 360.
3. Bruce G., 2011 - *Effect of a modular extensive green roof on stormwater runoff and water quality*. Ed. Elsevier, United States.
4. Cantor Maria, Pop Ioana, 2008 – *Floricultură - baza de date*. Ed. Todesco Cluj-Napoca.
5. Draghia Lucia, Chelariu Elena Liliana, 2011 - *Floricultură*. Ed. "Ion Ionescu de la Brad" Iaşi.
6. Higgins A., 2005 - *Nearly Perfect Plants, Hiding Up on the Roof. Ed.* The Washington Post, United States.
7. Sîrbu C., Oprea A., 2011 - *Plante adventive în flora României*. Ed. Ion Ionescu de la Brad, Iași, pp. 141-142.
8. Șelaru Elena, 2007 - *Cultura florilor de grădină*. Editura Ceres, București, p. 208.
9. Zheng Youbin, 2013 - *Optimal growing substrate pH for five Sedum species. Ed.* AMER Soc Horticultural Science, United States.

THE EVALUATION OF TOXICITY IN ORNAMENTAL PLANTS – ELEMENT IN ECOLOGICAL LANDSCAPE DESIGN

EVALUAREA TOXICITĂȚII PLANTELOR ORNAMENTALE – ELEMENT ÎN PROIECTAREA ECOLOGICĂ

MIRCEA (ARSENE) Cristina Cerasela[1], DRAGHIA Lucia[1]
e-mail: cris_arsene@yahoo.com

Abstract. *Ornamental plants can create allergenic, irritative and toxic diseases. The knowledge of potentially toxic plants is a public health issue. This paper aims to study the unwanted effects of ornamental plants on health, correlated with the presence of these plants in parks and gardens. The main research methods used were: bibliographical study, analysis and synthesis of data, collection and processing of statistical and legislative data, case-centered analysis. The information was provided by scientific literature. The analysis units were green urban spaces in Iasi. The paper centralizes the potentially pathogenic effects of ornamental plants, exemplifying with herbaceous and ligneous vegetation present in Romanian flora. Urban landscapes in Iasi were evaluated from an ambient health perspective. In the structure of landscape designs it is recommended to avoid the use of highly allergenic and toxic species. It is necessary for different designing programmes to consider the impact on health.*
Keywords: *proiectare, biosecuritate, plante toxice*

Rezumat. *Plantele ornamentale pot produce afecțiuni alergenice, iritative şi toxice. Cunoaşterea plantelor cu potențial toxic constituie o problemă de sănătate publică. Scopul lucrării este de a studia efectele nedorite ale plantelor ornamentale asupra sănătății, corelat cu prezența acestor plante în compoziții peisagere. Principalele metode de cercetare abordate au fost: studiul bibliografic, analiza şi sinteza datelor, colectarea şi analiza datelor statistice şi legislative, analiza centrată pe caz. Informațiile au fost furnizate de literatura de specialitate. Unitățile de analiză au fost reprezentate de spații verzi din Iaşi. Lucrarea centralizează efectele potențial patogene ale plantelor ornamentale, exemplificând cu vegetație erbacee şi lemnoasă prezentă în flora României. Amenajări urbane din Iaşi sunt evaluate din punct de vedere al sănătății ambientale. In compoziția spațiilor verzi se recomandă evitarea utilizării speciilor de mare alergenitate şi toxicitate. Este necesar ca diferitele programe de proiectare să ia în considerare impactul asupra sănătății.*
Cuvinte cheie: *landscape design, biosecurity, toxic plants*

INTRODUCTION

Wellbeing is a delicate balance between healthy human processes (psychological, physical, spiritual) and healthy environments (landscapes, weather, build environment, social circumstances). Carefully designed garden

[1]University of Agricultural Sciences and Veterinary Medicine Iaşi, Romania

environments can help maintain the balance necessary for the healthy life (Johnson and Johnson, 2012).

Vegetation is an important component used in landscape design that blends beauty with environmental benefits. However, most plants are toxic to a degree and planning a garden with only zero-risk plants would probably be impossible. Plant toxicity must be kept in perspective. Ornamental plants can act as hazardous when eaten, touched or inhaled. Basically, plants cause harm through some mechanisms: irritation (mechanical or chemical), toxicity and allergenicity. Sometimes, different mechanisms can act together (Crosby, 2004).

This paper aims to overview common ornamental shrubs and trees from Romania with hazardous potential. Urban landscapes in Iasi were evaluated from an ambient health perspective and potential dangerous plants were emphasized.

MATERIALS AND METHOD

This paper synthesized commonly hazardous outdoor plants, used and popular in Romania. Their risk potential to human health was discussed. Vegetal structure of landscape from Iasi was evaluated from a health perspective. The research methods used were bibliographical study, literature review and synthesis, collection and processing of data, comparative analysis. The biological material considered included representative trees, shrubs, climbers and some herbaceous ornamentals.

RESULTS AND DISCUSSIONS

Outdoor plants could be dangerous in special circumstances related to the plant, way of exposure and potential victim (Crosby, 2004; Piirilia, 1994). Usually, undesired effects are registered by accident. Testing on humans solely for hazard identification purposes is generally not acceptable.

Hazardous plant parts or plant constituents represent effective defence against predatory animals or an expression of environmental adaptation. Plants present protective structures which could be harmful for humans and animals. These structures are classified by botanists in four classes: thorns, spines, prickles and epidermal hairs. Chemical compounds from secondary metabolism act as chemical protection. These active principles belong to different chemical classes and are species related. Their role in plant life is complex and incompletely known (Capon, 2010).

Morphological structures (Capon, 2010) and chemical compounds (Ciulei *et al*., 1993) which can be harmful for humans and animals are tabulated (tab. 1). The basic effects on human health are mentioned.

Poisonings and deaths from ornamental outdoor plants are rare. However, as Johnson (2006) emphasized, it is better to know which plants are potentially dangerous. Small children are often at risk from coloured berries, leaves and petals. Gardening could be a risk factor for allergic sufferers and some plants are known to produce hay fever, asthma or allergic contact dermatitis (Crosby, 2004; Esch, 2001; Huntington, 1999).

Table 1

Common outdoor plant constituents hazardous for human health

Type	Class	Typical elicitors	Health effects
1. Morphological structures	thorns	*Crataegus* sp.	- wounds - irritant urticaria - irritant dermatitis
	spines	*Ilex* sp.	
	prickles	*Rosa* sp.	
	epidermal hairs	*Urtica* sp.	
	sharp leaves	*Cortaderia sp.*	
2. Chemical compounds: 2.1 Toxic	peptides	*Ricinus communis*	- systemic toxicity or target organ specific effect (neuro, cardio, vascular, nephro, hepato, gastrointestinal, immune, respiratory, blood, reproductive); - carcinogenic
	alkaloids	*Taxus baccata*	
	glycosides	*Prunus* sp.	
	tannins	*Quercus* sp. *Robinia* sp.	
	isoprenoides	*Chrysanthemum* sp.	
	phenols & derivates	*Salix* sp.	
2.2 Sensitizers	quinones	*Betula pendula*	- skin sensitization (allergic contact dermatitis/ urticaria); - eye damage; - respiratory sensitization (airborne allergens)
	lactones	*Tulipa* sp. *Chrysanthemum* sp.	
	urushioids	*Ginkgo biloba*	
	essential oils	*Rosa damascena;* *Lavandula angustifolia;* *Juniperus virginiana*	
	resins	*Thuya plicata*	
	acetylenic alcohols	*Hedera helix*	
2.3 Irritants	esters	*Daphne* sp.	- skin and mucosal damage; - irritant urticaria/ dermatitis; - eye irritation/ eye damage
	organosulfur-compounds	*Brassica* sp.	
	calcium oxalates	*Parthenocissus* sp.	
3. Pollen grains	trees	*Acer* sp., *Betula* sp. *Platanus* sp.	- respiratory allergy (asthma, hay fever) in sensitised people
	shrubs	*Buddleja* sp. *Ligustrum* sp.	
	grasses	*Cortaderia selloana*	
	herbaceous plants	*Amaranthus* sp.	

Male trees and shrubs of some species (*Fraxinus, Acer, Ilex*) produce large amounts of pollen. It is recommended to avoid them (Quyang et al, 2007). Hazardous common ornamental plants from Romania are listed in table 2.

Table 2

Hazardous common ornamental plants

Hazardous class	Potential risk	Typical species
Trees	toxic (ingested)	*Ailanthus altissima, Betula pendula, Thuya* sp., *Salix* sp.
	toxic and dermal sensitizer	*Acer* sp., *Fraxinus* sp., *Quercus* sp., *Juglans* sp., *Populus* sp., *Platanus* sp.
	allergenic through pollen (inhaled)	*Aesculus* sp., *Tilia tomentosa, Prunus* sp., *Robinia pseudoacacia, Laburnum sp.*
Shrubs	toxic (ingested)	*Berberis* sp., *Taxus baccata, Cytisus scoparius, Cotoneaster* sp., *Juniperus* sp., *Ilex* sp., *Ligustrum vulgare, Viscum album, Sambucus* sp.
	sensitizer (dermal/eye contact)	*Thuya plicata, Juniperus* sp., *Cornus* sp., *Hydrangea macrophylla, Ligustrum vulgare, Rhus* sp., *Ceanothus* sp.
	allergenic through pollen (inhaled)	*Ligustrum vulgare, Syringa vulgaris, Buddleya davidii*
	fragrances (inhaled)	*Rosa* sp., *Lavandula angustifolia, Philadelphus* sp.
Climber	toxic and sensitizer (ingested/contact)	*Hedera helix, Clematis* sp., *Parthenocissus* sp., *Wisteria sinensis, Lonicera japonica*
	allergenic (pollen, inhaled)	*Wisteria sinensis*
Ornamental grasses	allergenic (inhaled or dermal contact)	*Cortaderia selloana, Stipa gigantea, Phalaris arundinacea, Festuca glauca*
Herbaceous ornamentals	toxic (ingested)	*Ricinus communis, Colchicum autumnale, Delphinium* sp., *Convallaria majalis, Ipomoea* sp., *Anemone nemorosa, Arum maculatum, Lupinus* sp.
	sensitizers (contact/inhaled)	*Chrysanthemum* sp., *Primula* sp.
	allergenic through pollen	*Amaranthus caudatus*

Iasi city has approximately 912 hectars of planted green areas. Sandu, Trofin and Bernardis (2012) assess the structure of ornamental vegetation in Iasi in a 10-year study. According to the general structure's evaluation, the authors found 47% deciduous trees, 29% deciduous shrubs, 15% resin tree and 9% resin shrubs from existing ornamental plants. The dominant species for all green spaces in Iasi are listed in table 3.

According to the authors, street plantations are 99% composed of deciduous trees, especially from the following genus: *Tilia, Acer, Robinia, Quercus, Populus, Juglans*. Recreational areas have the following dominant

genus: *Acer, Quercus, Fraxinus (F. excelsior), Robinia (R. pseudoacacia), Tilia.* There are also frequently planted gymnosperm shrubs as *Taxus baccata*, which present ornamental and pollution resistance value. There is no mention about the sex of the plants registered.

Table 3

Dominant vegetal species in Iasi urban landscapes (after Sandu *et al.*, 2012)

Class	Genus	Common species
Trees	*Tilia*	*T. tomentosa, T. cordata, T. plathyphyllos* (lime, linden, basswood)
	Acer	*A. platanoides, A. pseudoplatanus* (maples)
	Quercus	*Q. robur, Q. pedunculiflora, Q. petracea* (oaks)
	Populus	*P. canadensis,P. alba* (poplars)
	Robinia	*R. pseudoacacia* (black locust)
	Pinus	*P. sylvestris* (forest pine), *P. nigra* (black pine)
	Thuja	*T. occidentalis 'Fastigiata'* (arbovitae)
Shrubs	*Thuja*	*T. orientalis*
	Spiraea	*S. vanhouttei* (honeysuckle)
	Rosa	different species and cultivars (roses)
	Cornus	*C. sanguinea* (silver carp)
	Buxus	*B. sempervirens* (boxwood)
	Syringa	*S. vulgaris* (lilac tree)
	Ligustrum	*L. vulgare* (privet)
	Forsythia	*Forsythia* sp. (forsythia)

The analysis of vegetal structures from Iasi landscape (tab. 3) emphasised the presence of highly allergenic species (*Fraxinus excelsior, Acer* sp., *Tilia tomentosa*, *Quercus* sp., *Populus* sp., *Juglans* sp., *Ligustrum vulgare*) (tab. 2). Toxic species are also used in public spaces: *Robinia pseudoacacia, Taxus baccata* (tab. 2). Other studies are needed to correlate the vegetal structures in Iasi green spaces and the incident of allergic respiratory diseases.

CONCLUSIONS

1. Undertaking research on the criteria used to select plants for urban environment is a public health problem. Nuisance factors must be considered (including shedding bark and other morphological structures, unpleasant odours and poisonous and allergenic properties).

2. Urban landscape strategy must exclude plants which are dangerous for children from spaces used by them. Moreover, allergenic trees and shrubs and allergenic herbaceous ornamentals which are known to cause asthma or hay fever must be avoided. Low allergenic species are recommended.

3. Vegetal structure in landscape from Iasi reflects a high presence of allergenic plants. Allergenic trees are dominant.

4. Landscape design must be friendly, low-allergenic and low-poisonous, with a minimum of hazardous elements taken into account, so as to ensure biosecurity in human environments.

Acknowledgements: *This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132*

REFERENCES

1. **Capon B**., **2011** - *Botany for Gardeners – Third edition.* Timber Press, Portland, London, 268 p
2. **Ciulei I., Grigorescu E., Stănescu U**., **1993** - *Plante medicinale, fitochimie şi fitoterapie*. Vol. I, II, Editura Medicală, Bucureşti, pp. 732-740
3. **Crosby D.G.**, 2004 - *The Poisoned Weed – Plants toxic to skin.* Oxford University Press, London, 266 p.
4. **Esch R., Hartsell C., Crenshaw R., Jacobson R.**, **2001** - *Common Allergenic Pollens, Fungi, Animals and Arthropods,* Clinical Reviews in Allergy and Immunology, Humana Press Inc, vol. 21: 261-292
5. **Huntington L**, **1999** - *Creating a low-allergen garden.* Mitchell Beazley, Octopus Publishing Group Ltd., China, 127 p
6. **Johnson A., Johnson S.**, **2006** - *Garden plants poisonous to people*. NSW DPI Primefacts, 359, pp. 1-12
7. **Piirila P.**, **1994** - *Occupational asthma caused by decorative flowers: review and case reports*. Int. Arch. Occup. Environ. Health. 66, pp. 131-136
8. **Quyang Z.Y., Xin J.N., Zeng H., Meng X.S., Wang X.K.**, **2007** - *Species composition, distribution and phenological characters of pollen allergenic plants in Beijing urban area.* Ying Yong Sheong Tai Xue Bao, 18(9), pp. 1953-1958
9. **Sandu Tatiana, Trofin Alina, Bernardis R.**, **2012** - *An analysis of ornamental woody vegetation existing in Iasi county's green spaces.* Lucrări Ştiinţifice, Seria Horticultură, anul LV, vol. 55, nr. 2, pp. 269-274
10. ******* *Common poisonous plants and plant parts,*Earth-Kind Landscaping, Texas A&M University, http://aggie-horticulture.tamu.edu/earthkind/landscape/poisonous-plants-resources/common-poisonous-plants-and-plant-parts/
11. ******* October 2012, *Safe and Poisonous Garden Plants*, University of California, http://ucanr.edu/sites/poisonous_safe_plants/
12. ******* *Poisonous Outdoor Plants,* Alberta Agriculture and Rural Development, 2010, http://www1.agric.gov.ab.ca/$department/deptdocs.nsf/all/agdex13348

COMMON MISTAKES IN DESIGNING ALLEYS AND URBAN RECREATION PLACES

GREŞELI COMUNE ÎN PROIECTAREA PEISAGISTICĂ A ALEILOR ŞI LOCURILOR DE ODIHNĂ URBANE

DASCĂLU Doina Mira [1]
e-mail: doinamira@yahoo.com

Abstract. *It has become a "normal" fact that many urban green spaces provide landscaping polluting both physically and visually. The paper aims to select and analyze the most "practiced" mistakes in design and execution of paths and places of urban rest. Identifying these mistakes could help to establish the causes and subsequently their eradication by proposing correction. The aim would be that the mayors of cities should become aware that there is a need for landscape specialists able to create harmonious compositions, providing alleys both for people hurry to reach their jobs, but also pleasant path, with resting places, judiciously placed.*
Key words: *landscape design, urban effects, common mistakes.*

Rezumat. *A devenit un fapt "obisnuit" ca multe din spaţiile verzi urbane să ofere amenajări peisagistice poluante atât fizic, cât şi vizual. Lucrarea îşi propune să selecteze şi să analizeze cele mai "practicate" greşeli din proiectarea şi execuţia aleilor şi locurilor de odihnă urbane. Identificarea acestor greşeli ar putea contribui la stabilirea cauzelor şi, ulterior, la eradicarea lor prin propuneri de corectare. Scopul ar fi ca edilii oraşelor să accepte faptul că este nevoie de specialişti peisagişti capabili de a crea compoziţii armonioase, care să ofere atât trasee pentru oamenii grăbiţi să ajungă la locurile de muncă, dar şi trasee plăcute, revigorante, cu locuri de odihnă confortabile, judicios amplasate.*
Cuvinte cheie: *proiectare peisagistică, efecte urbane, greşeli comune.*

INTRODUCTION

Currently landscaping encounters a "helplessness" to overcome stage words regarding actual implementation of proposals to improve the urban comfort, the aestheticization of degraded urban areas and, effectively, combat pollution in general, especially visual pollution (Dascălu, 2006). Landscaping of paths and places of rest constitutes one of the areas generally endangered, due to municipality neglect. Alarm signals have emerged as in the very frequent cases specialized training has been neglected: arrangements are often created by untrained people, having "subjective" ideas and opinions, or leaving out executions to the workers of green spaces.

[1]University of Agricultural Sciences and Veterinary Medicine of Iasi, Romania

MATERIAL AND METHOD

This study aims to identify and analyse the most common mistakes in designing and executing alleys and urban resting places.

The investigation tries to establish the causes of these mistakes, in order to help finding ways for judicious planning, design and execution for various situations.

The cases chosen and analyzed in this paper tried to highlight some negative aspects that contribute to visual pollution and urban discomfort, both by design and execution.

RESULTS AND DISCUSSIONS

The design and implementation of alleys and resting places seems to be very simple and easy to be done by anyone unspecialized in landscaping. In this illusion lies a great danger. As example, the most common "folk concept" for the creation of paths into a site, in order to create a future square, consists in the idea of "letting people move freely and then draw the paths according to their daily routes". This "folk" solution could, possibly, solve only the problem of daily intensive circulations towards important points in surroundings.

In terms of landscape, despite these "landscape populisms", walkways imply more complex features, simultaneously providing: direct circulation or promenade, opportunities for rest and socializing, a relaxing adjacent ambient, developing aesthetic sense and civic responsibility.

Studying sites in Romania and abroad, analyzing and reviewing numerous cases, we identified the following issues as being important and endangered: dimensions of traffic flow, alleys and places of recreation; location of rest places; ergonomics, style, color and materials of urban furniture items; structure and design of adjacent vegetation composition.

The aim of this presentation would be that the mayors of cities should become aware that there is a need for landscape specialists, able to create harmonious compositions, providing alleys both for people hurry to reach their jobs, but also pleasant path, with resting places, judiciously placed.

In the urban areas that can be created alleys and resting places in various landscape zones: from little squares to big parks, from sidewalks or footways to pedestrian esplanades, from outdoor institutions areas to residential gardens (Dascălu, 2006). The circulation appears to be the most important of the alleys functions. Therefore, studies of population segments, that cross daily the urban green areas, are helping to establish the correct traffic flows.

Depending on the type of movement and functionality of landscape areas, it can be designed various type of alleys: only for traffic or promenade, but also with resting places located along or in some dilatation created as adjacent spaces of traffic directions (Dascălu, 2011). Also, the route can be sprinkled with spaces expansions, treated as areas of dynamic landscape interest and/or passive recreation areas.

In the design of paths with resting places, the most frequent mistakes are those regarding the location of furniture (fig. 1).

Fig. 1 - If banks are located completely outside of paving
people are forced to keep their feet in the dust, or in the mud (photo D. Dascalu)

In these cases are necesary following recommendations:

- in front seating space must be created a space of min. 0.50 m for the person sitting on the bench, which should not be disturbed or touched by the persons circulating on the alley; we can call this as „vital individual space";
- the space occupied by banks and the "vital individual space" should not enter into the calculation of alley traffic flow;
- it stands to reason that the locations of rest places along the paths will increase the width of the walkways; consequently the resting places can be located also in some dilatation created as adjacent spaces of traffic directions;
- incorrectly placed in the alleys, banks may become obstacles, creating a traffic strangulation.

If we design an alley with resting places, banks must be placed on paving. Mistakes occur when banks are placed half on paving-half on green space, or completely outside of paving. In these cases, people are forced to keep their feet in the dust, or in the mud (fig. 1). Of course, some resting places can be placed in the lawn or green spaces, but these cases require a special study (fig. 2).

Fig. 2 - Some resting places can be placed in the lawn or green spaces,
but these cases require a special study (photo D. Dascalu)

Pavings should be anti-sliding, pleasant and comforting as a texture while walking, combined with harmonious materials and colors. Sliding pavings create problems especially in the rainy and snow season.

Edges can bring in the urban spaces a touch of elegance, if proportions, harmonies, materials and colors are used correctly (Dascălu D. M., 2006). Edges and borders can often generate problems due to lack of attention for their versatility. Sometimes redundancies occur, placing the bank in front of wide borders that can be used as sitting places. Sometimes edges are incoherent as route, generating confusion and visual pollution (fig.3).

Fig. 3 - The edge is interrupted by wrong position of public lighting columns (photo D. Dascalu)

In order to remove visual pollution, aestheticization proposals for degraded urban areas are involving the use of an important and well known principle of landscape design: unity in diversity.

Styles, colors and materials, used in many cases of alleys and recreation areas, offer many opportunities for violation of this concept. Nowadays there are many design catalogs for aesthetic urban furniture. Despite this fact, mistakes consist mostly in the wrong combination of colors, shapes and volumes, for the furniture of resting places, also for the paving (fig.4 and 5).

Fig. 4 - The unaesthetic effect due to joining of two banks with completely different and opposite styles (photo D. Dascalu)

Fig. 5 - Unaesthetic effect due to joining of many furniture objects with completely opposite styles (photo D. Dascalu)

The design of vegetation, as complex and harmonious compositions, can create an intimate and relaxing space, both for promenade along the alleys, also around the resting places (Trowbridge and Bassuk, 2004).

Unfortunately, the most frequent urban examples of plantations all along the alleys are either those eclectically composed, or that monotone composed. This is the result of a routine design, as a consequence of the lack of understanding of landscape design techniques regarding vegetation.

Trying to create diversity without unity, or without a harmonious composition of species, bring the danger of eclectic plantations, suffocated by a big density. The effects consist in visual stress or visual pollution (Dascălu, 2012). In the adjacent spaces, around alleys and resting places, another common mistake is the fragmentation or the dilution of images, either by using anaesthetic hedgerows, or by spreading singular shrubs or trees on large surfaces (fig. 6 and 7). This kind of design vegetation can generate visual monotony. The lack of personality and the insipidity of these vegetal images can create a less desire of meetings in such spaces.

Fig. 6 - Unaesthetic effect due to the unsightly aspect of the hedgerows, combined with the old bench wrong located (photo D. Dascalu)

Fig. 6 - Dilution of images by spreading singular shrubs or trees on large surfaces, around alleys and resting places (photo D. Dascalu)

CONCLUSIONS

Common mistakes in designing alleys and urban recreation places are most often related to the lack of information, routine, “folk concepts” and a false desire to "save public money" that hides the real problems.

The issues presented in this paper are intended to be an original contribution, necessary to improve the existing urban landscape studies which are dealing usually with general known informations, paying less attention to the details above mentioned and analysed.

Correction of these errors, through curative and preventive action, is absolutely needed.

REFERENCES

1. **Dascălu D. M., 2006** - *Peisagistica: o posibilă terapie pentru problemele mileniului al III-lea*, Editura Societății Academice „Matei - Teiu Botez”, Iaşi, pp.198-200.
2. **Dascălu D. M.**, **2011** - *Landscape effects of urban furniture textures*, Bulletin of USAMV Cluj Napoca, vol. 68 (1), Horticulture, pp. 324-331.
3. **Dascălu D. M., 2012** - *Critical analysis of some urban plantations in Iaşi citty,* USAMV Iaşi, Lucrări ştiințifice, vol.55, nr. 2, seria Horticultură, pp. 563-569.
4. **Trowbridge J. P., Bassuk L. N., 2004** - *Trees in the urban landscape*, John Wiley & Sons, USA, p.25.

WATER AND INDUSTRIAL ARCHITECTURE. FROM TECHNOLOGICAL PROCESS TO AESTHETIC MEANING

APA ȘI ARHITECTURA INDUSTRIALĂ. DE LA PROCES TEHNOLOGIC LA SEMNIFICAȚIE ESTETICĂ

ANDREI Radu[1]
e-mail: radu_and3@yahoo.com

Abstract.*Water has been one of the natural elements with a major influence in industrial development. Both as an energy source and as a cheap and effective transportation means for materials and products, natural and artificial water courses have influenced not only the layout of industrial buildings, but also their relationship with the urban environment, bringing radical changes in the outlook of cities. Once they cease to function, deserted industries left behind an impressive built heritage. Our study probes into the potential of this natural element as a premise of industrial architecture form, further exploring the possibility of redesigning the relationship between city and water through architectural conversion.*
Key-words*: water, industrial architecture, architectural conversion, urban regeneration.*

Rezumat. *Apa a constituit unul din elementele naturale cu o importanță majoră pentru dezvoltarea industrială. Deopotrivă sursă de energie și mijloc eficient și ieftin pentru transportul produselor și materiilor prime, cursurile naturale și artificiale de apă au influențat atât configurația clădirilor industriale, cât și dispunerea lor în mediul urban, schimbând radical imaginea orașelor. După încetarea funcționării, industriile părăsite lasă în urmă un fond construit impresionabil și totodată posibilitatea de a reface conexiunea orașului cu apa. Studiul explorează potențialul elementului natural ca premisă a formei arhitecturale industriale și totodată ca resursă de regenerare urbană.*
Cuvinte cheie: *apă, arhitectură industrială, conversie arhitecturală, regenerare urbană*.

INTRODUCTION

The layoutof industries in the territory is based on a system of merely practical constraints. Some of them are specific to each branch, deriving from the characteristics of the raw materials as well as of the end products. Others, of more general nature, are determined by the situation of the human resources, the position and evolution of the market and the transport required to link these industries to the place of origin of the primary resources.

In relationship to the means of transport and the production technologies,the natural elements have often had a major influence.

[1]„Gh. Asachi" Technical University of Iasi, Romania

In the decision to preserve and reuse industrial buildings, beside the economic or built heritage value, an important role was played by the favourable positioning in relation to the natural elements.

MATERIAL AND METHOD

This paper focuses on highlighting the relationship between the industrial buildings layout and their site, respectively the natural conditions. The study area was reduced to the specific situation shown by the proximity of water, initially strictly based on functional reasons and which is often a strong argument for the conversion to civil architecture programmes.

In order to define this aspect there have been identified and analysed some examples, significant in number as well as in typology regarding the historical evolution of production and the production specific, respectively by studying the positioning plan and the key elements of the architectural configuration: plans, sections, images of the building's volume.

This study takes into consideration the relationship between the landscape and the industrial architecture by analysing the different functional types – production buildings and storage buildings. The ideas are supported by plans and images from representative areas for the industrial development.

It is shown the influence of water on one side as a modelling factor in the concept of town planning and the industrial architecture from the 18th and 19th centuries and on the other side as an aesthetic factor in its ulterior conversion.

RESULTS AND DISCUSSION

The study has underlined two significant aspects:

1. The positioning of industrial buildings next to water flows has directly influenced their architectural features.
2. The positioning of former industrial buildings next to water fronts is a decisive argument, most of the times, in the conversion option.

1. The research made on the industrial halls from different stages in the technological development and belonging to different branches has shown that there are two categories of buildings directly connected to water: production buildings and warehouses.

1.1 Production buildings

At the beginning of the industrial revolution the positioning near the water of the first production buildings belonging to the textile industry was an essential condition for the production activity as water was the main source of energy as well as an efficient and cheap means of transport, mostly before the development of the railway transport.

Examples from this period show that the main principle which generated the solution for the multi-level layout is related to the source of energy used by the machines which were needed for the mechanization in textile production.The use of water energy implied connecting the machines to a big wheel through a system ofspur wheel and shafts.Thus, it was more efficient to place the industrial

equipment on different levels close to a single vertical axis.Starting from the functional and technical details of the production flow, the so called *cottonmills* were built as production halls on 3-6 levels.

Still, the interesting fact is that water has also influenced the structure of the industrial buildings due to its features as a landscape element. The level plan was thus elongated in order to obtain the best contact with the water. The subsequent extensions followed the same motivation for a linear structure. (fig. 1, fig. 2)

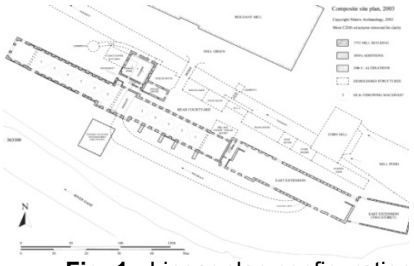


Fig. 1 - Linear plan configuration Old Mill, EastCheshire, half of the 18th century

Fig. 2 - Old Mill, EastCheshire, aerial view,half of the 18th century

The perspective of accomplishing a profitable activity led to the strategic connection between the production places and markets, either directly, by placing them inside town areas or indirectly, by getting closer to natural or artificial transport means.The 19th century brought the development of several water channels which enhanced these connections and contributed to the spreading of industrial buildings.

The continuous development of the energy resources added to the transport development flourished during the second half of the 20th century.This aspect was in favour to a new criterion in choosing the position of industrial buildings, namely the strategic element. The development of industrial buildings in the port area was based on strategic reason. The efficiency of this production system is explained by the spreading of these buildings in the neighbourhood of ports, areas which include a wide range of production branches and which cover wide areas. (fig.3)

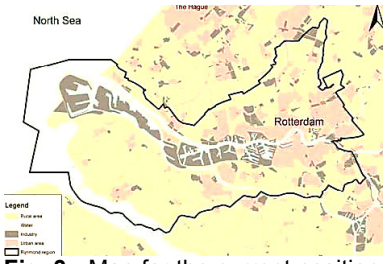


Fig. 3 - Map for the current position of industries in Rotterdam (the industrial area is marked in a darker shade)

1.1. Warehouses

The major changes from the industrial revolution which generated the large scale industrial production had as consequence the delivery of great quantities of raw materials, products and fuel which required the arrangement of storage buildings that would answer to the new demands.

During the industrialization period the distribution warehouses evolved simultaneously with the production buildings and the progress made in transport.

The storage buildings around the ports (docks) mainly thrived, the commerce on sea being initially the most efficient and accessible, until the land transport spread. The most efficient system of storage in wide spaces was to be found in the layout of docks.

Consequently, the docks spread and the waterfronts expanded through the development of inner channels and basins (fig.4, fig.5, fig.6). Maximum economicand land occupation efficiency were the base ideas of the planning , resulting areas of spectacular structure, with buildings in linear arrangement – warehouses along the artificial channels or lagoons with heights imposed by the level of the building technology and manipulation techniques for the products. One of the widest and most interesting docks spreading in the system of channel network is seen in Hamburg port (fig.5). London docks are included in the same category (fig.4).

Fig. 4 - Plan of the London docks from 1964

Fig. 5 - Hamburg in 1910

Fig. 6 - Liverpool in 1836

2. Water proximity as a convenience for industrial building conversion

The success of the urban development through the conversion of the deserted docks, supported by the authorities from the USA and UK during the 1980s proves the interest shown by the population and the business environment to reuse these places.

Beside the practical opportunity of accessibility which,under the new conditions, could be helpful to the civil programme functions, the natural element presents an obvious special psychic component which is interesting to the beneficiaries. Hence, some companies prefer to place their headquarters close to water, taking advantage of the high visibility and an underlined architectural image due to the water reflection effect, creating on one hand a favourable working environment (Warman, 1990)and on the other hand, an attractive spot for the clients, and for the product advertising (fig.7, fig.8, fig.9).

Fig. 7 - Offices designed in the former docks in Hamburg

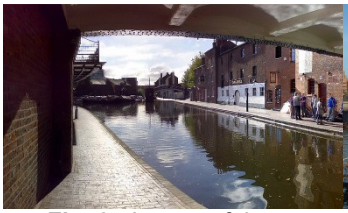


Fig. 8 - Layout of the channels from the industrial areas in Birmingham

Fig. 9 - offices in former docks - Bristol

Built under special technical and economic conditions, prohibitive for the civil architecture but necessary to the efficiency required by the industrial process, the docks and mills are objectives of high interest in the architectural conversion. The water surface brings in the congested and often rigid modern town design, an element of contrast due to the impression of an immaterial world, of permanent change and vibration.The subtle qualities of the water, its ability to reflect, its continuous movement and the living creatures it attracts, all these features become active parts in the complex perception of the architectural ensemble.

"It isthelure of water, its spells, its reflection, it sendless movement and change, that best captures man's imagination and provides a variety of applications from business to recreation, from calm to passive activities, the water's edge is where life is most diverse and unique" (Torre, 1989).

3. Significant examples

The Bankside building, built between1947 and 1963, is impressive due to its monumentality and expressivity of the volume.

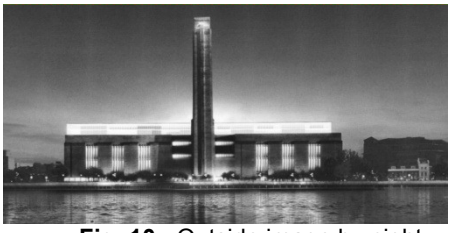


Fig. 10 - Outside image by night

Fig. 11 - View from the inside

The massive aspect, the symmetry and the dominant chimney give a distant personality to the building, visible from the opposite bank of the Thames. The special position on the river quays was a decisive argument for the conversion of the industrial building into a gallery of modern art. The change in function is announced by the extensions of luminous volumes on the roof and the chimney which are reflected on the Thames at night (fig.10). The same effect is desired for the interior, in a replica of the outer image of the water mirror, which is a clear proof given by the designers for its important presence, by suggesting the natural element in the architectural work (fig.11).

Through the minimum changes made during the conversion of the Menier chocolate factory from Noisiel-sur-Marne, France, there are underlined the aesthetic qualities of the buildings and of the natural environment, water being a dominant element in the landscape and a linking element for the buildings in the

ensemble, built along the times, in different styles and with different personalities (fig.12, fig.13, fig.14).

Fig. 12 - Menier chocolate factory, Noisiel-sur-Marne.Archive image before the conversion

Fig. 14 - Menier chocolate factory, Noisiel-sur-Marne after the conversion

Fig. 13 - View of the factory from Marne river

Also, most docks from the big ports are in full conversion process, as it is the case of the Liverpool docks, converted for the Tate Gallery, or the more recent example of the Hamburg Philharmonic.

CONCLUSIONS

The industrial building, a dwelling for the machine, was a machine itself, placed in the environment so as to directly use the resources, ignoring any other kind of relationship with it. The close connection between the water and the industrial buildings proves to be not only an example of the practical inclusion of the building in the context, but also a major advantage to conversion. Through adapting these places to new functions, new meanings are born. The building changes from machine into a living organism, populated by people who give it a new personality. The meaning of the water is transformed from a merely technological element to an aesthetic object. At the same time, the area around the converted building changes significantly. Those industrial urban areas which divided the city from the water are now conquered by the public space and a new equilibrium is reached.

REFERENCES

1. **Fletcher M.,2008** - *Old Mill, Congleton, Cheshire - Brindley's Grand Design?*, Industrial Archeological Review, XXX: 1,.
2. **Powell K., 1999** - *Architecture Reborn*, Laurence King Publishing, p. 224.
3. **Torre L. A.,1989** –*Water front Development.* Van Nostrand Reinhold, New York.
4. **Warman C.,1990** -*Business taken to working on water.* The Times, 25 July, p. 4.

LAND ART – THE HARMONY BETWEEN ART, NATURE, LANDSCAPE

LAND ART – ARMONIA DINTRE ARTĂ, NATURĂ, PEISAJ

ŞTEFĂNESCU M.[1], ŞTEFĂNESCU Mirela[2]
e-mail: mirstef68@yahoo

Abstract. *In this paper present one of the most interesting artistic experiences from the perspective of art/ nature/ landscape architecture called land art and how the artists managed to create an harmony between natural materials, working techiniques and lanscape. The art created with the specific area materials which is integrated in landscape has an aesthetic effect and also ecological aspects. Through their land works the artists seek to raise awareness of the destruction of nature by people and the effects of global warming on the environment. The interest of the artists for the unprocessed materials, the love for art and landscape has led to create wonderful works which have a special and great visual impact and from this point a view we can say that pure art we will find in the works of land art. The visual impact for these land works is very impressive both through works dimension and the harmony between the forms, the materials (plants, trees, stones, sand etc.) and the landscapes chosen for create these amazing land works.*
Key words: *land art, global warming, Robert Smithson.*

Rezumat*. În acest articol este prezentat unul din cele mai interesante experimente artistice din perspectiva artă/natură/ arhitectura peisagistă şi anume land art şi cum artiştii au reuşit să creeze armonie între materile naturale, tehnici de lucru şi peisaj. Arta, creată cu materiale specifice zonei, integrată în peisaj, are, în principal, impact estetic dar şi rol ecologic. Prin lucrările Land art artiştii vor să tragă un semnal de alarmă privind mediul înconjurător, distrugerea naturii, încalzirea globală. Interesul artiştilor pentru materialele folosite în starea lor pură, neprelucrate, dragostea pentru artă şi peisaj a condus la realizarea unor lucrări spectaculoase, cu efect deosebit şi impact vizual maxim, iar din acest punct de vedere putem afirma că arta pură o găsim în lucrările land art. Impactul vizual al lucrărilor land art este unul impresionant, atât prin dimensiunile lucrărilor, cât şi prin armonia dintre formele, materialele (plante, pietre, nisip etc.) şi peisajele alese pentru realizarea lucrărilor.*
Cuvinte cheie*: arta pământului, încălzire globală, Robert Smithson.*

INTRODUCTION

The contemporary artist, who looking for many plastic means of expression, it must fined those combinations of artistic language to express him self. The artist doesn't need a cliché to be explicit and meaningful.

[1] "George Enescu" University of the Arts of Iaşi, Romania

Contemporary artist's tendency is to release the designs and figurative traditions of the past, in order to consecrate the contemporary world, dynamic and constantly evolving. 20th-century art has gone through revolutionary changes. As a result of the countless influences were born new styles, themes have been used and new materials, resulting in creation of an astounding variety and strength.

Land art is an artistic movement which combines in the original way the landscape and human artistic creation. These artists are trying the potential of the landscape and the environment both through unprocessed materials and the landscape chosen for their land works (Farthing, 2011).

Land art emerged in America in 1968 shortly after the publication of Robert Smithson's essay 'The Sedimentation of the Mind: Earth Projects'. Robet Smithson wanted the people to realize their relationship with the nature and to involve in the landscape for create land works (Smithson, 1968).

The Robert's Smithson idea was that to escape from galleries of the arts from Manhattan and to give a geographic dimension between art and nature.

MATERIAL AND METHOD

The purpose of this paper is to show how the artists can create landscape architecture with simple means of nature, even in the place where the work will be placed. In this way they always try the potential of various materials, techniques, location places to create spectacular works.

Land art is one of the most interesting artistic experiences from the perspective of art/ nature/ landscape architecture. The art created with the specific area materials which is integrated in landscape has an aesthetic effect and also ecological aspects (Andrews M., 2006). Through their land works the artists seek to raise awareness of the destruction of nature by people and the effects of global warming on the environment. Robert Smithson, for example to protect nature he transformed a space where industrial waste were collected in one of the most famous works of art Land (fig. 1).

The most important artists who create land work and we will look on are: Robert Smithson, Andy Goldsworthy, Jim Denevan, Christo and Jeanne-Claude, Sonja Hinrichsen, Agnes Dumouchel, Richard Schilling.

RESULTS AND DISCUSSION

The most famous work in land art is Spiral Jetty create by Robert Smithson in 1970, who represents a long way (spiral) built of mud, rocks, salt crystals and algae of the Great Salt Lake, in Utah, and it has 1500 m (fig. 1).

Although his work was laborious work was dependent on the fluctuations in water level, being subject to the ephemeral, like many works of this kind, the nature of these fluctuations in water, he managed to acquire the work of art through the crust of white salt crystals deposited on rocks with such a spectacular work.

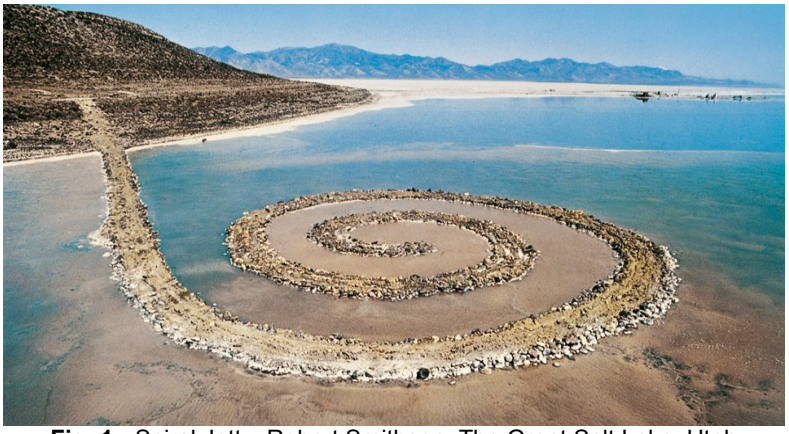


Fig. 1 - Spiral Jetty, Robert Smithson, The Great Salt Lake, Utah (http://en.wikipedia.org/wiki/Spiral_Jetty)

Because land works are created in nature with unprocessed materials they are ephemeral and this land works will keep in the art history with the help of the recordings and photos.

For exemple Sonja's Hinrichsen land works are so interesting, she used the snow to recreate the dram winter landscape (fig. 2) or Jim's Denevan land art in sand which reworks the beach (fig. 3).

Fig.2–Snow Drawings, Sonja Hinrichsen **Fig.3** - Surfers in Circles, Jim Denevan (https://www.google.ro/search Sonja Hinrichsen; https://www.google.ro/search Jim Denevan)

For their land works the artists use a lot of creativity, sensibility, respect for the nature also they have more knowledge in the other domains.

The best example is the land work creates in Sahara Desert by the artist Danae Stratou, the industrial designer Alexandra Stratou and the architect Stella Constantinides (fig.4). They work at this project two years.

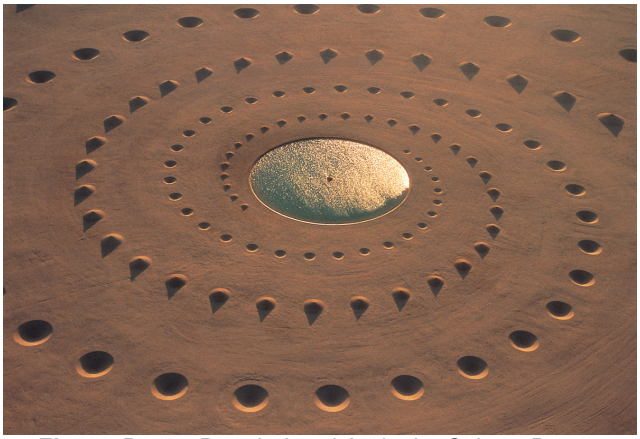


Fig. 4 - Desert Breath, Land Art in the Sahara Desert
(https://www.google.ro/search,Desert Breath,Land Art in the SaharaDesert)

The visual impact for these land works is very impressive both through works dimension and the harmony between the forms, the materials (plants, trees, stones, sand etc.) and the landscapes chosen for create these amazing land works (Grande, 2003). The land artists chose the special location for their works because this integration in nature of this works are more spectaculare. For instance the places: The Sahara Desert (fig. 4), The Salt Lack in Utah (fig. 1) or winter landscapes from Norway (fig. 2).

In their amazing land sculptures the artists are more intreresting about the creative process even than final result of their works. (fig. 5 and fig. 6).

Fig. 5 - Eve IL, Agnes Dumouchel
The creative process

Fig. 6 - Eve IL, Agnes Dumouchel.
The final work

(https://www.google.ro/search client)

The purpose of land-art - says the English land-painter, sculptor and photographer Richard Schilling - is rather a creative process then a final result. Using only what I could find in a certain place, I learned a lot about this place (fig. 7) (http://ecology.md /md/section.php).

Fig. 7- Richard Schilling (https://www.google.ro/search?client)

Besides the ephemeral sculptures in landscape using materials such as sand, seeking timber, snow, leaves, creating all sorts of shapes, Andy Goldsworthy created in 1997 the permanent sculpture /Storm King Wall/ in a modern sculpture park in New York State (fig. 8). The wall is made of a coil-shaped stones found there, 695 meters long, winds among trees, sink into a pond, stands out in the far plane and heading towards a deal without the trees, where it meets a highway. The Art and landscape is completed making from Andy Goldsworthy's work a symbol.

Fig. 8 – The Wall Storm King, Andy Goldsworthy
(http://greenskydesigns.com/art-in-the-landscape-goldsworthys-storm-king-wall)

A different concept about land art have Cristo and Jeanne- Claude by using aluminized polypropylene and string for wrapping the German parlament building (fig. 9). In another project Valley Curtain (fig. 10) they used an orange cloth, steel bars, many cables, concrete to hang this curtain in the Rocky Mountains. In their works, in general, not just in the two works presented here, the two artists do not use natural materials but rather plastic, concrete and steel. From this point of view we can say that the works of Christo and Jeanne Claude not adapt in landscape, we don't see that harmony between of materials, nature, art as the work of Robert Smithson or Andy Goldsworthy.

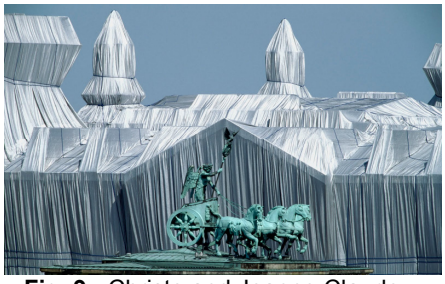


Fig. 9 - Christo and Jeanne-Claude Reicchstag packed

Fig. 10 - Christo and Jeanne-Claude Valley Curtain, Rifle, Colorado

(https://www.google.ro/search?q=christo si jeanne-claude)

Therefore natural materials, the special places of location, the creative process are important elements in land art creations through which is achieved a stunning landscape harmony."We appreciate the natural objects in terms of the work of art, which means that we embellish the nature" (Zaharia, 2007).

CONCLUSIONS

The interest of the artists for the unprocessed materials, the love for art and landscape has led to create wonderful works which have a special and great visual impact. We can consider the land art works a pure art both by the fact that the artists use unprocessed materials and by the choice of the special location for their works. As a result of the countless influences were born new styles, themes have been used the materials from the place where the work will be located, resulting a creation of an astounding variety and strength.

As a conclusion the particularity of this spectacular land art creations is the symbiosis between nature and human, also his place in the universe, they were created in the natural landscape with the traditional elements of nature being subject to degradation in nature, so it is a perfect harmony between art, nature and landscape.

REFERENCES

1. **Andrews M**., 2006 - *Land, Art: A Cultural Ecology Handbook*. London
2. Farthing St., 2011 - *Istoria Artei de la pictura rupestă la arta urbană*. Editura Rao, Bucureşti.
3. Grande J.K., 2003, *Balance: Art and Nature*
4. Smithson R., 1968 - *The Sedimentation of the Mind: Earth Projects*
5. Zaharia D. N, 2007 – *Estetica Analitică, Noi prefigurări conceptuale in artele vizuale,* Editura Artes, Iaşi.
6. http://en.wikipedia.org/wiki/Spiral_Jetty
7. https://www.google.ro/search Sonja Hinrichsen; https://www.google.ro/search Jim Denevan
8. https://www.google.ro/search,Desert Breath,Land Art in the SaharaDesert
9. https://www.google.ro/search client
10. http://ecology.md /md/section.php
11. https://www.google.ro/search?client
12. http://greenskydesigns.com/art-in-the-landscape-goldsworthys-storm-king-wall
13. https://www.google.ro/search?q=christo si jeanne-claude
14. https://www.google.ro/search?q=christo si jeanne-claude

RESEARCHES ON THE REDEVELOPMENT AND EXPANSION OF ZOO BEJAN DEVA

CERCETĂRI PRIVIND REAMENAJAREA ȘI EXTINDEREA GRĂDINII ZOOLOGICE BEJAN DIN MUNICIPIUL DEVA

BERAR C.[1], GHIURCA Andrada[1], SILIVĂȘAN M[1]., BĂLA Maria[1], TOȚA Cristina[1]
e-mail: cristianberar@yahoo.com

***Abstract**: Through this research is aimed at creating spaces solved optimally functional and aesthetically, with positive impact on the natural and social environment. By arranging complex design will be improving the quality of green spaces, increase the endowment of the area, increasing comfort and improving urban environmental quality. We propose the creation of facilities for the residents from Micro 15 neighborhood of Deva and others, which will increase the comfort and quality of life. This project aims to comply as much as the man and his problems, to respect nature and to help improve the environment. It also aims to have a positive impact on quality of life for residents and to increase biodiversity in the area.*
***Key words**: zoo, landscape planning, Bejan, Deva, animals*

***Rezumat**: Prin aceste cercetări se urmăreşte crearea unor spaţii optim rezolvate funcţional şi estetic, cu impact pozitiv deosebit asupra mediului natural şi social. Prin amenajarea complexă proiectată se va realiza ridicarea calităţii spaţiilor verzi, creşterea gradului de dotare a zonei, mărirea confortului urban şi îmbunătăţirea calităţii mediului. Propunem crearea de facilităţi locuitorilor din cartierul Micro 15 din municipiul Deva şi nu numai, ceea ce va conduce la creşterea confortului şi calităţii vieţii populaţiei. Acest proiect îşi propune să respecte cât mai mult omul şi problemele sale, să respecte natura şi să contribuie la îmbunătăţirea calităţii mediului. De asemenea, îşi propune să aibă un impact pozitiv asupra calităţii vieţii pentru locuitori şi să contribuie la creşterea biodiversităţii din zonă.*
***Cuvinte cheie**: grădină zoologică, amenajare peisageră, Bejan, Deva, animale*

INTRODUCTION

The Bejan Park covers a surface of approximately 2.4 hectares, most of it being occupied by the green area, flower patches, ornamental shrubs, trees, hedges, playing grounds for children. In the park one can find benches, drinking fountains, ecologic toilets, a water pool and a spring fountain as well as a gazebo, where during holidays the town brass band sings. During night time the park's lighting is insured by 30 small ornamental lamps and over 20 high ornamental lamps.

The Bejan Park is the largest in the town of Deva, and it includes a Zoo. Among the animals that can be seen there are wild boars, deer, ponies, ostriches and peacocks. The redevelopment of the park will attract the development of the

[1] Banat University of Agricultural Sciences and Veterinary Medicine of Timişoara, Romania

Micro 15 district as well, district placed in its immediate neighbourhood.

MATERIAL AND METHOD

The paper proposes to create facilities for the inhabitants of the Micro 15 district and more, something that will lead to the increase in the population's comfort and life quality. Its purpose is to respect the human being and his problems, to respect nature, to contribute to the improvement in environment quality, and to respect the general objectives of the National Programme for Environment quality Improvement. This project aims to have a positive impact on the inhabitants' life quality and to contribute to the biodiversity growth in the area.

And the Bejan Zoo, alongside with other zoos, offers its visitors the unique experience of living with animals, through adoption programmes or other special activities. The Zoo administrators wish for the public to appreciate nature and the animals and to get actively involved in their protection.

In time, things have evolved with regards to zoos as well, the bars have been replaced by glass fences, and the animal environment looks more and more like their natural habitat. Instead of concrete, they use natural stone and grass. Once these changes have occurred, the animals have started to change, and the aggressive behaviour has turned into a natural one. Gradually, people's attitude towards wild animals in zoos has also changed.

RESULTS AND DISCUSSIONS

The new norms in raising and taking care of wild animals in zoos forbids their keeping in improper conditions such as small and narrow spaces, and impose the creation of large spaces, of the „microbiotop or minihabitat" type, which should imitate as closely as possible the natural habitat of the respective species (caves, pools, rivulets, rocks, specific vegetation) and where the separation from the visitors to be achieved by level differences or water moats (Iliescu, 2003).

In the case of flying birds, we have built large bird houses, which should allow for a minimum of flying movement.

Fig. 1 – The Bejan Zoo – the situation plan of the area

Fig. 2 – The Bejan Zoo –general development plan

The woody and grassy vegetation proposed to cover important surfaces in order to complement the ambient, to mask unaesthetic walls, constructions or administrative areas serving for animal caretaking and maintenance of the respective installations.

Spaces destined for animals are set out with various tree, shrub or liana plantations in order to create an environment as close as possible to the natural environment of the respective species, to insure shadow in summertime, to increase the aesthetic value of the spaces and to mask shelters of some animal species.

The delimitations and the separation between different sectors were achieved by massive tree and shrub plantations, liana curtains or liana masked fences (Iliescu, 2002).

The placement of animal collections was made outside the building perimeter in a forest massive and close to a river, which is used as a water source, as well as for specific developments for the aquatic fauna. Inside the zoo there is also a drinking water source.

There are no industrial areas in the neighbourhood, nor garbage dust holes or installations with harmful influences on the sheltered fauna. There is a safe distance to the locality, so that bad smells or the noise produced by the animals do not disturb the population from the suburbs of the populated centre. Also, the distance does not present an impediment in visiting the facility for those visitors who choose to come on foot.

Access is also possible with public transport means, which insures the access of an important visitor segment. The number and types of zoo sectors varies according to the vastness of the territory, the number of species and the respective developments. Each sector will include spaces which shelter related animals (large herbivores, small herbivores, small carnivores, birds etc.) or animals living in the same habitat type (everglades, alpine and subalpine). The surface of a sector is correlated with the dimension and maximum number of animals which are to be sheltered.

Spaces reserved for animals are covered with vegetation, especially forests, as close as possible to the regions from where the animals originate.

The alleys are dimensioned according to the maximum visitor flow, in order to avoid crowding in the intensely solicited areas, and the indicated routes allow the public to see the exponents in a logical succession, without many overlappings or crossings.

In the main entrance area, there are large spaces, with landscape plannings proper for creating a pleasant atmosphere for the visitors.

Facilities necessary for resting (benches) can be found in every sector. In every area of the park, green spaces predominate, thus largely contributing to the zoo's beauty, regardless of the developments or the species that can be seen here.

The perimeter area presents a green belt, formed by the Bejan forest, with the main role to protect it against the dominant winds, possible pollution sources, to fade the noise coming from the zoo, to purify the air. There is a gazebo with food and refreshments, with products which do not harm the animals, since many visitors tend to offer animals food in order to attract them.

The Bejan Park is designed in a mixed style (Fig. 1). This style reunites characteristics of regular green spaces with landscaping ones, achieving in fact a merge of the two composition methods, inside the same green space.

In the central parts of the composition, straight, broad routes are used, which allow access for the large public, also sheltering the important buildings or edifices (Fig. 2). As a general rule, there is a main perspective axe, but also secondary axes, all of them shorter when compared to the perspectives of geometrically developed gardens.

In the park centre, there is a pool with water lilies and turtles, representing the centre of the composition. From all around it alleys start in all directions, some towards the zoo and others towards the children playing grounds, the resting and quiet leisure area, at the park's borders, where it merges with the forest margin.

Fig. 3 – The Bejan Zoo – panorama

The transit from the central area treated in a geometrical manner to the

marginal one treated in a landscaping manner is not done blatantly, but gradually, using certain transition elements characteristic for a certain area, but which can be used on a part of the other area, thus achieving a harmonious transition.

Green areas designed in a mixed style present a multifunctional character, comprising numerous equipment and secondary systemisings, predominantly in the case of large surfaces, with a high visitor receiving capacity, simultaneously allowing a greater freedom to adopt various solutions (Simonds and Starke, 2006).

Access to and circulation in the park was made possible in order to highlight functional and visual relationships between different elements and to differentiate between various degrees of their importance. The material used for the alleys is natural stone.

The woody vegetation is the main construction material of a green space, a material which changes its volume, colour, texture and shape in the course of a year or during a longer period of time. Through this material, the designer of the green space creates shapes, volumes, compositions, as it is also the harmonizing all anthropic elements, which will form a unity in the end (Rosemary, 2009).

The proposed vegetation is made up by species of deciduous trees, in order to contrast the resineferous species of *Picea abies* which cover a significant part of the park. Their choice was made following criteria of adaptation to the environment conditions and the creation of a pleasant, attractive ambient (Fig. 3).

The plants taken into account for the new development fulfil the following conditions:

- Resistance tested in the geostationary conditions of the site;
- Require minimum of maintenance efforts (natural resistance to physical-chemical factors of the visual placement);
- Good resistance to anthropic aggression 3 years from installation (the root system is extending underground quickly enough in order to insure tolerance for soil compaction and vandalizing);
- Do not bear risks regarding public health state (do not stain surfaces neighbouring to planting spot);
- Do not constitute temptations for thieves and children (through edible fruit or persistent element of ornamental value);
- Are not toxic for people or the animals present there;
- Do not present direct or indirect physiologic antagonisms to other planned species or to the neighbouring vegetation (do not have chemical or hormonal inhibition mechanisms against local competing vegetation, are not favourite hosts for vermin);
- In general, the selected species can insure shadowing shortly after plating;
- The aesthetic and social impact on the destination landscape is optimal on medium on long term.

Proposed deciduous trees: *Carpinus betulus, Platanus acerifolia*.

Proposed deciduous shrubs: *Buddleja davidii, Cornus sanguine, Chaenomeles japonica, Forsythia x intermedia, Spiraea biliardi, Spiraea*

bumalda, Viburnum opulus, Weigella florida, Rhododendron sp.

Proposed hedge: *Ligustrum ovalifolium*

In choosing the plant species planned for the development inside the Bejan park, we considered the analysis of conditions specific to the area: climate, soil, sunshine period, dominant winds, number of days of soil freezing, pollution degree, anthropic factors, the necessity to fulfil architectural-ambient requirements specific for the development project (pleasant ambient, shadowing of certain areas, growth rhythm, colour panel, contrasting effects, creating linking elements between spaces and area unity), biodiversity.

In order to solve these requirements, we took into consideration, alongside architectural criteria, the most adequate biologic and ecologic characters of plant species which could use to their advantage the area's potential and the specific pedo-climatic conditions.

CONCLUSIONS

The research regarding the landscape planning proposition referred to the following works:

- building alleys with ecologic materials (natural stone);
- creating new green spaces, which are currently not developed, with shrubbery and trees of various dimensions and species;
- placing urban furniture proper for the area;
- achieving public lighting with the help of photovoltaic lighting system;
- achieving an automatic irrigation system on the entire surface of the green space;
- placing ecologic toilets.

No species of great height were introduced, nor with special requirements regarding climatic conditions. All these criteria were taken into account in the landscape planning project so as to be better adapted to local conditions, in order not to raise the maintenance costs (free access to the garden), but also for the needs of the Deva town inhabitants.

The creation of spaces optimal from the point of view of functionality and aesthetics was sought after, with a special positive impact on the natural and social environment. Through complex landscape planning we will raise the quality of the green spaces, increase the equipment degree of the area, the urban comfort, and improve the environment quality. The maintenance of the green space is easily carried out, and the expenses allotted to this process are reduced.

REFERENCES

1. Iliescu A.F., **2003** – *Arhitectură peisageră*, Editura Ceres, Bucuresti

2. Iliescu A.F, **2002** - *Cultura arborilor și arbuștilor ornamentali*, Editura Ceres, Bucuresti

3. Rosemary A., **2009** - *The essential garden design workbook*, Timber Press;

4.Simonds J.O.,Starke B.W., **2006** – *Landscape architecture – a manual of environmental planning and design*, The McGraw and Hill Companies.

OCCUPATIONAL TRAINING FOR THE MENTALLY ILL THROUGH LANDSCAPING PROJECTS

PREGĂTIREA PROFESIONALĂ A PERSOANELOR CU PROBLEME ALE SĂNĂTĂȚII MINTALE PRIN PROGRAME DE AMENAJARE PEISAGISTICĂ

DUMITRAŞCU Aurora Irina[1], GAFIUC P.V.[2], NICA R.M.[1], CORDUBAN C.G.[1]
e-mail: aura.irina.dumitrascu@gmail.com

Abstract. *The integration of mentally ill citizens through professional reorientation constitutes an important issue for a society that encourages human rights. The active involvement through work is important for the mentally ill as part of their therapy, but also for the society that can benefit from their actions. With respect to these issues, landscaping projects represent a good opportunity that fits properly with the requirements of this group, creating a positive environment through an active lifestyle, as well as for the society, that needs well-trained employees in a domain that continues to develop. In a sustainability- oriented society, the focus is on rehabilitating and maintaining the existing green areas as well as developing new ones. The article presents general considerations on the topic of training people with mental illnesses, through landscaping projects, as well as a case-study of such a program successfully implemented in Romania.*
Key words: *training the mentally-ill, social program, landscaping competencies*

Rezumat. *Integrarea profesională a persoanelor cu probleme ale sănătății mintale constituie o temă importantă pentru o societate fundamentată pe respectarea drepturilor omului. Implicarea activă prin muncă este importantă pentru starea acestor persoane, ca parte integrantă a tratamentului cât şi pentru societate, care, poate beneficia de pe urma implicării active a acestora. Legat de aceste chestiuni, domeniul amenajărilor peisagistice constituie o oportunitate bună de punere în practică a acestor principii, prin crearea unui mediu de muncă favorizant terapiei, dar şi prin faptul că reprezintă un domeniu în continuă dezvoltare. Într-o societate care pune accentul pe dezvoltare sustenabilă, reabilitarea şi menținerea spațiilor verzi constituie un subiect de o deosebită importanță. Acest articol prezintă considerații generale pe subiectul implicării persoanelor cu probleme ale sănătății mintale în proiecte de amenajare peisagistică, precum şi un studiu de caz despre un astfel de program implementat în România.*
Cuvinte cheie: *integrarea profesională a persoanelor cu probleme ale sănătății mintale, programe sociale, competențe în amenajările peisagistice*

INTRODUCTION

Landscaping work has proved to be both "restorative" and "interventionist" for the mentally ill, beginning with gardening practices in the asylums a century

[1] „Gh. Asachi" Technical University of Iasi,"G.M. Cantacuzino" Faculty of Architecture, Romania
[2] Asociația Regională de Educare a Adulților (AREAS), Suceava, Romania

ago and reaching potentially new levels with the association of contemporary concepts such as social inclusion and equal opportunities in the latter decades. There is a therapeutic as well as a social dimension related to green work for the mentally ill, with focus on the agenda of social inclusion, of providing a sense of belonging to the mainstream community (Parr, 2007). Parallel to an appropriate activity that complements the therapeutic programs, there is an undisputed benefit for the community, considering the need to develop and enhance the quality of green areas in our cities for a sustainable approach to town planning.

During the last decades of the twentieth century, the tendency in western countries was towards the deinstitutionalization of people experiencing mental health problems, for better inclusion within the community, a system that in turn attracted criticism for the problems related to the provision of adequate health and social services. At the same time, there is a constant concern to identify the best jobs and work opportunities for people with mental health problems, so they can play a meaningful role in the community, just like everybody else (Parr, 2011).

There is a documented correlation between the quality of public open spaces and mental health, beneficial for everybody, as such developing training programs in landscaping for people with mental health issues facilitates contact with nature and develops an inter-determinate triangular relation between community, the mentally ill and public space (Francis et al, 2012).

MATERIAL AND METHOD

Training people with mental illnesses

People experiencing mental health problems, even with severe illnesses, are able to acquire and maintain new knowledge and skills. As such, occupational therapy interventions should identify the appropriate training for an individual to succeed professionally, as well as to implement step by step measures to ensure the program's sustainability. Occupational therapy practices must be "knowledgeable about evidence-based research and apply it ethically and appropriately to provide occupational therapy services consistent with best practice approaches." Research suggests that applications in real-world environments are more effective than the ones that focus on pre-training or preliminary skill building, but, depending on the severity of the mental condition, cognitive adaptation training and job accommodations are necessary in order to compensate some impairments that limit a successful occupational development.

Occupational practices for people with mental health problems should include a four stages approach:

1. Recommendation for professional services;
2. Evaluation
    - identifying an appropriate occupational structure,
    - analysis of performance through observation and assessment;
3. Following an intervention scheme
    - supported employment policies,
    - developing specific educational programmes,
    - training for real-life situations, including finance administration,
    - management of health care provision,
    - strengthening community living skills,

- focusing on exercise and outdoor activities,
- cognitive as well as emotional training;

4. Follow-up (Brown, 2012).

Considering the social context of Romania, a successful program should target issues related to the reluctance of occupational inclusion for the mentally ill in all the four stages of the training. In Romania, the current legislation, in particular Law no. 448 of 6 December 2006 on the protection and promotion of rights of people with disabilities, promotes principles and stipulations for equal opportunities for people with disabilities including fiscal facilities for the employer. Referring to the training of persons with disabilities, according to law, these are to be organized programs that pursue qualification, training and specialization. Authorities need to create the conditions for people with disabilities to have the right to choose and practice their profession, trade or occupation, to gain and maintain employment, in order to advance professionally. Unfortunately, the norms for the application of this law, approved by Government Decision no. 268/2007 of 14/03/2007 do not specify anything about practical methods of achieving these goals of the Act. Most programs for training people with mental health problems are carried by NGO's that focus on the social inclusion and equal chances of disabled citizens. AREAS NGO in Suceava, has a long history of occupational training for people with mental illnesses, including 4 workshops of occupational therapy for the patients of the Psychiatric Hospital in Burdujeni. The project for training 20 people with mental health problems in the Citadel Park of Suceava in the field of landscaping, that is presented in this article, represents an initiative of AREAS NGO.

RESULTS AND DISCUSSIONS

Description of the Citadel Park of Suceava

The green area that links the city centre of Suceava with the seat citadel of the Musatini dynasty has been transformed into the town's green lung in the 1970's with the effort of architect and city planner Nicolae Porumbescu, in parallel to the design of the House of Culture and main square. The concept of the project was inspired by the San Marco Plaza in Venice, the city square opening to the forest and the citadel in the same manner as the Piazza opens itself to the lagoon. For decades, the park represented an area animated by people enjoying open-air activities.

What could be the most important place of recreation for Suceava, at the foot of the Fortress of Prince Stephen the Great, is currently one of the most disagreeable and dangerous areas of the town, with destroyed alleys, nonexistent public lighting, uncovered manholes, broken stairs and permanent floods. This is a situation that persists for years without anyone able to invest in the rehabilitation of the park. And all this for the simple reason that the whole forest area located near the Citadel is owned by Suceava Forestry Department, specifically in the Forestry District of Pătrăuți. Under these circumstances, the management of Suceava City Hall claims that it doesn't have the ability to invest in the rehabilitation and redevelopment of the park, "an enclave in the city" and in terms of the Forestry Department, the institution has responsibilities only in protecting the forest fund.

Considering these premises, the aim of the project proposed by AREAS NGO is to rehabilitate the urban green space from the centre city of Suceava through involvement of the local community and people with mental illnesses in educational environmental activities. One of the dangers of bringing new life to an area with great arboretum value would be the insertion of spaces for leisure that would perturb the natural state. The best practices, on the other hand, include the involvement of the community in maintaining and restoring the quality of the space.

Project detailing

Capitalizing on the potential for green rehabilitation of the park described in the previous chapter, the project envisioned by AREAS NGO aimed at training 20 people with mental health problems in the field of landscaping work on the premises of the Citadel Park of Suceava. Through training activities in the open-air that took place so close to the city centre, the organization intended to tackle the issue of discrimination by demonstrating practically and bringing awareness to the public that the mentally ill can be integrated in the society with equal occupational chances. A secondary objective was to rehabilitate parts of the park, especially alleys and to implement public furniture in order to bring new life to an area that needs to find a renewed sense of belonging to the local community

Based on the four stages previously described, the programme was phased in such manner to include theoretical and practical courses with the involvement of qualified social workers. After an evaluation stage, the selection and assessment of the trainees, the year-long training course included:

- theoretical courses,
- workshops for urban furniture,
- gardening and tree caring,
- repaving of alleys.

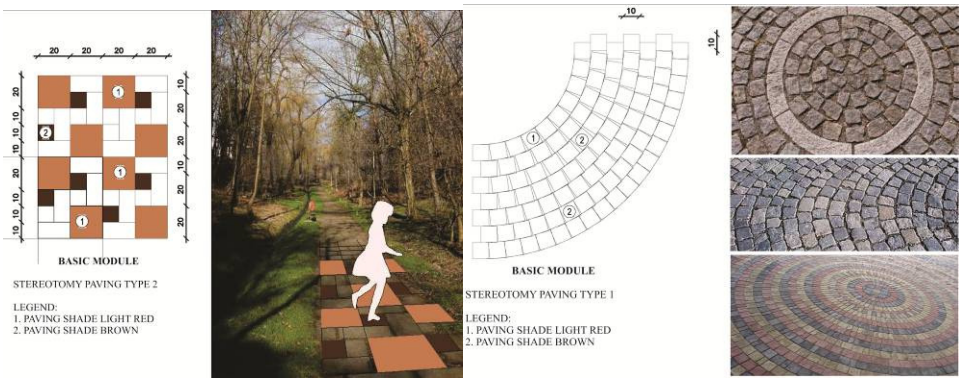


Fig. 1 - Stereotomy paving types

The structure of the program provided the possibility of the theoretical courses to run in parallel to the practical applications, with the possibility to

conduct the courses on urban furniture and parts of the practical courses on gardening in a workshop. The authors identified the paving schemes of the existing alleys and proposed designs with respect to the original configurations, (Fig. 1).

The authors proposed a series of designs for public furniture, that the people with mental health problems could learn to assemble using simple modulated wood elements. Considering that the problems of the trainees ranged from mild to serious, the designs ranged in turn from simple to complex. The concept was unity, producing architectural pieces that would reunite people and community with nature, (Fig. 2).

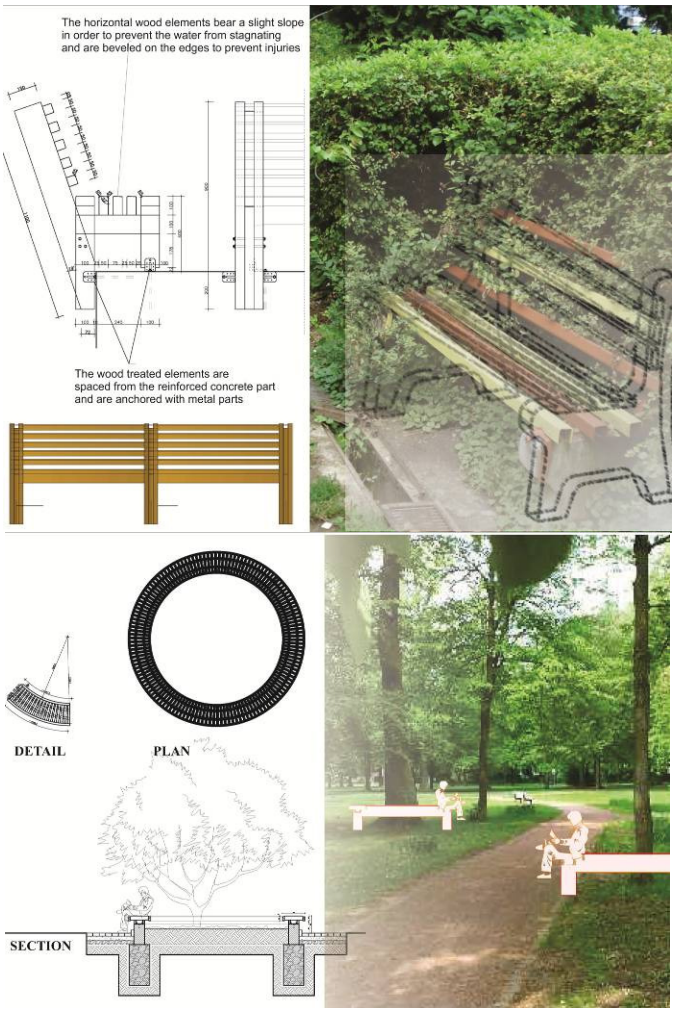


Fig. 2 - Proposed park benches - details

CONCLUSIONS

1. Unfortunately, the initially conceived project, presented in this article, did not receive founding, because the financiers considered that the focus had to be on training persons with mental health problems and secondary objectives were not part of the program. With a positive and creative attitude, the members of AREAS NGO managed to incorporate the ideas of this project in two different programs, one that focused on training the mentally ill in landscaping and gardening and the other involving volunteers to rehabilitate urban green spaces from the Citadel Park through engagement of the local community in educational environmental activities.

2. The training of the persons with mental health problems was done in partnership with the landscaping company Rompeisaj and involved courses for 14 people, prepared to work in gardening activities: some trainees practiced at the green house, others practiced in open-air works in the parks and the rest received their professional formation at a do-it-yourself goods hypermarket.

3. The activity of rehabilitation of the park engaged graduates and students from all high schools in the city, which under the guidance of an architect in the city hall and the environmental education officer planted propagating material. Location of trees, shrubs and flowers was projected theoretically (with models made on paper) and then placed in the field, designed as a harmonious whole between urban architecture and nature, intended to give new life to the public green space.

4. Although both projects proved successful, the authors hope that the program as it was first conceived could be implemented in the future with multiple benefits for the mentally ill as well as the community to which they belong.

REFERENCES

1. **Brown C., 2012 -** *Occupational therapy practice guidelines for adults with serious mental illness*, Bethesda (MD): American Occupational Therapy Association, Inc. (AOTA), pp. 115.
2. **Francis J., Wood L.J., Knuiman M., Giles-Corti B., 2012 -** *Quality or quantity? Exploring the relationship between Public Open Space attributes and mental health in Perth, Western Australia*, Social Science & Medicine, Volume 74, Issue 10, May 2012, Elsevier Ed., pp. 1570–157
3. **Parr H, 2011 -** *Mental Health and Social Space: Towards Inclusionary Geographies*, John Wiley & Sons.
4. **Parr H, 2007** - *Mental health, nature work, and social inclusion*, Environment and Planning D: Society and Space 25(3), pp. 537 – 561.
5. *****, 2006 -** *Law no. 448 of 6 December 2006 on the protection and promotion of the rights of persons with disabilities*, Official Monitor of Romania

THE PUPYES SERIES BY JEFF KOONS

SERIA „PUPPYES" CREATĂ DE JEFF KOONS

ŞTEFĂNESCU M.[1]
email: mirstef68@yahoo.com

Abstract. *In this paper we will show how Jeff Koons has managed to combine the landscape sculpture and architecture realizingt an interesting series of the floral starting from 1992 until 2002. The novelty brought by the Koon's works is given by the shape and size of the entire series. These works represent the form of the pet puppies, the Terrier race, oversized up to a 13 meter height. The flower pots are supported by metal frames. Although the flowers are perennial, these floral arrangements look wonderful and they need a lot of care by qualified personnel. The visual effect of such floral arrangements is special and for this it is worth the effort. Until now, the artist Jeff Koons has created several of these floral arrangements located in different contries in the world, such as: The Schloss Arolsen, Germany during Documenta IX, 1992, Guggenheim Museum, Bilbao, Spain, 1997, The Brant Foundation, Greenwich, Connecticut, 2002. The artistic quality of this series of pet puppies is controversial because the art critics have a different opinion about it. Desipe this, art collectors are still interested in floral arrangements. Placed in different locations these works get on different meanings aesthetic and managed to impose itself as an argument in support of the contemporary artistic approach.*
Key words*:* floral arrangements, Guggenheim Museum, Bilbao

Rezumat. *In acest articol vom arăta cum Jeff Koons a reuşit să îmbine arhitectura peisageră cu sculptura, realizând în perioada 1992-2002 o serie de aranjamente florale. Ca element de noutate, aceste aranjamente sunt volumetrice, redând forma unor căţeluşi de companie, rasa Terrier, supradimensionat până la 13 m înălţime. Ghivecele de flori sunt susţinute pe schelete metalice. Ţinând cont de caracterul peren al florilor, aceste aranjamente necesita o îngrijire permanentă, asigurată de personal calificat. Efectul vizual deosebit al unui asemenea aranjament merita efortul de întreţinere. Până în prezent, au fost realizate cinci asemenea aranjamente la Schloss Arolsen, Germany pe parcursul Documenta IX, 1992, Guggenheim Museum, Bilbao, Spain, 1997, The Brant Foundation, Greenwich, Connecticut, 2002 (instalaţie permanentă). Calitatea artistică a acestei serii este discutabilă, criticii având păreri diferite, dar în acelaşi timp, a trezit interesul colecţionarilor. Situate în diferite locaţii, lucrările capătă diferite valenţe estetice, reuşind să se impună ca argument în sprijinul demersului artistic contemporan.*
Cuvinte cheie: aranjamente florale, Muzeul Guggengeim, Bilbao

[1] „George Enescu" University of the Arts of Iaşi, Romania

INTRODUCTION

The 20th century remain in the history of art through constant redefinition of the field of Visual Arts. Creative energies were channeled more on searching of new mediums of expression than on deepening and plastic styles claimed by various artistic groups.

Born in New York, Pennsylvania, Jeff Koons studied at the Art Institute of Chicago and Maryland. Before he has a reputation as an artist, he has enjoyed success as a broker on Wall Street. He also worked in a Department that was collecting funds for the Museum of Modern Art in New York.

Building on the style of Pop-art and conceptual ideas, in particular his concept of the "readymade" Duchamp 's, presented as found art object, he discovered new areas as regards the content, the production, and the artist's relationship with the celebrity and the market art. He created beauty and challenging sculptures with a technical refinement and deserves the most critical and commercial success and his popularity (Kaufman, 2014).

MATERIAL AND METHOD

One of the famous artists who managed to merge and then revolutionize with success two completely different disciplines at first glance, sculpture and landscape architecture, it's the artist Jeff Koons. The series of works entitled "Puppy" (fig. 1) illustrates very well the aforementioned.

Fig. 1 - Puppy The Brant Foundation, Greenwich, Connecticut, 2002 - the permanent instalation (http://www.jeffkoons.com/site/pup_3.html)

The artist performs volume that defines these innovative projects, through the combination of different colored flowers supported on a metal armature.

To carry of the plants over an entire season, he designed an internal network of pipes providing water quantity needed of flowers, and if necessary some pots can be replaced by qualified personnel providing assistance to maintain the life of the high volume of plants.

With an instinct of roasting vs. consumer desires and enjoying the challenge, former broker cheerfully dissolved any authority itself of aesthetics and replaced it with fun — little girls who embrace the Bavarian porcelain pink Panthers, huge covered with flowers, puppies are just two examples of the topics addressed by the artist.

Toward the realization of a dream scenery classical sculptor Jeff Koons did not focus on its deployment on a flat surface, as usually happens, but simply turns the plan down, as a result of this transformation resulting in a form of a puppy that stands upright and is awaiting an order from calm to his master.

In most of cases the quality of a landscape, or a green space is achieved by bringing together all the elements of decor in a whole landscape that produces pleasure, delight, or unconditioned admiration, which exercises a spontaneous attraction (Wunenburger, 2004), but in this case the lawn increases its outline and the result was one full volume effect, due to the Association of clusters happy with the familiar image of a pet puppy.

RESULTS AND DISCUSSION

Contemporary multimedia artist Jeff Koons is one of the better quoted (in financial terms) but also controversial artists from Andy Warhol till now.

Fig. 2 - Puppy Guggenheim Museum, Bilbao, Spain, 1997 - the permanent instalation (http://www.jeffkoons.com/site/pup_2.html)

Influenced by his predecessors pop art such as Claes Oldenburg, Koons is known for his neo-pop kitsch, exemplified by works such as "Michael Jackson and Bubbles"-1988, made from porcelain and gold, or giant reproductions of banal objects such as "Puppy" (fig. 2), Guggenheim Museum Bilbao,1997, or "Balloon Dog", a giant steel replica of banal forms of puppy made of the combination of several balloons (Metropolitan Museum of Art).

It is important to point that his kitsch works aforementioned are part of a commercial strategy well thought preparation and coordinated in order to capture the attention of the consumer public art, so as to benefit from a media audience needed to bring in the auction halls as well many customers. In support of this claim stands as proof that the artist aware of lack of genuine artistic value he destroyed some of his works from the beginning.

Critics are skeptical when it comes to the work of Koons, although foals were sold at auction for astronomical amounts. In 1991, a version of "Michael and Bubbles" sold for 5.6 million dollars, at that time a record for a living artist. In 2008, the "Balloon Flower" was sold at Christie's in London for record amount of 25.7 million dollars, shortly before the onset of the global economic crisis.

Personally I don't think that financial success is synonymous with artistic value of a work of art, but in the case of Puppyes series I agree that we have to do with the high-quality contemporary art created in the spirit of the ephemeral and consumerist style which characterize the way of living of our days.

Fig. 3 - Puppy Schloss Arolsen, Germany during Documenta IX, 1992.
(http://www.jeffkoons.com/site/index.html)

In 1992, he managed to obtain financing from the Art Exhibition in Bad Arolsen, Germany. Koons has made what was to be one of the most popular of his works, "Puppy" reproduced in fig. 3, a sculpture of 13 meter drop in flower arrangements supported on a steel structure, which gives the image of a Terrier puppy.

The work was bought by the Guggenheim Foundation in 1997 and installed in the courtyard at Guggenheim Museum, in Bilbao. A faithful replica of the puppy has been performed for the renowned businessman, Peter Brant of Connecticut (fig. 1).

Critics have different opinions when it comes to the value of Koons as an artist. Adherents of traditional art taking into account the importance of an artist's skills in the craft of sculptor Jeff Koons and say it has no value because in his works appeal to the help of assistants. Others considered that only certain topics may benefit from the quality and aesthetic themes of Koons are far too "kitsch" to be considered art.

CONCLUSIONS

Located in various locations, such as the naturally, his works acquires different aesthetics meanings in musicology and, and with it the way which it is perceived is automatically diversify.

Being a work of contemporary art the most proper place to show his work is in front of the Guggenheim Museum in town Bilbao, Spain, where the relationship with the contemporary style of deconstructivist (Ortega y Gasset, 2000) and feature architecture creates a new reality suprarealisto-futuristic, that changed the classic perception which art consumers used to it (Marleau, 2001) if we refer to the classical relationship existing for thousands of years between sculpture and architecture.

The location of the work in the front of Bad Arolsen Castle, wants to create a contrast between straight lines and walls free of vibration, and round shapes life full of gaiety of living forms created by plants, the contrast does not annul but rather accentuates and detect each piece as compose the picture even though we have the temptation to regard as a picture frame and the castle on the right composition of Puppy work resulting from the proximity of the two works.

Other outdoor locations are not so happy chosen because it can easily be confused with the environement not being any other way to highlight the work than through the stain color that creates a strong contrast between the work and the environment.

The contemporary artist have the emblematic desire to distance themselves completely from classical art crossing the kitsch from subculturale genres, among classical art genres (Zaharia, 2007), but when it comes to aesthetic value, Puppyes series manages to impose itself as

one of the few artistic moments full of plastic and aesthetic meanings, as an argument in support of the contemporary artistic approach.

REFERENCES

1. Marleau Ponty M., 2001 – *Fenomenologia percepției*. Ed. Aion, Bucureşti.
2. Ortega y Gasset J., 2000 – *Dezumanizarea artei*. Ed. Humanitas, Bucureşti.
3. Zaharia D.N., 2007 – *Estetica analitică.* Ed. Artes, Iaşi
4. Wunenburger J.J., 2004 – *Filosofia imaginilor*. Ed. Polirom, Iaşi.
5. www.artphaire.com/delightful-perplexing-art-jeff-koons/ by Jason Edward Kaufman, 5 August 2014
6. http://www.jeffkoons.com/site/pup_3.html
7. http://www.jeffkoons.com/site/index.html
8. http://www.jeffkoons.com/site/pup_2.html

THE SYMBOL OF GRAPEVINE IN THE ARCHITECTURE OF THE SACRED SPACE

SIMBOLUL VIȚEI DE VIE IN ARHITECTURA SPAȚIULUI SACRU

CEHAN Mihaela Agata[1], GHEORGHIȚĂ Carmina Constanța[1]
e-mail: agata.cehan@yahoo.com

Abstract. *The symbol is an emblem or an image associated with a few words or abbreviations on the item it represents. Religious symbols, the oldest symbols of the world, have always helped to retrieve or to identify certain values, beliefs, ideas and confessions of faith, having the primary goal of gathering together those who share the same religious or cultural beliefs. To survive, the symbols must be recognized as such and be properly interpreted within the context that generated them. In modern times the question of the meaning and existence of these symbols arose. To understand this symbolism, the history of the construction types is not enough and references to biblical culture, which renders the origin and development of symbols, are needed. This paper identifies different representations of the grapevine being presented as decorative motifs in sacred art and architecture. The grapevine appears from ancient times represented in the drawings of the catacombs, in the Byzantine mosaics or carved on the walls of the medieval cathedrals.*
Key words: *sacred space, symbol, grapevine*

Rezumat. *Simbolul este o emblema sau o imagine asociată cu câteva cuvinte sau prescurtări referitoare la elementul pe care-l reprezintă. Simbolurile religioase, cele mai vechi simboluri din lume, au ajutat întotdeauna la regăsirea sau identificarea anumitor valori, convingeri, idei sau mărturisiri de credință, având drept prim scop adunarea la un loc a celor ce împărtășeau aceleași convingeri religioase ori culturale. Pentru a supraviețui, simbolurile trebuie să fie recunoscute ca atare și să fie corect interpretate în cadrul contextului care le-a generat. În epoca modernă s-a pus problema sensului și existenței acestor simboluri. Pentru a înțelege aceasta simbolistică, istoria constructivă nu este de ajuns și pentru aceasta, referiri la cultura biblică, ce redă originea și dezvoltarea simbolurilor sunt necesare. Lucrarea de față identifică diferite reprezentări ale viței de vie prezente ca motiv decorativ, în arta și arhitectura sacră. Aceasta apare din cele mai vechi timpuri reprezentată în desenele din catacombe, în mozaicurile bizantine sau sculptată pe fațadele catedralelor medievale.*
Cuvinte cheie: *spațiu sacru, simbol, viță-de-vie*

INTRODUCTION

The Christian sacred spaces have an architectural component and in the same time a symbolical one.

[1]„Gh. Asachi" Technical University of Iasi,"G.M. Cantacuzino" Faculty of Architecture, Romania

The word „symbol", derives from the Latin word „*symbolum*" which means „representation". The symbol is often an emblem or an image associated with a few words or abbreviations on the item it represents.

Religious symbols are among the most popular, most used and oldest symbols in the world. Symbols have always helped to retrieve or identify certain values, beliefs, ideas, or professions of faith. They had the main purpose of gathering together those who share the same religious or cultural beliefs.

In order to survive, the symbols must be recognized as such and be properly interpreted within the context that generated them. In the modern age questions about the meaning and existence of these symbols were raised. When contemplating the decorations or shapes of the facades of cathedrals, one asks himself what those are, which is their symbolic significance that often escapes to the viewer. The history of architecture and constructions is not enough for understanding this symbolism, references to the biblical culture, which renders the origin and development of symbols are needed.

MATERIAL AND METHOD

This paper identifies different representations of the grapevine, being presented as decorative motifs in sacred art and architecture. As research methods were used: theoretical and photographic documentation, observation and case study.

RESULTS AND DISCUSSIONS

The image of the grapevine is often seen as a decorative motif (Fig. 1). In both art and sacred architecture its image appears from ancient times represented in the drawings of the catacombs, in the Byzantine mosaics or carved on the facades of medieval cathedrals. Vines and its fruits, grapes, are often considered the symbol of Christ and his sacrifice, as well as the symbol of the Christian faith. The symbolism of (grape) vine extends to every human soul.

In their spiritual meaning symbols may be fitted in allegorical (referring to a historical reality), anagogical type (referring to eschatological meaning, in times of angels and saints) or tropological (referring to moralizing spiritual sense) (Herea, 2013).

Grapevine as a symbol is very common in the art of the early centuries. It is a clear transposition of the words of Christ:" *Abide in me, and I in you. As the branch cannot bear fruit of itself, except it abide in the vine; no more can ye, except ye abide in me. I am the vine, ye are the branches: He that abideth in me, and I in him, the same bringeth forth much fruit: for without me ye can do nothing. If a man abide not in me, he is cast forth as a branch, and is withered; and men gather them, and cast them into the fire, and they are burned. If ye abide in me, and my words abide in you, ye shall ask what ye will, and it shall be done unto you*." (John 15, 4-5). These words and the image have both ecclesiological and sacramental sense. When the vine and the branches are represented, it is about Christ and His Church: „I am the vine, ye are the branches".

Fig.1 - The interior of Santa Agatha Church, Queige – Beaufortain © Savoie Mont Blanc
(http://www.savoie-montblanc.com)

Fig. 2 - Harvesting Grapevine, Vault Mosaic, Santa Costanza, Rome, 35

But more often, the image of the vine is complemented by that of harvesting the grapevine (Fig. 2) or of birds that feed on grapes (Fig. 3). In this case, the vine reminds Christians especially the central mystery of the Church, the Eucharist. 'Vine gives us the wine as the Word gave His blood, „says Clement of Alexandria (Clement Alexandrinul, 1982). People who harvest and birds that feed on grapevine represent Christians who share the body and blood of Christ.

Fig. 3 - Birds feeding on grapevine

a) **b)**

Fig. 4
a) - Capitel Biserica Saint-Priest
http://www.flickr.com/photos/29223544@N08/8067494053/

b) - A man holding a glass of wine in his right hand, and clusters of grapes in his left hand.
Bas-relief, Church of the Holy Cross, Akdamar Island, Turkey
http://travelswithsheila.com/outside_of_church.htm

The decorative motif of the grapevine still exists today in the sacred art of the Orthodox Church, preserving its symbolic interest (Fig. 4 - a, b).

Grape-vine is considered to be the image of Christ (Fig 5). As lively branches of grapevine that are grafted on the stem, so Christians are having life only as long as they live a genuine life in Christ. This plant is chosen by Christ, with great understanding, grapevines cannot reach maturity and fruition on its own, thus it should be helped out. Therefore, if this plant is not nurtured by human hands and is not helped even by a prop or a tree, on which to climb, it cannot give its fruit.

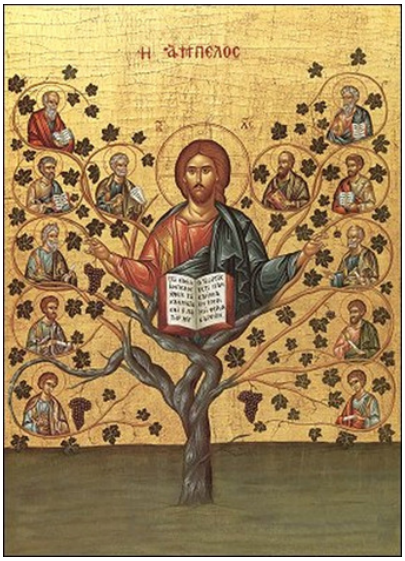


Fig. 5 - Grapevine, tree and Christ
„I am the true vine, and my Father is the husbandman.”. (John 15,1).

a) **b)**

Fig. 6 - a), b) - Jesus and the grapevine
(http://icoaneromanestipesticla.blogspot.ro/2008/11/
-http://www.icoane-taranesti-pe-sticla.ro/?attachment_id=156)

As Grape vine needs to be cut in time, trimmed and bound to yield its fruit, so, one needs to be directed, when wrong, to be able to bear fruit forever.

For its wonderful significance, the grapevine was immediately embraced by church painters, both as a decorative element, its leaves and branches adorning the

edges of icons and church vaults, and as icon itself, it depicting the Lord Jesus Christ (Gherghe and Ciuca, 2010).

The grapevine appears in icons depicting the genealogical tree of Jesse (Fig 5), Abraham or any noticeable founder. Also, grapevines appear on many iconostases belonging to old churches, its branches embracing icons of Jesus, of the Apostles and of other saints (Fig. 6).

However, the most prominent representative of this symbolic plant remains one in which Christ is depicted as strain and the Apostles are represented as branches (Fig. 5). Dionysius of Furna, in his famous book of Erminia of Byzantine Painting states: "*Christ blessing with both hands and having at his chest the Gospel, says: I am the vine, ye are the branches! And out of Him coming out coiled grapevine branches, and apostles sitting among them*." (Dionisie din Furna, 2000).

CONCLUSIONS

The decorative motif of grapevine and grape has three states of appearance in an ornamental register of a sacred space:

- as a branch with vine leaves;
- as a rope vine leaves, grapes and tendrils on it;
- as a cluster repeated on an ornamental register;
- as vine (shoot) of climbing plant.

REFERENCES

1. **Clement Alexandrinul, 1982** – *Scrieri, Partea I,* Colectia "Părinți şi scriitori bisericeşti", Editura Institutului Biblic şi de Misiune al Biserici Ortodoxe Române, Bucureşti
2. **Danalache T., 2014** - *Vita-de-vie, chip al lui Hristos,*on http://www.crestinortodox.ro/liturgica/pictura/vita-vie-chip-lui-hristos-127502.html, accessed on May 2014
3. **Dionisie din Furna, 2000** – *Erminia picturii bizantine,* Editura Sophia, București, p. 129
4. **Gherghe F., Ciuca R., 2010 -** *Vița de vie şi strugurele – simboluri pe ştergarele colecției muzeului național al agriculturii,* în revista „Studii şi comunicări", vol. III/ 2010, pp.. 375-394, disponibil online la data http://studii.crifst.ro/doc/2010/2010_28.pdf, accesed on June 2014
5. **Herea G., 2013** - *Mesajul eshatologic al spațiului liturgic creștin,* Editura Karl A. Romstorfer, Suceava, pp. 19-59

THE ALLERGENICITY OF ORNAMENTAL PLANTS IN THE *ASTERACEAE* FAMILY

ALERGENITATEA SPECIILOR FLORICOLE DIN FAMILIA *ASTERACEAE*

MIRCEA (ARSENE) Cristina Cerasela[1], DRAGHIA Lucia[1]
e-mail: cris_arsene@yahoo.com

Abstract. *This paper aims to synthesise data regarding the allergenic potential of ornamental plants from the Asteraceae family, both from spontaneous and cultivated flora in Romania. The groups of allergenic substances, their presence in plants and the factors influencing them were analysed. Methods used were: bibliographical study, analysis and synthesis of data. The information was provided by national and international scientific literature from various fields (botany, plant physiology, phytochemistry, floriculture, dermatology, public health). Ornamental plants from the Asteraceae family can contain allergenic substances. These compounds present variability depending on the species, soil, climate or part of the plant. The most important allergens belong to the sesquiterpene lactones. The risk to health imposes health and safety regulations, the need to inform the general population and the evaluation of natural remedies and cosmetic products from plants from an allergenic perspective.*
Key words: *Asteraceae, allergenicity, sesquiterpene lactones*

Rezumat. *Lucrarea îşi propune să sintetizeze datele referitoare la potenţialul alergenic al plantelor floricole din familia Asteraceae, atât din flora spontană cât şi cultivată din România. Sunt analizate: clasele de substanţe alergene, prezenţa acestora în plante şi factorii care le influenţează. Metodele utilizate au fost: studiul bibliografic, analiza şi sinteza datelor. Informaţiile au fost furnizate de date din literatura de specialitate naţională şi internaţională, din domenii multiple (botanică, fiziologica plantelor, fitochimie, floricultură, dermatologie, sănătate publică). Plantele floricole din familia Asteraceae pot conţine substanţe alergene. Aceşti compuşi prezintă variabilitate în funcţie de specie, sol, condiţii climatice sau părţi din plantă. Alergenii cei mai importanţi aparţin sesquiterpenlactonelor. Riscul pentru sănătate impune măsuri de protecţia muncii, necesitatea informării populaţiei şi evaluarea remediilor naturiste şi a produselor cosmetice din plante prin prisma alergenităţii.*
Cuvinte cheie: *Asteraceae, alergenitate, lactone sescviterpenice*

INTRODUCTION

Asteraceae family represents more than 10% of floral species (Crosby, 2004). Asteraceae are notorious for producing skin allergies. In Europe, the former "*Compositae*" allergy (now *Asteraceae*) is among the top ten contact sensitivities. Jovanovic and Poljacki (2003) concluded that among cultivated Asteraceae plants, *Chrysanthemum* is considered to be a major sensitizer (60% in Europe). Human

[1]University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania

susceptibility varies with occupation, age and lifestyle. Cross-reactions add to the problem (Crosby, 2004).

The most important allergenic compounds in Asteraceae plants are present in different parts of the plants, especially the leaf, the stem, the flower, in seeds and even in the pollen (Gordon, 1999). Plant allergens are remarkably widespread and exposure is possible both directly by contact and indirectly through contaminated clothing, tools, pets. Skin exposure to plants or topical application of products like herbal medicines or cosmetics may cause sensitization to allergenic compounds resulting in allergenic contact dermatitis – ACD (Crosby, 2004; Gordon, 1999). Exposure via the skin is the most prevalent, but exposure via eyes (ocular), mouth (oral) and lungs (respiratory) are all possible.

Sesquiterpene lactones from Asteraceae plants appear to be a resource of many biologically active compounds. Some of them are already used for insecticidal activity. Others may give new therapeutic and agricultural products of commercial importance (Kumar et al, 2005).

This study presents a brief overview of the most common allergenic ornamental plants belonging to the Asteraceae.

MATERIAL AND METHOD

The study was based on literature data collection, analysis and synthesis. Ornamental plants from Asteraceae families (spontaneous and cultivated flora) were investigated in relation to their allergenic compounds. Basic data were centralized. Very allergenic weeds were not included (*Ambrosia, Artemisia, Taraxacum*).

RESULTS AND DISCUSSIONS

Allergic contact dermatitis (ACD) is more common than most people realize. Typical symptoms include inflammation (redness), pruritus (itching) and eruptions (blysters). Asteraceae allergens and their relatives are found worldwide in flowers, herbs and vegetable gardens, urban and rural weed population and native vegetation. "*Compositae*" (redefined as Asteraceae) plant extracts are present in many cosmetics, massage oils, shampoos, herbal creams, herbal remedies and tonics, aroma lamps (Gordon, 1999). Paulsen (2002) emphasized that the risk of elicitation of dermatitis by using Asteraceae-containing products is by-and-large unknown.

Exposure to plants and plant products is commonly by direct contact, via skin. Allergens are able to become airborne – as particles of dry leaf materials, fine sawdust and smoke from burning plant material. The airborne particles can settle on unprotected skin or, worse, be inhaled. *Chrysanthemum*, *Dahlia*, *Parthenium* and *Achillea* are some plant sources of airborne allergens. Also, pollen is a possible carrier of airborne allergens (typically for *Ambrosia* sp. and *Parthenium* sp.).

Certain members of the Asteraceae are notorious for producing allergies other than ACD. The pollen of the ragweed (*Ambrosia* sp.), golden rod (*Solidago*)

and sneeze weeds (*Helenium*) is responsible for the widespread sneezing, coughing and congestion of hay fever and related respiratory maladies (Crosby, 2004).

The most important allergens in the Asteraceae family are sesquiterpene lactones with a α-methylene group (circled) on the γ-lactone ring (fig. 1); without it, there is no allergenicity (Crosby, 2004).

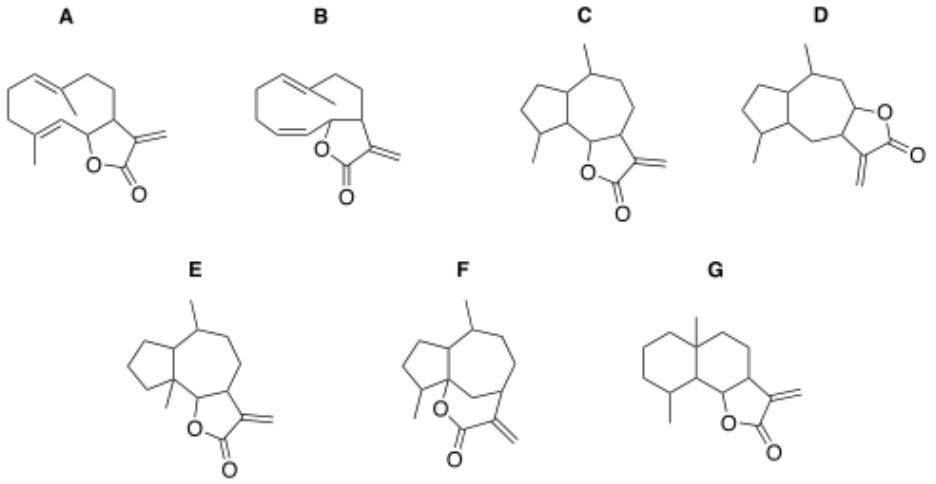


Fig. 1 - Chemical structures of some sesquiterpene lactones:
A: Germacranolides, **B**: Heliangolides, **C**+**D**: Guaianolides, **E**: Pseudoguaianolides, **F**: Hypocretenolides, **G**: Eudesmanolides.
(http://en.wikipedia.org/wiki/Sesquiterpene_lactone)

Sesquiterpene lactones (SQL) constitute a "large and diverse group of biologically active plant chemicals which have been identified in several plant families. However, the greatest number are found in Asteraceae family with over 3,000 reported different structures" (Moufid and Eddouks, 2012). The SQL are usually stored in different plant parts. The diversity and distribution of SQL in different tissues are genetically related (Ramirez et al, 2013). It was proven by different research studies that SQL often display potent bioactivities and are sequestered in special organs such as lactifers, resin ducts and trichomes (Göpfert et al, 2009). They are mostly found in leaves and flowering heads of plants. Chadwick et al (2013) emphasized that SQL are functional compounds and are therefore liable to change in concentration during plant development according to the plant's needs. This means significant changes occur in the plant in a spatial, temporal and species-dependent manner.

Interestingly, there are large differences in chemotypes of sesquiterpene lactone constitution between countries and regions according to the properties of geographical location, soil and climate (Foster et al, 2006; Mohamed et al, 2010). The phytochemical studies have clearly demonstrated that there is "an intraspecific variability of these constituents depending on the geographical area" (Moufid and Edolouks, 2012).

Sesquiterpene lactones are natural compounds which have a broad range of biological activities: antimicrobial effects, antihelmintic properties, analgesic and anti-inflammatory actions, antihyperlipidic activity, effects on plant growth, insecticidal, insect antifeedant properties, mammalian antifeedant properties, acute mammalian toxicity, cytotoxic and antitumor properties. The level of efficacy cannot be generalized. Each compound has individual properties and therapeutic and economical significance.

The main Asteraceae ornamental plants which contain SQL are listed in table 1. Representative chemical compounds were tabulated.

Table 1

Ornamental Asteraceae with allergenic sesquiterpene lactones

Scientific name	Common name	Allergens
Aster sp.	Daisy	dihydrogriesenin ivalin
Arnica montana	Hillside arnica	dihydrohelinalin, helenalin, acetydihydrohelinalin
Arnica longifolia	Deep-spring arnica	carabron
Anthemis cotula	Mayweed	anthocotulidae
Anthemis nobilis	Camomile	nobilin
Achillea millefolium	Yarrow	germacren D, azulena
Chrysanthemum cinerariaefolium	Pyrethrum daisy	pyrethrosin
Calendula officinalis	Marigold	calendin
Centaurea cyanus	Corn flower, basket flower	salograviolide A
Cosmos bipinnatus	Garden cosmos	costunolide, germacrene D, sabinene, farnesene
Coreopsis grandiflora	large-flowered tickseed	perezone, parvifoline
Dahlia variabilis	Dahlia	difensin
Dendranthema grandiflorium (Chrysanthema morifolium)	Chrysanthemum, florists' chrysanthemum	arteglasin A
Echinacea purpurea	Purple coneflower	α-humulene α-copaene, allo-aromadendrene
Gaillardia sp.	Fireweed	pulchellin, neopulchellin, gailla
Helianthus annuus	Sunflower	Niveusin A
Helenium autumnale	Sneezeweed	helenin, helenalin, flexuosin A, B, sulferalin, carolenalone
Inula helenium	Elecampane	helenalin, helenin

Matricaria/ Chamomilla recutita (German chamomile)	Chamomile	desacetylmatricarin, matricarin
Parthenium hysterophorus	Wild feverfew	parthenin, ambrosin, coronopilin, lymenin
Rudbeckia hirta	Black Eyed Susan	pulchelin, rudmolin, dihydrorudmolin
Senecio sp.	Ragworts, groundsel	6-hydroxy-eremophilenolide, eremophilane
Solidago sp.	Goldenrod	germacrene A, D
Tagetes sp.	French marigold	patuletin, patulitrin
Tanacetum vulgare	Tansy	arbusculin A
Tanacetum parthenium	Feverfew	parthenolide

Most exposure and its consequences are highly individual and rely on age, occupation, location and many other variables. Occupation is a prime factor, especially for gardeners, florists, outdoors men (Crosby, 2004).

Table 2

Occupational dermatitis from ornamental Asteraceae plants

Occupation	Plants or plant products involved
Horticulturists, florists, nurserymen, floriculturists, flower handlers	*Chrysanthemum, Dahlia, Helenium*
Gardeners and groundskeepers	*Chysanthemum, Tagetes*
Plant/plant product handlers (cosmetician, masseur, pharmacists)	*Parthenium, Echinacea, Matricaria, Arnica, Calendula*
Farmers	*Helianthus*
Herbal medicine users	*Chamomile, Arnica*

Composites and other lactone-bearing plants likewise tend to stand in for each other's allergenicity. Cross-reaction is a real problem. It can occur between flowers, weeds, herbs and vegetables.

CONCLUSIONS

1. Ornamental plants from the Asteraceae family have a great allergenicity potential in humans. Cross-reactions between ornamentals, weeds and even vegetables are frequent and often underestimated.

2. The most important allergenic compounds from Asteraceae plants are sesquiterpene lactones (sesquiterpenoids). SQL structures and levels are species and local-related.

3. Many sesquiterpene lactones are highly biologically active compounds and could have different economic uses (medical, industrial, agricultural).

4. Florists and horticulturists are highly exposed to Asteraceae ornamentals contact and could develop occupational contact dermatitis.

5. The risk to health imposes health and safety regulations at work, the need to inform population and the evaluation of natural remedies and cosmetic products from plants from an allergenic perspective.

Acknowledgements: *This paper was published under the frame of European Social Fund, Human Resources Development Operational Programme 2007-2013, project no. POSDRU/159/1.5/S/132765.*

REFERENCES

1. **Chadwick M., Trewin H., Gawthrop F., Wagstaff C.**, **2013** - *Sesquiterpeneoids lactones: Benefits to Plants and People*. Int. J. Mol. Sci., 14, pp. 12780-12805
2. **Crosby D.G., 2004** - *The Poisoned Weed – Plants Toxic to Skin*. Oxford University Press, New York, 266 p
3. **Foster J.G., Clapham WM, Belesky D.P., Labriveux M., Hall M.H., Sanderson M.A.**, **2006** – *Influence of cultivation site on sesquiterpene lactone composition of forage chicory (Cichorium intybus L)*. Agric. Food. Chem., 54, pp. 1772-1778
4. **Göpfert J.C., MacNevin G., Ro D.K., Spring O.**, **2009** – *Identification, functional characterization and developmental regulation of sesquiterpene synthase from sunflower capitate glandular trichomes*. BMC Plant Biology, 9:86/ doi: 10:1186/ 1471-2229-9-86
5. **Gordon L.A.**, **1999** – *Compositae dermatitis.* Australia's J. Dermatol. 40(3), pp. 123-128
6. **Jovanovic M., Poljacki M., 2003** – *Compositae dermatitis.* Med. Pregl., 56(1-2), pp.43-49
7. **Kumar A., Singh S.P., Bhakuni R.S.**, **2005** – *Secondary metabolites of Chrysanthemum genus and their biological activities*. Current Science, Vol. 89, No. 9, pp. 1489-1501
8. **Mohamed A.H.M., El-Sayed M.A., Hegazy M.E., Helaly S.E., Esmail A.M., Mohamed N.S.**, **2010** – *Chemical constituents and biological activities of Artemisia herba-alba.* Rec. Nat. Prod., 4, pp. 1-25
9. **Moufid A., Eddouks M.**, **2012** – *Artemisia herba-alba: a popular plant with potential medicinal properties.* Pakistan Journal of Biological Science, 15, pp. 1152-1159
10. **Ramirez A.M., Saillard N., Yang T., Franssen MCR, Bouwmeester H.J., Jongsma M.A.**, **2013** – *Biosynthesis of sesquiterpene lactones in Pyrethrum (Tanacetum cinerarifolium)*. PLOS ONE 8 (5): e65030. doi:10.1371

THE ACCUMULATION OF HEAVY METALS IN RHUBARB (*RHEUM RHABARBARUM* L.)

ACUMULAREA UNOR METALE GRELE ÎN REVENT (*RHEUM RHABARBARUM* L.)

IPĂTIOAIEI D.C.[1], MUNTEANU N.[1], STOLERU V.[1], SELLITTO V.M.[2], COJOCARU A.[1]
e-mail: vstoleru@uaiasi.ro

Abstract. *Rhubarb (Rheum rhabarbarum L.) is a perennial plant, adapted to cold and temperate climate, less spread crop in Romania. Due to the fact, in some areas of our country, rhubarb found favorable conditions for growth and development (Transylvania, Moldavia), the culture of this species require special attention in terms of sustainability. Through the application of differentiated technology, the content of organic acids, minerals, carbohydrates, proteins, vitamins and contaminants differs of the cultivar and the harvesting period. Based on these considerations, the aim of the present study was to highlight the content of any heavy metals (Cu, Cd, Zn and Pb), in two cultivars of rhubarb. In all samples, the Cd content was below the detection limit of the device (<10 ppm). The highest accumulation of heavy metals in the petioles of rhubarb was achieved in cultivar Glaskins perpetual which was planted at distances of 1,1 m x 0,75 m, with a density of 12120 pl/ha, as: Cu – 36 ppm/100 fresh weight; Zn – 166 ppm/100 g fresh weight and Pb – 18 ppm/100 g fresh weight.*
Key words*: rhubarb, heavy metals, planting distances, cultivars*

Rezumat. *Reventul sau rhubarba (Rheum rhabarbarum L.) este o plantă perenă, adaptată climatului rece şi temperat, foarte puţin răspândită în cultură, in România. In acest sens, reventul găseşte condiţii bune pentru creştere şi dezvoltare în câteva zone din ţara noastră (Transilvania, Moldova), insă cultura acestei specii necesită o atenţie deosebită din punct de vedere al sustenabilităţii. Prin aplicarea diferenţiată a tehnologiei de cultivare, conţinutul de acizi organici, minerale, carbohidraţi, proteine, vitamine dar şi contaminanţi, diferă în funcţie de cultivar şi de perioada de recoltare. Bazându-ne pe aceste consideraţii, scopul studiului de faţă a fost acela de a scoate în evidenţă prezenţa unor metale grele (Cu, Cd, Zn şiPb), la două cultivare de revent, în funcţie de tehnologia aplicată. În toate probele, conţinutul de Cd a fost sub limita de detecţie a aparatului (<10 ppm). Cel mai ridicat conţinut de metale grele în peţiol a fost întâlnit la cultivarul Glaskins perpetual care a fost plantat la distanţe de 1,1 m x 0,75 m, cu o densitate de 12120 pl/ha, astfel: Cu – 36 ppm/100 subst. proaspătă; Zn – 166 ppm/100 g subst. proaspătă şi Pb – 18 ppm/100 g subst. proaspătă.*
Cuvinte cheie*: revent, metale grele, distanţe de plantare, cultivare*

[1] University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania
[2] SACOM ,Termoli, Italy

INTRODUCTION

The rhubarb (*Rheum rhabarbarum* L.) is a perennial vegetable species adapted to cold and temperate climate, known and spread least crop in Romania (Ciofu et al., 2003; Indrea et al., 2007).

Through the application of differentiated technology, the content of organic acids, minerals, carbohydrates, proteins and vitamins differs of the cultivar and the harvesting period (Beceanu, 2002; Butnariu, 2012; Patras, 2013).

Botanically speaking, rhubarb is a vegetable because it has both leaf and stem. Fresh rhubarb is generally available across the country from April to October. Hothouse rhubarb appears from December through March and may be available year-round in some markets. Rhubarb originated in the Himalayas, where its root was an important medicine believed to purge the body of ill humans.

Heavy metal pollution is an issue of global importance which, although associated particularly to intensely industrialized areas, has become highly typical for farm land as well (Munteanu et al., 2010; 2012). Considering at least 90 pollutant metals, zinc (Zn), copper (Cu) and lead (Pb) are most commonly detected (Hura, 2007; Patras et al., 2013; Hura et al., 2013; Gavrilescu, 2009; Butnariu et al., 2014).

In the same time, heavy metals are the most significant pollutants of the natural environmet, by their negative effects on the plants, animals and men health (Butnariu et al., 2005; Hlihor et al., 2009;Stoleru, 2013; Stefan, 2008).

The level of heavy metals in the agricultural ecosystem depends by soil pH, type of plant, organic matter, technology applied and vegetation plants period.

The main goal of this paper ist o present the result of a comparative study regarding the level of hevy metals in rhubarb petiole depending by cultivars and planting distances (Stoleru et al., 2012; Samfira et al., 2013).

MATERIAL AND METHOD

The research has been carried out at U.A.S.V.M. Iasi where was organized an experience in a rhubarb crop, established during 2013, on a research plot of 1800 square meter, used two varieties (Glaskin's perpetual cv. (cultivar) and a Local population cv. from Moldova) at densities of 9.090 pl./ha (1.1 m x 1.0 m) and 12.120 (1.1 m x 0.75 m) pl./ha. For setting up of experience was used for both cultivars, rhizomes from the crop of 2012.

The determinations revealed that the density underlying the variety and technology are two factors significantly influence the content of organic acids in the leaf petioles, and the harvest time was on 27 July 2013.

The analyses carried out at U.A.S.V.M.B. Timisoara. Samples have been dried in an oven at 105°C, then weighed 1 g of each sample. They were submitted digestion for bringing them into solution. For determination of mineral elements and heavy metals in the samples studied using atomic absorption spectrometry AAS atomic absorption spectrometer AAS Contra 300, Analytik Jena. The significance of the differences was analyzed using the Analysis of Variance test (Butnariu, 2012).

Analytical data were compared with admitted maximum limits (AML), according to Romanian Regulation (Order 756/1997) and European Regulation (EC Regulation 1881/2006).

RESULTS AND DISCUSSION

In correlation with the interaction between the two factors resulted in four experimental variants (V_1-V_4), which were organized in three replications (R_1-R_3), as presented in Table 1.

The table 1 shows the data on the content of heavy metals varied in each replication.

Table 1.

The content of heavy metals in the petiols from rhubarb (µ/kg fresh weight)

Variants	Heavy metal contents			
	Cu ppm	Cd ppm	Zn ppm	Pb ppm
V_1R_1	42	<10	195	55
V_1R_2	37	<10	235	0
V_1R_3	28	<10	162	0
V_2R_1	27	<10	146	0
V_2R_2	28	<10	161	22
V_2R_3	25	<10	169	28
V_3R_1	38	<10	163	18
V_3R_2	30	<10	188	8
V_3R_3	26	<10	161	23
V_4R_1	26	<10	148	22
V_4R_2	32	<10	181	13
V_4R_3	31	<10	201	0
□	**30.83**	<10	**175.83**	**15.75**

V_1-Glaskin's perpetual cv. X 12.120 pl./ha; V_2-Glaskin's perpetual cv. X 9.090 pl./ha; V_3-Local population cv. X 12.120 pl./ha; V_4- Local population cv. X 9.090 pl./ha., R1, R2, R3 – replications.

Regarding tothe content of Cu in rhubarb, it ranged from25 ppm in V_2-Glaskin's perpetual cv. X 9.090 pl./ha till to 42 ppm in V_1- Glaskin's perpetual cv. X 12.120 pl./ha, compared to the average value that is30.83 ppm. In all variants analyzed, the average contentis less than the maximum limit, which shows tha in the crop of rhubarb, no treatments have been applied based on Cu or Cu $(OH)_2$.

Cadmium (Cd) is another chemical that can be found in soil and plants in several forms: Cd^{2+}, Cd^{3+}, Cd^{6+}, but in high quantities can be toxict o humans.

In all variants studied, the content was lower than the detection limit of the device that is below 10 ppm.

Zinc content limits lower than AML has favorable effects on plant growth and development, but if it exceeds the AML, can be toxic for both plants and especially to humans.

In the crop of rhubarb, the average content was175.83 ppm, but in variants increased from 146 ppm in V_2- Glaskin's perpetual cv. X 9.090 pl./ha; to 235 ppm in V_1- Glaskin's perpetual cv. X 12.120 pl./ha (Fig. 1).

Lead (Pb) as a toxic chemical in the soil,varies within very wide limits, in the study carried out from undetectable if V_1- Glaskin's perpetual cv. X 12.120 pl./ha; to 55 ppm in the same variant in R1.

In the case of Pb, we conclude that Pb accumulationin petiole is not conditioned by variety or planting distances.

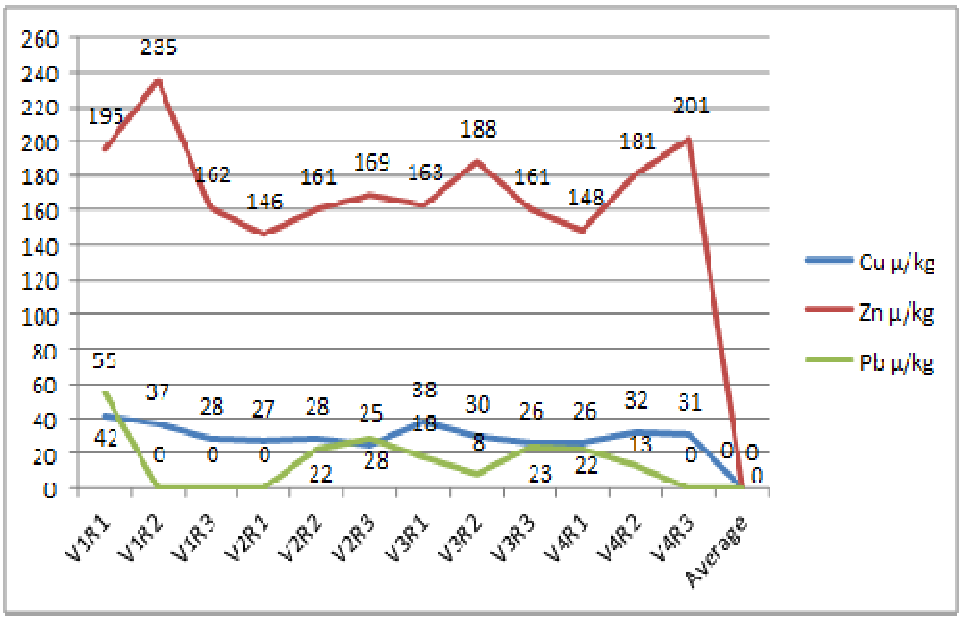


Fig. 1 - Graphical representation of the content of heavy metals in the petiols from rhubarb (µ/kg fresh weight)

Average content in toxic element shad higher values in variants planted at densitie shigher than densities of 9090 pl./ha.

From the data presente in Table 2 it can be seent hat all values of toxic elements are below the maximum limits of EC regulation 1881/2006

Table 2.

The average content of heavy metals from rhubarb (µ/kg fresh weight)

Variants	Heavy metal contents			
	Cu ppm	**Cd ppm**	**Zn ppm**	**Pb ppm**
V_1	35.66	<10	197.34	18.34
V_2	26.67	<10	158.67	16.66
V_3	31.33	<10	170.66	16.33
V_4	29.67	<10	176.67	11.67
□	**30.83**	<10	**175.83**	**15.75**

V_1-Glaskin's perpetual cv. X 12.120 pl./ha; V_2-Glaskin's perpetual cv. X 9.090 pl./ha; V_3-Local population cv. X 12.120 pl./ha; V_4- Local population cv. X 9.090 pl./ha.

The lowest content of heavy metal, was found in the Glaskin's perpetual cultivar has been planted at a density of 9.090 pl./ha (Fig. 2.).

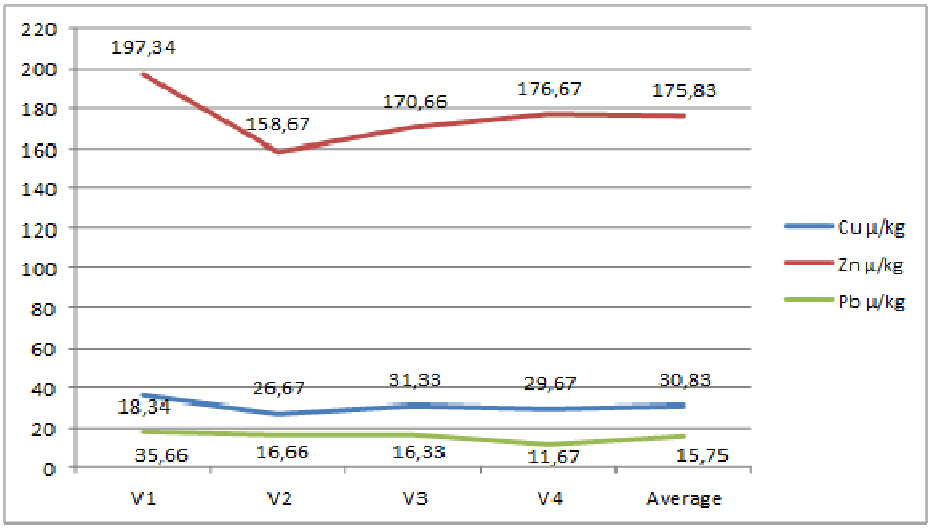


Fig. 2 - Graphical representation of the average content of heavy metals from rhubarb petiols (µ/kg fresh weight)

CONCLUSIONS

Inside most soil samples and vegetable products comming from the lands chosen for measurements, the main chemical contaminants were within the maximum accepted limits according to European and national regulation, 293/640/2001-1/2002.

The high content of metals analyzed has been recorded in Zn, average content was 175.83 µ / kg fresh weight.

In the case of Cd, the metal content was below the detection limit of the device fits in AML.

REFERENCES

1. **Beceanu Dumitru**, **2002** - *Tehnologia produselor horticole*. Vol. I., Editura Pim, Iasi. pp. 53-60.
2. **Butnariu Monica**, **2012** - *Biochimie vegetală*. Editura Agroprint, Timișoara.
3. **Butnariu Monica, Goian M., Ianculov I., Gergen I., Negrea P**., **2005** - *Studies about CO^{2+} ion influence on soy plants development and acumulation of other chemical elements (Iron, magnesium, calcium, potassium and phosphorus),* Revista de chimie, vol. 56(8), pp. 837-841.
4. **Butnariu Monica, Rodino S., Petrache P., Negoescu C., Butu M**., **2014** - *Determination and quantification of maize zeaxanth in stability*. Digest journal of nanomaterials and biostructures, vol. 9 (2), pp. 745-755.
5. **Ciofu Ruxandra, Stan N., Popescu V., Chilom Pelaghia, Apahidean S., Horgos A., Berar V., Lauer K. F., Atanasiu N., 2004** - *Tratat de legumicultura*. Ed. Ceres Bucuresti.
6. **Gavrilescu M**., **2009** - *Behaviour of persistent pollutants and risks associated with their presence in the environment - integrated studies*, Environmental Engineering and Management Journal, vol. 8, pp. 1517-1531.
7. **Hlihor R.M., Apostol L.C., Smaranda C., Pavel L.V., Căliman F.A., Robu B.M., Gavrilescu M., 2009** - *Bioavailability processes for contaminants in soils and their use in risk assessment*, Environmental Engineering and Management Journal, vol. 8, pp. 1199-1206.
8. **Hura Carmen**, **2007** - *Chemical Contamination ofFoodinRomaniain 2006* (in Romanian), vol. 6, CERMI Publishing House, Iaşi, Romania.
9. **Hura Carmen, Munteanu N., Stoleru V**., **2013** - *Heavy Metals Levels in Soil and Vegetables in Different Growing Systems.* E3S Web of Conferences. DOI: 10.1051/C _published by EDP Sciences, 2013
10. **Indrea D., Apahidean S., Apahidean Maria, Maniutiu D., Sima Rodica**, **2007** - *Cultura legumelor*. Ed. Ceres, Bucuresti.
11. **Munteanu N., Bireescu L., Bulgariu D., Hura C., Stoian L., Stoleru V**., **2010** - *The Monograph of Organic Vegetable Production in Northeastern Romania: Opportunities and Risks* (in Romanian), Publisher Arhip Art, Iasi, Romania.
12. **Munteanu N., Stoleru V., Hura Carmen**, **2012** – *Assessment of heavy metals control from soi land vegetable plants in different growing systems*. Journal of Agricultural Science and Technology A 2, pp. 716-722.
13. **Patras Antoanela**, **2013** - *Chimie organică*. Editura Pim, Iași.
14. **Patras Antoanela, Luchian Camelia Elena, Niculaua Marius, Stoleru Vasile**, **2013** - *Effects of some Abiotic Factors on Brassica Oleracea Var. Capitata Sprouts*. Bulletin UASVM Horticulture, 70 (1), pp. 172-179.
15. **Samfira I., Butnariu M., Rodino S., Butu M**., **2013** - *Structural investigation of mistletoe plants from various hosts exhibiting diverse lignin phenotypes*. Digest journal of nanomaterials and biostructures. vol. 8 (4), pp. 1679-1686.
16. **Stoleru Vasile**, **2013** - *Managementul sistemelor legumicole ecologice*. Editura "Ion Ionescu de la Brad", Iasi.
17. **Stoleru Vasile, Munteanu Neculai, Stoleru Carmen Maria, Rotaru Liliana**, **2012** - *Cultivar Selection and Pest Control Techniques on Organic White Cabbage Yield*. Not. Bot. Horti. Agrobot., 40(2), pp. 190-196.
18. **Ştefan Marius**, **2008** - *Biologia microorganismelor rizosferice - aplicații biotehnologice*, Ed. Tehnopress Iaşi, p. 369.

SEEDS GERMINATION AND ROOTS LENGTH IN CADMIUM POLLUTED SOILS

GERMINAREA SEMINȚELOR ŞI ALUNGIREA RĂDĂCINILOR ÎN CONDIȚIILE SOLURILOR POLUATE CU CADMIU

POHONȚU C.M.[1]
e-mail: profuldebio@yahoo.com

Abstract*: The most frequently soils pollution consist the heavy metal ions contamination, having extremely serious effects both on ecosystems and human health. In this context, a method for decontamination of soils contained heavy metal ions is phytoremediation, which consist in the use of plant species in order to extraction, stabilization and/or neutralization of soil pollutants. For biomonitoring and establishing the parameters of this method, first, the phytotoxicity tests are needed. This causes the maximum dose of heavy metal ions which do not cause a negative effect on plants, even from germination process. Phytotoxicity tests measure the decrease or absence of germination and root length, in just a few days of exposure seeds to contaminated soil, in comparison with unpolluted soil. This study aimed to determine the Cd^{2+} toxicity in seeds germination and root length of bioaccumulating plants such as white mustard (Sinapis alba), rape (Brassica juncea) and triticale (Triticosecale rimpau), at different Cd^{2+} concentrations.*
Key words*: polluted soil, cadmium, germination seed, phytotoxicity, root length*

Rezumat*: Cea mai frecventă formă de poluare a solurilor o constituie contaminarea cu ioni de metale grele, având efecte deosebit de grave atât asupra ecosistemelor cât şi a sănătății umane. În acest context, o metodă de depoluare a solurilor contaminate cu ioni ai metalelor grele este fitoremedierea, care constă in folosirea unor anumite specii de plante cu scopul extracției, stabilizării şi/sau neutralizării substanțelor poluante aflate în soluri. Pentru biomonitorizarea şi stabilirea parametrilor acestei metode, mai intai, sunt necesare testele de fitotoxicitate. Acestea determină doza maximă de ioni de metale grele care nu cauzează un efect negativ asupra plantelor, chiar de la nivelul procesului de germinare. Testele de fitotoxicitate măsoară scăderea sau absența germinării şi creşterea rădăcinilor, in doar câteva zile de expunere a semințelor la soluri contaminate, în comparație cu un sol nepoluat. Acest studiu a avut ca scop determinarea toxicității Cd^{2+} asupra germinării semințelor şi alungirii rădăcinilor unor plante bioacumulatoare cum ar fi muştarul alb (Sinapis alba), rapița (Brassica juncea) şi triticale (Triticosecale rimpau), la concentratii diferite de Cd^{2+}.*
Cuvinte cheie*: sol poluat, cadmiu, germinatia semintelor, fitotoxicitate, alungirea rădăcinilor*

INTRODUCTION

Soils polluted by heavy metals ion is a worldwide problem. That soils is becoming one of the most significant environmental hazard with negative impact

[1] "Stefan cel Mare" University of Suceava, Romania

on biodiversity, surface and groundwater, agricultural sites and finally human health. This is the result of increasing population and industrial technology, finally increased waste quantity on earth`s terrestrial, atmospheric and aquatic systems. While some organic compounds in waste can be directly degraded (Salt et al., 1995), the heavy metal ions are nonbiodegradable. They accumulate in the environment and are mutagenic, carcinogenic and teratogenic (Hazrat et al. 2013). The inorganic pollutants, including heavy metal ions, can be degraded in the soil, for the first, these must either be stabilized (Pohontu, 2013). One of the most serious contamination of the ground is produced by heavy metal, which have a particularly badly both on terrestrial ecosystem vegetation physiology, and on humans and animals coming into directly or indirectly contact with contaminated sites. As a result of this situation, science and technology in environmental world has developed a number of methods and techniques preventing and / or combating pollution with heavy metal ions.

Cadmium is one of the common heavy metals affecting the soil quality. The sources of cadmium in the soil are especially from industrial operation, including steel, alloy, electroplating, motor vehicles, pigments, aircraft paint, chemicals and textiles, from combustion and from phosphate fertilizers (Mani and Freitas, 2008).

The biogeochemical transformations in soil including more processes such as: translocation, transformation, chelation, immobilisation, solubilisation, precipitation, volatilization and complexation of heavy metals (Alkorta and Garbisu, 2001). Several methods and technologies have been used and improved for rehabilitation, decontamination and removed pollutants from soils. These methods consist in physical removal of soil through excavation, uncovering, or extraction by chemical means. These technologies are unfortunately more expensive and have a negative impact on soil physical, chemical, biological properties. The classical soil treatments are most expensive. Better option and an alternative cost effective for decontaminated soils polluted by heavy metal ions is phytoremediation through hyperaccumulating plants (Hazrat et al. 2013). To control this process easier, are necessary phytotoxicity tests, one of the standard methods to determine the maximum dose of heavy metal ions, that not causing a negative effects on plants, even in the germination and seedling, consists in using phytotoxicity tests. These tests are the easiest method of biomonitoring of environmental and may be important in environmental engineering, to establish methods of phytoremediation, in agriculture for the decontamination of polluted soils. The phytotoxicity tests measure the decrease or absence of germination and root length. (Bialowiec and Randerson, 2010) Phytoremediation using the plants to remediation polluted soils is relatively inexpensive, efficient and potentially clean techniques. The major processes of phytoremediation including: phytodegradation, phytostabilisation and bioaccumulation in the rizosphere through root-microbes interaction. (Wild et al. 2005; Xiaoe et al. 2005)

White mustard (*Sinapsis alba*), rape (*Brassica juncea*) and triticale (*Triticosecale rimpau*) is a species of vascular plant with considerable capacity to

grow in a heavily polluted soils as well its capacity for metal ions hypeaccumulation. (Blaylock and Huang, 2000)

The present work aims to investigate the effects of Cd^{2+} in different soil concentrations for germination process of white mustard (*Sinapis alba*), rape (*Brassica juncea*) and triticale (*Triticosecale rimpau*) seeds.

MATERIAL AND METHOD

The experiments was performed at laboratory scale, after a few days of exposure of the seeds to the contamined soil, compared with non-polluted soil representing the control of the experiments.

Phytotoxicity kit consists of plastic plates, transparent, with dimensions of 21,00 cm x 15,50 cm x 0,80 cm and two compartments according to figure number 1. In the bottom compartment of the plate sits soil making sure the entire surface is covered, using a spatula for smoothing.

Fig. 1 - Kit for phytotoxicity determination

The soil used in these experiments was a ground reference OECD (*Organisation for Economic Co-operation and Development*) which is used frequently for standard phytotoxicity tests, provide by MicroBioTests Inc, Belgium. The reference soil composition is presented in table number 1. The initial analysis of OECD reference soil didn`t contain heavy metal ions, which facilitated the accuracy results.

Table 1

Soil composition

Soil compounds	Quantity (%)
Dry quartz sand	85
Kaolin	10
Peat moss *Sphagnum*	5
Calcium carbonate ($CaCO_3$)	To obtain a pH of 6,5 - 7

A second type of substrate used in germination tests was sand. Sand initial analyzes do not contain traces of heavy metal ions, leading to higher accuracy of results.

The palnt seeds used in this study was white mustard (*Sinapis alba*), rape (*Brassica juncea*) and triticale (*Triticosecale rimpau*). Selected seeds have been used, with high germination rate capacity, provided from the Bank of Vegetable Genetic Resource Suceava.

Soil was polluted in a controlled mode by adding a cadmium Cd^{2+} solution. For preparing the pollutant solution was used $3CdSO_4 \cdot 8H_2O$, finally obtained *50 mg/kg* concentration. The soil used in the experiments was placed in the bottom of the plate and saturated with cadmium solutions of various dilutions: 1/1(100%); 1/2 (50%); 1/4 (25%); 1/8 (12.5%) and 1/16 (6.25%). After that, soil was covered with a filter paper.

Distilled water was used for dilution an control the process, representing 0% cadmium concentration plate. On the filter paper was placed 10 seeds of plants at a distance of 1 cm from each other. The seeds of the plants used were white mustard (*Sinapis alba*), rape (*Brassica juncea*) and triticale (*Triticosecale rimpau*). After closing the transparent cover plates, it was placed vertically in a thermostat and incubated at a temperature of 25 ± 1 °C for 3 days. At the end of incubation, the plates were photographed, for measurement of seedlings with ImageTool 3.0 soft.

Each group of experiment was carried out in three times for results reproductibility, and calculated root elongation.

RESULTS AND DISCUSSIONS

After three days of incubation of analyzed plant seeds it could be seen that the pollutants affected seed germination and root elongation. Germination rate of seeds for each plant, at different Cd^{2+} concentrations, in OECD soil and sandy substrate can be observed in figures 2 – 7. As it can be seen, seed germination rate decreases with increasing concentration of the pollutant and depend on the type of soil used.

In the case of rape and white mustard seeds, the germination rate was Higher reaching 70.66% and 65.00% for sandy substrate, than in the case of using OECD soil which was 62.83% and 55.50%, according to figures 2 - 5.

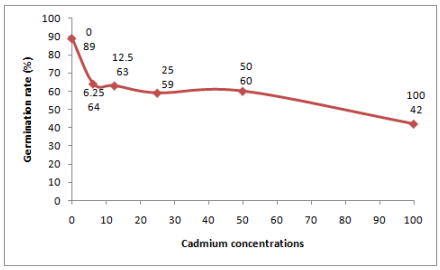


Fig. 2 - Variation of germination rate for white mustard seeds on OECD soil with different Cd^{2+} concentrations

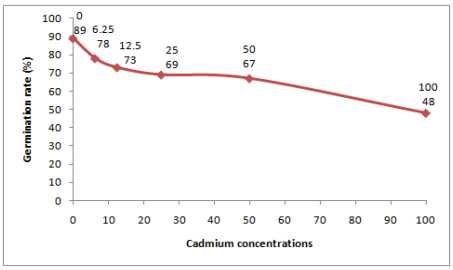


Fig. 3 - Variation of germination rate for white mustard seeds on sand with different Cd^{2+} concentrations

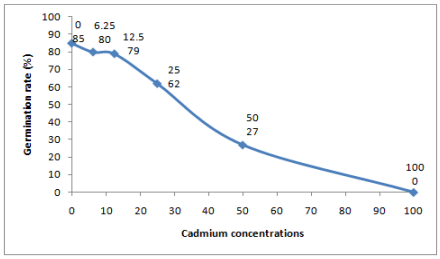


Fig. 4 - Variation of germination rate for rape seeds on OECD soil with different Cd^{2+} concentrations

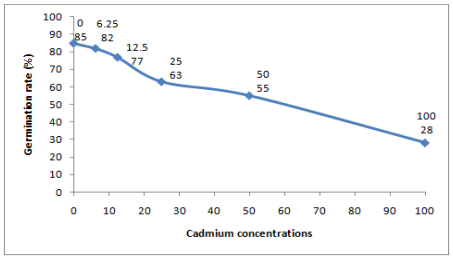


Fig. 5 - Variation of germination rate for rape seeds on sand with different Cd^{2+} concentrations

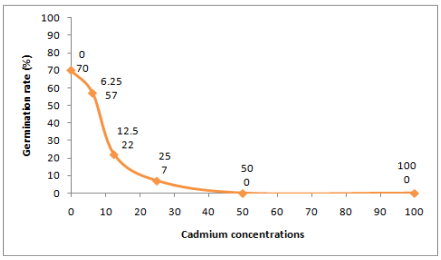


Fig. 6 - Variation of germination rate for triticale seeds on OECD soil with different Cd^{2+} concentrations

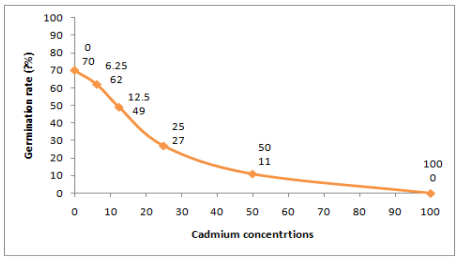


Fig. 7 - Variation of germination rate for triticale seeds on sand with different Cd^{2+} concentrations

Analyzing the figures 6 and 7, we may notice that triticale seeds germination rate was lower when we used OECD soil, it was 26,00% while when we used sand, germination rate reaching we used 36,50 %. Triticale seeds have not germinated in a sand at most concentration of cadmium [1/1 (100%)], while on the OECD soil seeds have not germinated starting at a concentration of cadmium [1/2 (50%)].

Inhibition of plant roots by pollutant, was calculated with the equation number 1 (Bialowiec et al. 2010):

$$I = \frac{C - T}{C} \cdot 100(\%) \quad (1), \text{ where:}$$

C - mean of root length of the control, blank (0%);

T - average root length for each pollutant concentration applied.

According to equation 1 was calculated inhibition rate for each situation (type of plant – type of soil), the values are presented in table 2.

Table 3

Inhibition rate

Plant type	Substrate	Inhibition (%)
Sinapis alba	OECD soil	41,64
	sand	28,23
Brassica juncea	OECD soil	35,28
	sand	24,71
Triticosecale rimpau	OECD soil	75,42
	sand	57,42

Inhibition values can determine the lowest effective concentration of cadmium ions in the soil which not causing a toxic effects in a seeds germination process. Thus, can find the optimum solution would be suitable plant for phytoremediation process.

CONCLUSIONS

1. The germination rate of plants seeds studied and their root elongation depend on soil type and decreased with increasing concentration of the pollutant. All seeds had a higher rate of germination on sandy soil compared with the

substrate consists of reference soil OECD, because the capacity of sand drainage, actually pollutant has no contact with seeds surface.

2. When using as germinative substrate OECD reference soil, germination rate was lower than sand, in fact due to both retention in the upper layers of the cadmium pollutant solution and because its complexation with organic substances in the soil.

3. White mustard seeds had a germination rate for OECD soil polluted by cadmium 55,50%, while the use of sand, germination rate is slightly increased, reaching 65,00%, but germinated at every concentration of cadmium in the soil.

4. Rape seeds do not germinate in the OECD soil have the most concentration of cadmium [1/1(100%)]. The average length of the roots began to decline significantly from the pollutant concentrations of 25% corresponding to 12.5 mg / kg Cd, both the OECD soil and the sand.

5. Triticale seeds were most affected by the cadmium toxicity, the germination rate of the sand was of 36,50% and on OECD soil 26,00%. The seeds have not germinated in a sand at most concentration of cadmium [1/1(100%)], while on the OECD soil have not germinated starting at a concentration of cadmium [1/2 (50%)].

6. Considering the inhibition rate, the least affected by the toxicity of cadmium in soil was rape and white mustard seeds.

REFERENCES

1. **Alkorta I., Garbisu C., 2001 -** *Phytoremediation of organic contaminants in soils*, Bioresource Technology, Vol. 79, pp. 273 – 276;
2. **Bialowiec A., Randerson P.F., 2010 -** *Phytotoxicity of landfill leachate on willow – Salix amygdalina L.* , Waste Management, Vol. 30, pp. 1587–1593.
3. **Blaylock, M.J., Huang, J.W, 2000 -** *Phytoextraction of Metals. In: Raskin, I., Ensley, B.D. (Eds.), Phytoremediation of Toxic Metals—Using Plants to Clean-up the Environment*, John Wiley & Sons, Inc., New York;
4. **Hazrat Ali, Ezzat Khan, Muhammad Anwar Sajad, 2013** - *Phytoremediation of heavy metals – Concepts and application*, Chemosphere, Vol. 91, No. 7, 2013, pp. 869-881;
5. **Mani Rajkumar, Helena Freitas, 2008 -** *Effects of inoculation of plant-growth promoting bacteria on Ni uptake by Indian mustard*, Bioresource Technology, Vol. 99, pp. 3491–3498;
6. **Pilon-Smits E., 2005,** *-Phytoremediation*, Annual Review of Plant Biology, vol. 56, pp. 15-39;
7. **Pohontu C.M., 2013** - *Rehabilitation of degraded soils containing Lead (Pb 2+) ions based on phytoremediation with Fagopyrum esculentum Moench in presence of Ethylene-diamine-tetracetic acid (EDTA),* Advances in Environment, Ecosystems and Sustainable Tourism, pp. 84 – 88;
8. **Salt D.E., Blaylock M., Kumar N.P.B.A., Dushenkov V,. Ensley B.D., Chet I., et al**., **1995** - *Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants*, BioTechnol, Vol. 13, No. 4, pp. 68–74;
9. **Wild E., Dent J., Thomas G.O., Jones K.C., 2005** - *Direct observation of organic contaminant uptake, storage, and metabolism within plant roots*, Environmental Science and Technology, Vol. 39, pp. 3695 -3702;
10. **Xiaoe Yang, Ying Feng, Zhenli He, Peter J., Stoffella**, **2005 -** *Molecular mechanisms of heavy metal hyperaccumulation and phytoremediation*, Journal of trace elements in medicine and biology, Vol. 18, pp. 339 – 353.

STUDIES UPON THE QUALITY STATUS OF A TERRAIN OCCUPIED BY A SUGAR MANUFACTURING WASTE DEPOSIT

STUDII PRIVIND STAREA DE CALITATE A UNUI TEREN OCUPAT DE UN DEPOZIT DE DEŞEURI PROVENITE DE LA FABRICAREA ZAHĂRULUI

***COJOCARU Paula*[1], *STĂTESCU F.*[1]**
e-mail: paula.cojocaru@yahoo.com

Abstract *The paper presents the established studies in order to determine the quality and reliability of the soils for agriculture purpose from the "decantation lake" belonging to a Romanian sugar industry. Towards these it were made 3 soil profiles and it were assayed 3 soil samples from each profile in order to determine the physic-chemical characteristics. Each profile was investigated "in situ" through specific pedological methods being identified two soil classes and two types of soil. It were also determined the bonity notes and the soil fertility showed by the trophicity index. The obtained results indicated the analyzed soils have a low trophicity index that means that they are into oligotrophic and extremely oligotrophic classes. The area is into the 5th class of pretability that comprise terrains with very severe limitations, unpretable for agriculture purpose in terrain undevelopment conditions and for valorification there are necessary the application of agropedireliability works.*
Keywords: *soil, sugar, pretability, fertility, troficity index.*

Rezumat. *În lucrare se prezintă studiile realizate în vederea stabilirii stării de calitate şi de pretabilitate, pentru arabil a solurilor din zona "iazului de decantare" aferent unei fabrici de zahăr din România. În acest scop, au fost executate 3 profile de sol iar din fiecare profil au fost recoltate câte 3 probe de sol în vederea determinării principalelor caracteristici fizico-chimice. Fiecare profil a fost cercetat direct "in situ" prin metode pedologice specifice, fiind identificate 2 clase de sol şi 2 tipuri de sol. Au fost determinate de asemenea, notele de bonitare precum şi fertilitatea solului redată prin indicele de troficitate. Rezultatele obţinute au arătat faptul că solurile analizate au un indice de troficitate scăzut încadrându-se în clasele oligotrofic şi extrem oligotrofic. Zona se încadrează în clasa a V-a de pretabilitate, cuprinzând terenuri cu limitări foarte severe, nepretabile pentru arabil în condiţii de neamenajare iar pentru valorificare se impun aplicarea de lucrări agropedoameliorative.*
Cuvinte cheie: *sol, zahăr, pretabilitate, fertilitate, indice troficitate.*

INTRODUCTION

The Romania adhesion to U.E. had imposed the harmonization of its politics with European ones in all fields of activities. Towards this the Romanian

[1]"Gheorghe Asachi" Technical University of Iasi, Romania

standards, the projects, the studies and the investigations from environmental protection field, of the natural resources, of infrastructure, etc. constitutes country integration measures into the European system of values.

One of the most important objectives of the European objectives is constituted by the conservation and the turning into advantage of the natural resources of each country. The rehabilitation of the degradation terrains and the turning into advantage to high cotes of the existent ones there are line up as a strategic importance objectives for our country.

The alimentary industry which use, most of the time, raw materials without a pollution character and there are, also presently, a recuperation – turning into advantage system of the raw material (most of it of organic nature) constitutes the technological sludge: the deposit in order to dehydrate and compost in so cold "fields" or "lakes" of sludge. After dehydration and compost (decomposed), the deposit sludge can be used as a agricultural fertilizer.

In our country and also in the world it exists a large number of terrain and laboratory investigations which aimed the solutions finding for reliability for agricultural purpose of the ash and cinder deposits of the electro – thermal central Rovinari (Fodor and Lazar, 2006; Racoceanu et. al., 2012), of deposits of mining tailings (Dunca and Ciolea, 2013).

The paper comes with another problem: the deposit which contain these kind of sludge, which were not turned into advantage, through time, can they be rehabilitate today for agricultural purpose?

For the application of one of the methods of rehabilitation we must accuracy know the physic – chemical characteristics from pedologic point of view, and to make a soil bonity exposed to the rehabilitation. This main objective is carried on in the paper for an auxiliary sludge deposit (lake) of a sugar beet factory.

MATERIAL AND METHOD

The studied area is placed to western of Cordun town, Neamt district, being characterized by continental climate, with temperate nuances and belonging, from geomorphologic point of view, to the Moldavian chute. From hydrographic and geomorphologic point of view the area is placed on the second terrace of Moldova river, left tributary of Siret river. The studied area can't be flooded even to overflows at overturn probability of 1%. From geologic point of view the area belongs to the central sector of Moldavian platform. From hydrogeologic point of view the phreatic water is placed to variable depths depending on the distance to the river: nearby the river the phreatic water depth is 5 m and in Cordun locality the phreatic water depth is 3 m.

From agricultural point of view the area is used as a paddock.

In this area there exist a 24.45 ha (fig. 1) area which belongs to S.C. AGRANA ROMANIA S.A. which was used for 50 years long for depositing the soil sludge, a result of the washing the sugar beet and the carbonate sludge, a result of the sugar

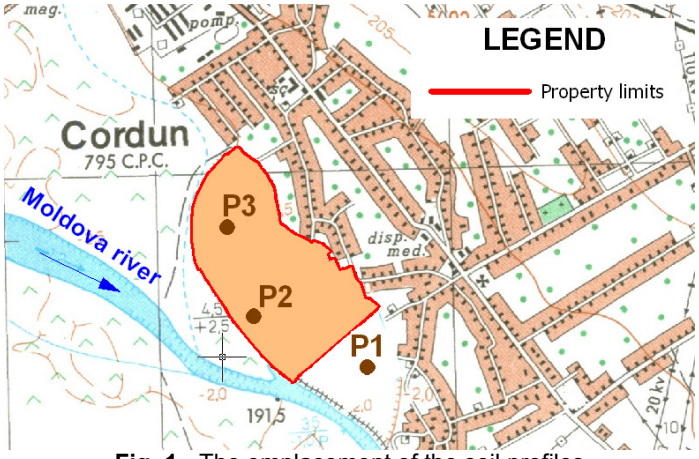


Fig. 1 - The emplacement of the soil profiles

For this terrain (actually a depositing lake), it was taking the problem of determining the classes and the types of soils, for which, finally, within the physic – chemical characteristics analysis determined in laboratory to be indicated the fertility of the soils, synthetic expressed by the trophicity index.

Towards this, as it can be observed in fig. 1, it were made 3 soil profiles (P1, P2 and P3), and from each profile were assay 3 soil samples as it follows:

- profile 1 (P1) placed in the control area – from depths of 10, 25 and 45 cm;
- profile 2 (P2) placed on the south – west area of the deposit - from depths of 20, 55 and 80 cm;
- profile 3 (P3) placed on the north area of the deposit – from depths of 15, 40 and 80 cm.

Each profile was investigated "in situ" through specific pedologic methods and the assayed nine samples of soil from the terrain were analyzed in laboratory and it were determined the following parameters: pH, carbonates, humus, total nitrogen, phosphorus, mobile potassium, aggregate grading and apparent density.

It was also determined the trophicity index (T_p), being used the following equation (Chirita, 1974):

$$T_p = \Sigma t_p = \Sigma (H \cdot d \cdot V \cdot 0.1 \cdot R_V)$$

In which: H is the humus content (%);
d – the horizont thickness (dm);
V – the base saturation level (%);
R_V – the ratio between the fine soil volume (without skeleton and roots etc.) and the total soil volume.

RESULTS AND DISCUSSIONS

The made terrain studies have marked out the following pedologic characters aspects:

- *In profile 1 area (P1) - control*

Type of soil: Aluviosol calcurus - prundic.
Thichness: superficial moderate (symbol d_2/MS, cod 035).
Identified hirizonts: *Aok (0-10 cm), ACRk (10 – 35 cm), CRk (sub 35 m).*

It is characterized by the following equation:

$$ASka - pr\frac{K_1 - d_1 - \frac{s}{sq_2} - \frac{Tfmg}{NB} - A}{C - OC - P_{01} - 0 - X_3 - Q_6}$$

- *In profile 2 area (P2)*

Type of soil: Technosoil mixtic – clinogleic - calcic

Thickness: profundity moderate (symbol d_4/MP, cod 088).

Identified horizonts: *Sludge (0-30 cm), A1 (30–60 cm), AC (under 60 cm).*

It is characterized by the following equation:

$$TTmi - cl - ca\frac{G_6 - W_6 - K_1 - \frac{t}{a} - \frac{Tfa}{NB} - A - C_{63}}{C - OC - P_{01} - 0 - Q_6}$$

- *In profile 3 area (P3)*

Type of soil: *Technosol mixtic-clinogleic-calcic.*

Thickness: *Profundity moderate* (symbol d_4/MP, cod 088).

Identified horizonts : *Ao (0 – 18 cm), $CaCO_3$ (18 – 24 cm),ACk (24 – 45 cm),$CaCO_3$, (45 – 63 cm),Ck (63 – 70 cm),$CaCO_3$ (70 – 90)* şi *Cca (under 90 cm).*

It is characterized by the following equation:

$$TTmi - cl - ca\frac{G_6 - W_3 - K_1 - \frac{s}{u} - \frac{Tfm}{NB} - A - C_{63}}{C - OC - P_{01} - 0 - X_3 - Q_6}$$

Laboratory tests performed on the 9 (nine) of soil samples revealed the physical and chemical characteristics shown in Table 1.

To calculate the bonity notes from the many environmental conditions that characterize each field unit (UT or TEO) defined in the pedological studies were chosen only those considered most important, easier and more accurately measurable that is (in brackets show the indicator according to Teaci, 1980: 1-the average annual temperature, corrected values, (3C); 2-the average annual precipitation, corrected values, (4C);-the gleyzation, (14);-the pseudogleyzation, (15);-the salinization or alkalization, (16) or (17);-the texture in Ap or in the top 0-20 cm, (23A);-the pollution, (29);-the gradient (33);-the landslides, (38);-the groundwater depth, (39);-the inundability, (40);-the total porosity in the restrictive horizon, (44);-the total $CaCO_3$ content in the 0 + 50 cm, (61);-the reaction in Ap or in the top 20 cm, (63);-the degree of base saturation in Ap or in the top 0-20 cm, (69);-the edaphic volume, (133);-the humus reserve in the 0-50 cm layer, (144);-the excess surface moisture, (181).

The detailed analysis of all these environmental conditions led to the following results regarding the trophicity:

- *the territorial unit from the control area (P1):* the potential trophicity index has the value 19.03 and the soil comply into *oligotrophic* class (OL simbol, 023 cod), because of the thinness of the soil profile and the low quantity of humus;

Table 1

The physico-chemical properties of the soil profile

Profile	Horizon	Depth (cm)	pH	$CaCO_3$ (%)	Humus (%)	Nt (%)	P (ppm)	K (ppm)	Sampling humidity (%)	Granulometry					DA
										Sand		Dust (%)	Clay		
										Coarse sand (%)	Sand (%)		Clay (%)	Natural clay (%)	
1	Ao	1 - 10	7.79	3.32	10.00	0.618	524.6	300	24.50	10.8	48.10	24.00	17.1	30.6	1.15
	ACR	15 - 25	7.32	2.61	4.36				22.45	25.7	46.50	12.50	15.3	22.1	
	CR	35 - 45	7.83	7.35	3.76				19.75	14.7	48.50	17.30	19.5	29.9	
2	Sludge	10 - 20	7.95	2.69	14.36	0.680	303.5	2750	78.30	0.1	34.00	28.60	37.3	55.9	
	Al	45 - 55	7.97	16.84	6.48				36.20	0.1	23.10	25.50	51.3	68.9	
	AC	70 - 80	8.35	39.42	4.24				40.00	0.9	48.90	19.40	30.8	43.2	
3	Ao	5 - 15	8.14	40.32	4.42	0.227	381.9	700	31.75	0.1	63.8	20.10	16.0	25.8	1.13
	AC	30 – 40	8.42	34.94	2.12				33.00	0.8	80.0	10.10	9.1	12.5	1.12
	$CaCO_3$	70 - 80	8.42	68.10	1.70				47.25	shows no silicate solid phase					0.79

- *the territorial unit from the profile 2 area (P2):* the potential trophicity index has the value 12.15, and the soil comply into *extremely oligotrophic* class (EO simbol, 0008 cod), because of the thinness of the soil profile and the very low quantity of humus;

- *the territorial unit from the profile 3 area (P3):* the potential trophicity index has the value 5.64, and the soil comply into *extremely oligotrophic* class (EO simbol, 0008 cod), because of the thinness of the soil profile and the very low quantity of humus.

CONCLUSIONS

As a result of the studies presented in this paper, we came to the following conclusions:

1. On the analyzed site were identified two soil classes, respectively, protisoils and antrisoils class, and two types of soil, respectively, aluviosoil and tehnosoil according to Romanian System of Soil Taxonomy (SRTS) (Florea et al., 2012).

2. The trophicity index in the deposit area of the earth sludge and the carbonate sludge is very low determining the soil class as *extremely oligotrophic.* And the soil from the control area is characterized as being *oligotrophic.*

3. The area is into the 5th class of pretability that comprise terrains with very severe limitations, unpretable for agriculture purpose in terrain undevelopment conditions.

4. In order to valorificate the soil are necessary the application of agropedireliability works (Cojocaru and Statescu, 2014) that will determine the switching field tested in a higher class of pretability.

REFERENCES

1. **Chiriță C., 1974 -** *Ecopedologie cu elemente de pedologie generală.* Ed. Ceres, Bucureşti.
2. **Cojocaru P., Stătescu F., 2014 -** *Ecological rehabilitation of a terrain occupied by a waste deposit from manufacturing of the sugar.* The 14th International Multidisciplinary Scientific Geoconference, Albena, Bulgaria, June 16 – 22, Conference Proceedings, pp. 229-236.
3. **Dunca E., Ciolea D., 2013 –** *Composting of the green waste also its use under the ecological reconstruction of waste dumps.* Recent Advances in Energy, Environment and Geology, pp. 101-106.
4. **Florea N., Munteanu I., 2012 –** *Sistemul Român de Taxonomie a Solurilor.* Ed. ESFALIA, Bucureşti.
5. **Fodor D., Lazar M., 2006 –** *Ocuparea şi reabilitarea ecologică a terenurilor din zona Olteniei.* Buletinul AGIR, 3, pp. 27-31.
6. **Racoceanu C., Popescu L., Popescu C., Filip V., 2012 –** *Research regarding environmental risks due to complex operation Rovinari.* The 12th International Multidisciplinary Scientific Geoconference, Albena, Bulgaria, June 16 – 22, Conference Proceedings, pp. 715-723.
7. **Stătescu F., Măcărescu B., 1997 -** *Elemente ale complexului ecologic din sol.* Ed. Sam Son´s, Bucureşti.
8. **Teaci D., 1980 -** *Bonitarea şi caracterizarea tehnologică a terenurilor agricole.* Ed. Ceres, Bucureşti.

INFLUENCE OF NEEDLING PROCES PARAMETERS ON NONWOVENS USED AS IRRIGATION SUBSTRATES

INFLUENȚA PARAMETRILOR PROCESULUI DE INTERȚESERE ASUPRA NEȚESUTELOR FOLOSITE CA SUBSTRATURI DE UDARE

***LUPU G. Iuliana*[1]*, HRISTIAN L.*[1]*, HOGAŞ H. I.*[1]**
e-mail: iuliana68lupu@yahoo.com

Abstract. *Nonwovens used as watering substrate distibute water uniformlyand act as slight water buffer owing to the absorbent capacity. So, the irrigation solution is brought directly to the root zone. At the same time, using of nonwovens with higher water holding capacity affects the frequency of irrigation which depends by existing environmental conditions. In this study, we present the influence of the needling process parameters on the functional characteristics of nonwovens used as irrigation substrate. The aim is to determine optimal processing parameters using a central, composite design for second-order model. Our results show that the needling process parameters have a significant influence on functional characteristics. The higher of needle depth penetration and needle board frequency, higher is the compactness of nonwoven (higher fabric density) due to the strong fiber peg formation as more number of fibers are arranged vertically. Also, a less porous nonwoven strauture has a lower water holding capacity.*
Key words: *irrigation substrate, nonwoven**,** central composite design*

Rezumat. *Neţesutele utilizate ca substraturi de udare distribuie apa uniform şi acţionează uşor ca un tampon datorită capacităţii de reţinere a apei. Astfel, soluţia de irigare este adusă direct în zona rădăcinii. În acelaşi timp, folosire de neţesute cu capacitate mare de reţinere a apei influenţează frecvenţa de udare ce depinde de condiţiile de mediu existente. În acest studiu se prezintă infşuenţa parametrilor procesului de interţesereasupra caracteristicilor funcţionale ale neţesutelor utilizate ca substraturi de udare**. Scopul lucrării este dea determina parametrii optimi de proces folosind un model matematic compus, central rotabil de ordinul doi. Rezultatele indică faptul că parametrii de interţesere au o influenţă semnificativă asupra caractericilor funcţional. Odată cu creşterea adâncimii de pătrundere a acelor şi a frecvenţei păcii cu ace creşte compactitatea neţesutului datorită punctelor de consolidare mai solide ca urmare numărului mai mare de fibre aranjate vertical o structură mai puţin poroasă are o capacitate de reţinere a apei mai mică..*
Cuvinte cheie: *substrat de udare, neţesut, model central compus*

INTRODUCTION

The possibility to create and manufacture nonwovens at lower cost can contribute to increase environmental protection. Nonwovens are used effectively for optimizing the productivity of crops, gardens and greenhouses. Their

[1] "Gh. Asachi" Technical University of Iaşi, Romania

protective nature means that the need for pesticides is reduced and manual labour is kept to a minimum.

Water absorptive capacity is a very important property and an important criterion for the performance of needle-punched nonwovens used as irrigation substrate in horticulture (Sengupta, 2009).

Nonwovens used as watering substrate distribute water uniformly and act as slight water buffer owing to the absorptive capacity. So, the irrigation solution is brought directly to the root zone. At the same time, the using of nonwovens with higher water holding capacity affects the frequency of irrigation which depends by existing environmental conditions. Nonwovens can have a higher water absorbency if contain in the composition cellulose-based fibers. The advantages of using in the fibrous blend of PP fibers include lighter weight, high wet strength, resistance to rot and chemicals and quick wicking action.

Needle punching is a process for converting webs of fiber into coherent fabric structures, normally by means of barbed needles, which produce mechanical bonds within the web (Purdy, 1980). In order to understand more about the influence of needling process parameters on nonwoven water absorptive capacity it is essential to use mathematical modelling which is an investigation method of technological processes based on experimental data collection and processing.

The present study is investigating the effect of two parameters on functional characteristics of nonwoven that can contribute to reducing the irrigation frequency and of course the costs with water, labour etc. For this purpose, a central composite design for second-order model has been employed.

MATERIAL AND METHOD

All fabric nonwovens have been prepared from blends of 50% viscose of 3.3dtex/38 mm and 50% polypropylene of 6.7dtex/50 mm. A high percentage of viscose (cellulose-based fibers) has been used due to high capacity to hold water.

Web of polypropylene/viscose fibers was formed by carding and lapping process, respectively. The basis weight of the web was controlled as 150 g/m^2. The web was fed to the needling zone on a needle loom type Automatex having 15x18x42x3CBA Foster needles.

The needle-punched fabrics were produced by the penetrating action of barbed needles which reorientation and intermingles the fibers from a horizontal to a vertical direction (Rusell, 2007).

The experiments took place under pilot unit condition. Before performing the measurements, the samples were conditioned at 65%, relative humidity and 20^{0}C temperature for 24 h.

The samples were tested to determine their weight (W) and thickness (T) according to EDANA standards (ERT 40.-90, 1999, ERT 30.5-99, 1999, respectively). Then, the density of the nonwoven was calculated using the following relation:

$$\rho_N = \frac{W}{T}\left[kg/m^3\right] \quad (1)$$

The fabric water absorptive capacity was tested according to ISO 9073-6. The water absorptive capacity in (%) was calculated using the following relation:

$$C_a = \frac{M_d - M_w}{M_w} x100(\%) \quad (2)$$

where: M_d: mass in g of the dry test sample:
M_w: mass in g of the wet test sample at the end of test.

RESULTS AND DISCUSSIONS

To study the individual and interactive influence of needle board frequency, expressed in cycles/min and needle depth penetration, expressed in mm, a central composite surface factorial design of two variables (Taloi, 1987), was used.

Estimation of the response from this factorial design model equation is suitable only when the independent variables are within the range for which the model has been developed. The useful limits of the two variables were selected by conducting a number of preliminary experiments. The limits and the actual and coded values of independent variables are given in Table 1.

Table 1

Actual and coded values of independent variables

Variable	Symbol	Code				
		-1.414	-1	0	1	+1.414
Needle board frequency (cycles/min)	x_1	94	115	165	215	236
Needle depth penetration (mm)	x_2	3	4	6	8	9

The design developed matrix had 13 sets of experimental combinations in which 8 sets were distinguished and 5 sets referred to the central point. These 5 sets of experimental combinations from the central point were performed in order to establish the value of experimental errors.

The design matrix so developed with the coded values of independent variables and measured values of fabric density (kg/m3) and water absorptive capacity (%) are shown in Table 2.

To correlate the effect of independent variables and response, the following second-order standard polynomial was considered (Cojocaru et.al, 1968):

$$Y = b_0 + b_1x_1 + b_2x_2 + b_{11}x_1^2 + b_{22}x_2^2 + b_{12}x_1x_2 \quad (3)$$

where Y represents the response and $b_0, b_1, ..., b_{12}$ are the coefficients of the model.

The coefficients of main and interactive effects were determined using the standard method (Cojocaru et. al., 1968). The regression coefficients of the proposed model for different parameters can be calculated.

To establish the relationship between the independent variables (x_1) and (x_2) and the dependent variable (Y), a regression analysis was performed as describe above. The regression coefficients were used in the quadratic-proposed polynomial model (Table 3) to determine the predicted response values. These

coefficients have either positive or negative value, and accordingly have an effect on the experimental results.

Table 2

Constructional details of experimental fabrics

Sample No	Needle board frequency, cycle/min (x1)	Needle depth penetration, mm (x2)	Fabric density (kg/m^3)	Water absorptive capacity (%)
1	-1	-1	44.296	1894
2	1	-1	49.580	2148
3	-1	1	47.954	1979
4	1	1	50.147	2017
5	-1.414	0	46.892	1850
6	1.414	0	51.673	1974
7	0	-1.414	47.944	2190
8	0	1.414	53.665	2102
9	0	0	46.936	2119
10	0	0	44.296	1994
11	0	0	44.500	2053
12	0	0	45.992	2081
13	0	0	45.223	2059

.

Table 3

Quadratic proposed polynomial models

Quadratic proposed polynomial models	
(1) Fabric density Y_1=	$45.401+1.78x_1+1.539x_2+1.425x_1^2+2.185x_2^2-0.733x_1x_2$
(2) Water absorptive capacity Y_2=	$2061.69+58.417x_1-21.304x_2-79.856x_1^2+37.109x_2^2-54x_1x_2$

The coefficients of multiple correlation R^2 and the F-values together with the response surface equations of the factorial design for second-order models after testing the regression coefficients by employing the student test are shown in Table 4. To check the significance of multiple correlation coefficients, we used F test (Taloi, 1987). Accordingly, F-ratios were calculated for 95% level of confidence and two degrees of freedom (f_1 =2 respectively, f_2=10) and then compared with the corresponding tabulated value. If the calculated values of F-ratios exceed the corresponding tabulated value, then the independent variables have a significant influence on the dependent variable. The tabulated value of F-ratio at 95% level of confidence is found to be 4.1 (Cojocaru, et.al, 1968).

The values of multiple correlation coefficient (Table 4) between the experimental data and the predicted values illustrate a very good and significant correlation. The fabric density equation Y_1 (Table 4) reveals that the needle board frequency (x_1) and needle depth penetration (x_2) have a significant influence on the characteristic described by the equation. Moreover, the coefficients of the first degree term have the same sign (b_1>0, b_2>0) indicating the effect of independent variables to be the same.

Table 4

Response surface equation after testing of regression coefficients and evaluation of multiple correlation coefficient

Response	Response surface equation	Coefficient of multiple correlation	F -ratios
Y_1=	$45.401+1.78x_1+1.539x_2+1.425x_1^2+2.185x_2^2$	0.9126	24.91
Y_2=	$2061.69+58.417x_1-79.856x_1^2+37.109x_2^2-54x_1x_2$	0.9312	32.63

The presence of second degree-term coefficients indicates a well-defined response surface. The effect of independent variables on fabric density is shown in Figure 1. As can be seen from Figure 1, the mathematical model Y_1 describes an elliptical parabolic dependency with a minimum point having the following coded values: x_1= -0.625 and x_2= -0.352. The actual values of the critical point obtained by optimization are x_1=134 cycles/min and x_2=5 mm for a fabric density Y_1=44.575 kg/m^3.

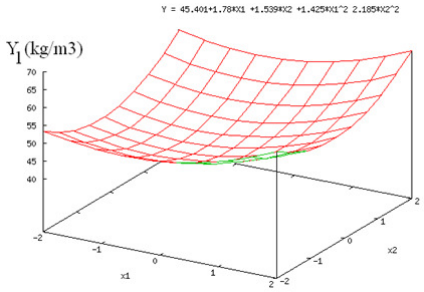


Fig. 1. Effect of needle board frequency (x_1) and needle depth penetration (x_2) on fabric density Y_1 (kg/m3)

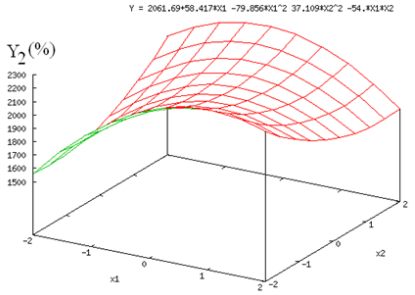


Fig. 2. Effect of needle board frequency (x_1) and needle depth penetration (x_2) on water absorptive capacity Y_2 (%)

The higher of needle board frequency and needle depth penetration, the higher is the fabric density due to the strong fiber peg formation as more number of fibers are arranged vertically (Sengupta and Sengupta, 2013). A higher fabric density means a higher compactness of the nonwoven and hence the number of pores (amount of voids) is decreasing. Therefore, the critical point which is a minimum point can be considered an optimum point for the nonwoven used as irrigation substrate.

Inspection of the equation of Y_2 (Table 4) indicates a significant influence of needle board frequency on water absorptive capacity through first–degree term. The coefficients of the second-degree terms also influence the water absorptive capacity and indicates a well-shaped response surface. A considerable influence has the interaction term. The negative sign of the interaction term coefficient indicates a decreasing tendency of the water absorptive capacity to the cumulative action of both parameters. The effect of independent variables on water absorptive capacity is presented graphically in Figure 2. The mathematical model of Y_2 describes a hyperboloid with saddle point having x_1= 0.294 and x_2 = 0.214 as

coded values. The actual values of the critical point are x_1= 180 cycles/min and x_2= 6.6 mm for a water absorptive capacity Y=2057.80%.

The increase in needle board frequency over a certain value and needle depth penetration are responsible for a better entanglement of fibers resulting in a more compact fabrics structure, with less number of voids. Thus, the water absorptive capacity is decreasing due to the lower amount of air present in a more compact fabrics structure which can be replaced by the water.

CONCLUSIONS

Second-order polynomials with two independent variables have been proposed with a good correlation for fabric density and water absorptive capacity of needle-punched, cross-laid nonwoven, with respect to mass/unit area of the web. From this model, one can understand the effects of different parameters on fabric density and water absorptive capacity and can also predict the water absorbency approximately knowing the values of parameters.

The information available from contour diagrams regarding the interaction of parameters on water absorbency and fabric density is very much useful to design a needle-punched nonwoven fabric for agricultural applications. It is known that water absorbency of nonwoven increases with the increasing of cellulose-based fibers proportion. Also, the needling process parameters can increase water absorbency until certain values. As needle depth penetration decrease for a particular needle board frequency, fabric density decrease due to weak fiber peg formation. An optimum fabric density of 44 kg/m^3 for a nonwoven used as irrigation substrate has been achieved at around 134 cycles/min and 5 mm needle depth penetration.

Water absorptive capacity is highly correlated with fabric density. So, as needle board frequency increase, for a particular needle depth penetration, the water absorptive capacity increases and after reaching to maximum, it decreases due to a less porous structure of nonwoven.

Using nonwovens in irrigation practices it is recommended based on following advantages: are less expensive and easy to install, allows for complete flexibility, keep foliage dry to minimize diseases and no runoff.

REFERENCES

1. **Cojocaru N., Clocotici V., Dobra D., 1968** – *Metode statistice aplicate în industria textilă*, Editura Bucureşti, România
2. **Purdy A.T., 1980** - *Needle punching*, The Textile Institute Manchester, North Carolina State University, ISBN 10: 0900739320/0-900739-32-0
3. **Rusell S.J., 2007** - *Handbook of nonwovens*, Woodhead Publishing Limited, Cambridge, England.
4. **Sengupta, S., 2009** - *Water absorbency of jute needle-punched nonwoven fabric,* Indian Journal of Fibre & Textile Research, Vol.34, No.4, pp. 345-351
5. **Sengupta S., Sengupta A., 2013** – *Electrical resistance of jute needle-punched nonwoven fabric – effect of punch density, needle penetration and area density*, Journal of The Textile Institute, Vol. 104, No.2, pp.132-139
6. **Taloi D, 1987** – *Optimization of metallurgical processes*, Editura Bucureşti, România

APPROACHES FOR BIOREMEDIATION OF PESTICIDE CONTAMINATED SOIL: COMPLEX POLLUTION PROBLEMS

PROCEDEE DE BIOREMEDIERE A SOLULUI POLUAT CU PESTICIDE: PROBLEMELE POLUĂRII COMPLEXE

RASTIMESINA Inna[1], CINCILEI A.[2], POSTOLACHI O.[1], TOLOCICHINA S.[1], MAMALIGA V.[1], STREAPAN N.[1]
e-mail: oleseap@yahoo.com

Abstract. *A strongly pollution with trifluralin and DDT of soil samples collected nearby an old pesticides storehouse was revealed. Two main approaches of treatment were used to remediate this complex contamination – creation strictly anoxic conditions, and alternating anoxic and oxic conditions. In both cases, trifluralin content in soil decreased by more than 4 times and degradation rate reached 95-96%. The cycled anoxic and oxic treatment of soil amended with phosphates and peptone, promoted the DDT and DDTs degradation in compare with the anoxic treatment. The prolongation of the experiment in aerobic conditions resulted in complete mineralization of DDT.*
Keywords: *bioremediation, soil, pesticides, trifluralin, DDT.*

Rezumat. *A fost depistat că solul din apropierea unui depozit vechi de pesticide este puternic poluat cu trifluralina si DDT. Pentru remedierea acestei contaminari complexe, s-a recurs la doua procedee fundamentale de tratare – crearea condiţiilor strict anaerobe si alternarea condiţiilor anaerob-aerobe. În ambele cazuri continutul trifluralinei a scazut cu mai mult de 4 ori, iar procentul degradării atingea 95-96%. Alternarea conditiilor anaerob-aerobe cu adăugarea în sol a fosfaţilor şi peptonei, a favorizat degradarea DDT şi DDTs, comparativ cu tratamentul anoxic. Continuarea experimentului în condiţii aerobe s-a soldat cu mineralizarea completă a DDT.*
Cuvinte cheie: *bioremediere, sol, pesticide, trifluralina, DDT.*

INTRODUCTION

Recent years the using of pesticides in the national economy of Republic of Moldova is significantly reduced. However the import, storage and application of harmful substances still is a current issue (National Report, 2011). According to the National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants, during the years 1991-2003 about 60% of deposits were destroyed and liquidated and only 20% of the remaining ones are in a satisfactory condition (National Implementation Plan, 2004; National Report, 2011). Considerable amounts of dangerous chemicals and pesticides have been abandoned in the fields and the devastated deposits cause a negative impact on public health and the

[1] Institute of Microbiology and Biotechnology, ASM, Chisinau, Republic of Moldova
[2] The State Center for Certification and Registration of Phytosanitary Means and Fertilizers, Ministry of Agriculture, Chisinau, Republic of Moldova

environment, since some of them are located near residential areas or near water resources.

Bioremediation of contaminated natural environments, soil in particular, is an innovative technology, highly topical, that uses biological systems in the pollutant elimination, promotes rehabilitation of contaminated sites and preservation of soil suitable conditions for further cultivation of plants (Matsumoto, 2009). The most efficient way for decomposition of organochlorine pesticides POPs is a reductive bioremediation, which promotes dehalogenation of organochlorine compounds. Creation of anaerobic conditions and supplementation with specific nutrients for anaerobic/facultative anaerobic soil microorganisms leads to efficient removal of residues of persistent compounds (Sasek et al., 2003; Phillips et al., 2004; Kantachote et al., 2004; Reference Guide, 2004). The bioremediation methods permit to value in particularly the amazing ability of microorganisms for adapting to changing environmental conditions and to decompose a wide range of xenobiotic compounds, including pesticides.

The purpose of our research was to identify the most effective methods for activation of biodestructive capacity of indigenous microorganisms to remediate the soil contaminated for a long time with complex pesticide mixture.

MATERIAL AND METHODS

Soil samples were collected near the storehouse of persistent organic pollutants, located in the center of the Republic of Moldova, Chisinau city, Singera village. Previously the collected soil samples were cleaned of roots and other impurities, sieved (mesh No. 2) and air-dried at 22-23^{0}C. Soil moisture content (SMC), water-holding capacity (WHC), soil pH and organic matter content were determined using standard methods (Arinushkina, 1970; Kozlova, 2009).

The extraction of DDTs and trifluralin from soil has been performed in four repetitions per option according (Klisenko and Alexandrova, 1983). The determination of pesticide residues (PR) in soil was confirmed by gas chromatography with mass spectrometry GC/MS multiresidue method, at the gas chromatograph "Agilent Technologies" 6890N coupled with MSD mass selective detector "Agilent Technologies" 5973. Percentage of degradation was calculated according to Bento (Bento et al., 2003) using the expression: % of degradation = [(PR control – PR experience) / PR control] $\times$ 100.

The bioremediation was established in plastic jars, each containing 1,000 g of contaminated soil. The experiment was carried out in two main directions: strictly anaerobic conditions (Section 1) and alternating of anoxic/oxic conditions (Section 2).

Section 1: Creating of strictly anaerobic conditions by saturating the polluted soil with water (up to 80% of WHC) in the dark plastic jars sealed with Parafilm, in the dark and at temperature 22-24^{0}C. The experiment duration – 112 days.

1. The soil was saturated with water (80% of WHC);
2. Amendment of soil with zero-valent Fe 0.5% w/w, twice during the experiment;
3. The soil was amended with mono- and dipotassium phosphate (1.0%) and peptone (0.5%) at the begining of the experiment;
4. The soil was amended with dipotassium and diammonium phosphate (1.0%) and peptone (0.5%) at the begining of the experiment.

Section 2: alternating of anoxic and oxic conditions. Each cycle consists of two phases – anaerobic (for 21 days) and aerobic (for 7 days). Anaerobic conditions were created by saturating the contaminated soil with water (80% of WHC) in the dark plastic jars sealed with Parafilm, and stored in the dark at 22-24°C. At the beginning of the aerobic phase parafilm was removed, soil mixed with a metal spatula and gradually brought soil moisture up to 60% of WHC. At the start of each anaerobic phase mineral and organic (peptone) amendments were added to the soil, soil humidity was maintained at 80% of WHC.

5. The soil was amended with mono- and dipotassium phosphate (1.0%) and peptone (0.5%) at the begining of the experiment, the experiment duration – 112 days;
6. At the beginning of the experiment, there were added dipotassium and diammonium phosphate in concentration of 1.0% by weight each, and peptone in concentration of 0.5%, the experiment duration – 135 days;
7. Alternating anoxic and oxic conditions as in the variant 6 for 63 days, than passing to the aerobic phase followed by stimulation of microorganisms by applying sawdust, phytobioremediation with oat (*Avena sativa L.*), monthly plants were cut, mixed with the soil and new seeds were planted, for 72 days.
8. The soil was amended with dipotassium and diammonium phosphate (0.5%) and peptone (1.0%) at the begining of the experiment, the experiment duration – 112 days.

As a control were used: (1) contaminated soil befor remediation, (2) variant 1 without variable factors of remediation.

RESULTS AND DISCUSSIONS

At the start of the experiment, the soil pH was 8.0 and the air-dry soil moisture content was 1.84%. Water holding capacity was 33.60% and soil organic matter content was 2.06%. Soil type was determined as carbonated chernozem.

The total content of organochlorine compounds was 21.00 mg/kg soil, which demonstrates that the level of pollution near the deposit exceeds the national standard. Soil pollution by trifluralin exceeded 195 times the Maximum Residue Limit (MRL) and made up 19.52 ± 0.22 mg / kg soil. ∑ DDTs (DDT, DDE, DDD) was 14.8 MRL (Normativele igienice, 2003). These data clearly indicates that the studied site was long-term and complex polluted and the major component is presented by the fluorine organic herbicide trifluralin and the minor component by organochlorine insecticide DDT and its metabolites.

After the soil bioremediation experiment in laboratory conditions was completed, soil samples were analyzed by GC/MS and the percentage of pesticides degradation was calculated.

The decrease of trifluralin concentration in polluted soil to 24.6% was managed merely by creating anaerobic conditions by saturation of soil with water up to 80% of WHC (trifluralin sanitary and toxicological MRL – 0.1 mg / kg soil). The decomposition of trifluralin in the Section 1 was favoured by anoxic conditions, and, in particular, by the soil amendment with peptone and dibasic phosphates of potassium and ammonium. These conditions ensured the mineralization of 76.6% and 95.8% of herbicide in the experimental variants 3 and 4 (tab.1).

In the Section 2 alternation of anoxic / oxic conditions along with the amendment of phosphates of potassium and ammonium, and peptone (variants 5-8) has facilitated the trifluralin degradation (tab. 1). The conditions created in the experimental variant 8 have allowed the mineralization of 95% of trifluralin. Thus, the most important factor for trifluralin biodegradation in carbonated chernozem is the creation of anoxic / oxic conditions, favourable for the development of anaerobic / facultative anaerobic microflora.

Table 1

The impact of bioremediation processes on general amount of trifluralin in polluted soil

Experimental variants	Trifluralin	
	mg/kg	% of degradation
Control	19.52 ± 0.22	-
1	14.71 ± 0.18	24.6
2	14.87 ± 0.85	23.8
3	4.57 ± 0.53	76.6
4	0.81 ± 0.03	95.8
5	4.76 ± 0.26	75.6
6	4.50 ± 0.37	76.9
7	7.76 ± 0.13	60.2
8	0.95 ± 0.19	95.1

It is known, that organochlorine pesticides are highly persistent, having at the same time relatively low environment mobility. The type and duration of degradation are determined by the chemical structure and stability of the pesticide. The ratio between DDT and its metabolites allows to roughly estimate the time of occurrence and degradation rate of pesticide residues in polluted soil. Thus, the ratio **(DDE + DDD) / DDT > 1** indicates an old pollution with DDT, exposed to active microbial transformation, and the ratio **(DDE + DDD) / DDT < 1** testifies about a recent pollution and low degradative activity of soil microorganisms (Galiulin and Galiulina, 2008; Kulikova-Khlebnikova, 2011). After the ratio between the soil concentration of DDT metabolites and DDT has been calculated, according to indicated expression, the value 3.92 > 1 was obtained, which indicates an old pollution and the pesticide degradation by the microbial biota.

Microbial degradation of POPs depends on several factors: temperature, pH, redox potential, humidity, aeration, the „special" microorganisms etc. (Phillips, 2004). Practically the optimal combination of these factors absents in the environment, therefore the transformation of pesticides is limited in its initial stages. The most effective bioremediation methods targeting DDTs contaminants, according our experimental data, was amendment of soil with peptone and dibasic phosphate of potassium and ammonium (variants 4 and 8), that have led to a decrease of ΣDDTs up to 64.6 and 61.3% respectively, comparing with non remediated control (tab.2).

Table 2

Residue of DDTs metabolites and DDT in soil befory and after bioremediation procedures

Experimental variants	DDT		DDTs	
	mg/kg	% of degradation	mg/kg	% of degradation
Initial (control)	0.32 ± 0.02	-	1.48 ± 0.07	-
1	0.23 ± 0.02	28.1	1.07 ± 0.10	27.7
2	0.23 ± 0.01	28.1	1.10 ± 0.09	25.7
3	0.14 ± 0.02	56.3	1.15 ± 0.12	22.3
4	0.21 ± 0.04	32.9	0.52 ± 0.11	64.6
5	0.10 ± 0.01	68.8	1.01 ± 0.09	31.8
6	0.09 ± 0.01	72.2	1.12 ± 0.03	24.4
7	0.00	100.0	0.97 ± 0.02	34.7
8	0.22 ± 0.04	31.4	0.57 ± 0.12	61.3

Amendment of soil with phosphates and peptone in Section 2 ensures a deep reductive cleavage of the DDT (up to 0.09 mg / kg soil) and further phytoremediation favours total degradation of this pesticide in the variant 7. An important increase of the metabolite DDD and an essential decrease of DDT – up to 68.7 and 72.2% – were observed in bioremediation variants 5 and 6, due to the alternation of anaerobic / aerobic cycles and soil supplementation with peptone and phosphates (tab.2). A deep reductive cleavage of investigated organochlorine pesticides occurred under these conditions, manifested by the disappearance of DDT and intensive accumulation of degradation products, especially *o,p′*-DDD.

CONCLUSIONS

Thus, the eight procedures of bioremediation of complex soil pollution with trifluralin and organochlorine compounds POPs have been examined, and the decrease of their amount in all experimental variants was determined, that indicated a good selection of remediation procedures tested.

The main factor for the trifluralin decomposition in carbonated chernozem is soil microbiota, especially the anaerobic / facultative anaerobic, and its stimulation with nutrient supplements substantially improves decomposition of the trifluralin herbicide in soil. The degree of trifluralin mineralization after an application of the anoxic treatment was at the same level as after a combination of the anoxic / oxic cycled condition and stimulation of indigenous microflora with phosphates and peptone. In both cases the concentration of trifluralin decreased by more than 4 times and degradation rate reached 95-96%.

Amendment of soil with phosphates and peptone under anaerobic conditions provides a deep reductive cleavage of organochlorine pesticides DDT and intensive accumulation of degradation products, especially of *o,p′*-DDD. The alternation of anaerobic-aerobic conditions favour degradation of DDT and DDTs metabolites, compared with an anoxic treatment. To reduce the amount of

extremely persistent metabolites DDD and DDE the prolongation of the experiments for bioremediation of the contaminated soil is necessary.

REFERENCES

1. **Arinushkina E.V., 1970** – *Guidance on chemical analysis of soils.* Editura MGU, Moscow, p. 488.
2. **Bento F.M., Camargo F.A.O., Okeke B., Frankenberger Jr. W.T., 2003** – *Bioremediation of soil contaminated by diesel oil.* Brazilian Journal of Microbiology, 34 (1), pp. 65-68.
3. **Galiulin R.V., Galiulina R.A., 2008** – *Ekologo-geokhimicheskaya otsenka «otpechatkov» stoykikh khlororganicheskikh pestitsidov v sisteme pochva – poverkhnostnaya voda.* Agrokhimiya, No. 1, pp. 52-56.
4. **Kantachote D., Singleton I., Naidu R., Mcclure N., Megharaj M., 2004** – *Sodium Application Enhances DDT Transformation in a Long-Term Contaminated Soil.* Water, Air, and Soil Pollution, 154 (1-4), pp. 115-125.
5. **Klisenko M.A., Alexandrova L.G., 1983** – *Determination of pesticide residues.* Kundiev Yu.I., eds., Editura Health, Kiev, p. 174.
6. **Kozlova A.A., 2009** – *The educational practice on soil physics: methodical handbook.* Editura Irkutsk State University Press, Irkutsk, pp. 52-53.
7. **Kulikova-Khlebnikova E.N., Robertus Yu.V., Kivatskaya A.V., 2011** – *Osobennosti metabolizma khlororganicheskikh pestitsidov v ob"yektakh okruzhayushchey sredy v usloviyakh Gornogo Altaya.* Vestnik Altayskogo gosudarstvennogo agrarnogo universiteta, 10 (84), pp. 50-53.
8. **Matsumoto E., Kawanaka Z., Zun S., 2009** – *Bioremediation of the organochlorine pesticides, dieldrin and endrin, and their occurrence in the environment.* Appl.Microbiol.Biotechnol., 84, pp. 205-216.
9. **Phillips T., Lee H., Trevors J.T., Seech A.G., 2004** – *Mineralization of hexachlorocyclohexane in soil during solid-phase bioremediation.* J.Ind.Microbiol.Biotechnol., no. 31, pp. 216-222.
10. **Sasek V., Glaser J.A., Baveye P., 2003** – *The Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions.* Proceedings of the NATO Advanced Research Workshop on the Utilization of Bioremediation to Reduce Soil Contamination: Problems and Solutions, Prague, Czech Republic, 14-19 June 2000. Editura Springer, Nature, p. 417.
11. **Takashi O., Ryota Y., 2004** – *Improvement of simazine degradation by inoculation of corn and soybean plants with rhizobacteria.* Soil Sci.and Plant Nutr., 50 (8), pp. 1295-1299.
12. *****, 2003** – *Normativele igienice privind reziduurile preparatelor de uz fitosanitar în obiectele mediului înconjurător din 21.08.2003.* Editura Ministerul Sănătății, Chişinău, Monitorul Oficial 248-253/359, 19.12.2003.
13. *****, 2004** – *Republic of Moldova. National Implementation Plan for the Stockholm Convention on Persistent Organic Pollutants. Ministry of Ecology and Natural Resources.* World Bank, Editura Stiinta, Chisinau, p. 80. http://www.pops.int/documents/implementation/nips/submissions/moldova.pdf (accessed March 29, 2014).
14. *****, 2011** – *State of the Environment in the Republic of Moldova*, 2007-2010 (National Report – Synthesis). Editura "Nova Imprim" SRL, Chisinau, p. 88. ISBN 978-9975-4224-3-7.

STUDY ON THE ANTIRADICAL ACTION OF ASEA (FOOD SUPPLEMENT) IN CASE OF SUBACUTE ACRYLAMIDE INTOXICATION

STUDII PRIVIND ACȚIUNEA ANTIRADICALARĂ A UNUI SUPLIMENT ALIMENTAR (ASEA) PE FUNDALUL INTOXICAȚIEI SUBACUTE CU ACRILAMIDĂ

PRISĂCARU Cornelia[1], PRISĂCARU Anca-Irina[2], ROTARU Liliana[1]
e-mail: corneliapris@uaiasi.ro

Abstract. *ASEA is "the first stable mixture with redox signaling molecules (RSM)" with the highest ORAC index (free radical absorption capacity). It is not a drug intended to cure a certain disease, but a mixture of redox signaling molecules (RSM) with high addressability, high efficacy level (100%) and zero toxicity. Considered to be a food supplement, ASEA acts on every cell stimulating the intracellular balance disrupted after the appearance of oxidative stress (OS) which is characteristic to most acute and chronic disease. This contraindication free food supplement is recommended for the disorders of all organs, apparatus and to all ages, its efficacy being remarkable in the case of autoimmune diseases. ASEA, this stable mixture of redox signaling molecules was developed and put on the American market from 2009. It is obtained of clean water, after a three-phase process: the dissolution of a certain amount of pure sodium chloride (NaCl) in water (I), the solution obtained goes through a special electrolysis process where the bonds between chloride, sodium, hydrogen and oxygen atoms are broken and they are transformed into free, floating radicals (II) and their recombination and the formation of stable molecules with redox signal (III). The present experiment tries to compare the antioxidant potential of this stable mixture with the antiradical capacity of certain phytopreparates as Pycnogenol (maritime pine bark extract), a phytopreparate with an ORAC index lower than that of ASEA, but with recognized efficacy, well tested in the European therapy. The experiment was conducted on 4 groups of Wistar rats and lasted 6 weeks. The results were assessed using the biochemical evaluation of certain oxidative stress indices.*
Key words*: redox signaling molecules (RSM), free radicals (FR), antioxidants (AO), superoxide dismutase (SOD),* glutathione peroxidase *(G-Px), free sulfhydryl groups*

Rezumat. *ASEA este „primul amestec stabilizat cu molecule de semnalizare redox (MSR)" care posedă cel mai înalt indice ORAC (capacitatea de captare a radicalilor liberi). Nu este un medicament care vindecă o anumită boală, ci este un amestec de molecule de semnalizare redox (RSM) care prezintă o adresabilitate mare, eficiență ridicată (100%) şi toxicitate zero. Considerat supliment alimentar, ASEA, acționează la nivelul fiecărei celule, stimulând instalarea echilibrului intracelular perturbat în urma producerii stresului oxidativ (SO), caracteristic majorității maladiilor acute şi cronice. Acest*

[1] University of Agricultural Sciences and Veterinary Medicine of Iaşi, Romania
[2] S.C. Fiterman Pharma, Iaşi, Romania

supliment alimentar lipsit de contraindicaţii se recomandă în patologiile tuturor oraganelor şi aparatelor, tuturor vârstelor, eficienţa sa fiind remarcabilă în maladiile autoimune. ASEA, acest amestec stabilizat de molecule de semnalizare redox, este elaborat şi comercializat în SUA din 2009. Este obţinut din apă curată în 3 faze: dizolvarea unei anumite cantităţi de clorură de sodiu pură (NaCl) în apă (I), supunerea soluţiei obţinute unui proces special de electroliză, proces ce presupune ruperea legăturilor dintre atomii de clor, natriu, hidrogen şi oxigen cu transformarea acestora în atomi liberi, flotanţi (II) şi recombinarea acestor cu formarea de molecule stabile cu semnal redox (III). Experimentul prezent încearcă să compare potenţialul antioxidant al acestui amestec stabilizat cu capacitatea antiradicalară a unor fitopreparate de tipul Pycnogenolului (extract de scoarţă de pin maritim), fitopreparat cu indice ORAC inferior celui declarat pentru ASEA, dar cu eficienţă incontestabilă, bine testatată în terapia europeană. Experimentul s-a efectuat pe 4 loturi de şobolani albi, linia Wistar, timp de 6 săptămâni, iar rezultatele au fost apreciate prin evaluarea biochimică a unor indicatori de stres oxidativ.
***Cuvinte cheie**: molecule de semnalizare redox, radicali liberi, antioxidanti, superoxid dismutaza, glutation peroxidaza, grupări sulfhidril lbere*

INTRODUCTION

In the previous century, somewhere around the 1950s, proving the existence of free radicals as a result of all oxidation processes, including the ones in living organisms, represented a turning point for the manner in which pathology and therapy were approached. The expression "oxidative stress" (OS) appeared (Olinescu and Greabu, 1990; Halliwell and Gutterridge, 1989; Percival, 1998; Burlacu and Prisăcaru, 2006) and the phenomenon was initially associated to chronic diseases and it was characterized by the presence of FR, mainly chemically reactive species of oxygen (RS O_2), in the cell, their attack on the key components of cells leading to cellular apoptosis. The action of free radicals in severe diseases as cancer, diabetes, arthrosis, Parkinson and Alzheimer disease etc. is obvious (Prisăcaru and Burlacu, 2009; Prisăcaru, 2010; Prisăcaru et al., 2011). Immediately after, it appeared the problem of finding the antioxidants, those chemical substances capable of preventing the formation of FR or inhibiting their high reactivity level; the focus fell initially, according to the tendencies of the time, on chemically synthesized antioxidants. When the organism's unexpected answer to synthetic antioxidants pointed out in some of the cases their toxicity and in other cases, their inefficiency, vegetal antioxidants started being sought, according to the model of the ones in living organisms (glutathione, flavonoids, vitamins C, A, E etc.).

The combination of the results obtained from phytochemical, pharmacognostic and toxicological studies with the results from phytotherapeutic studies and medical clinique have led to the apparition of numerous vegetal pharmaceutical forms with antioxidant action (Burlacu and Prisăcaru, 2006; Prisăcaru and Burlacu, 2009; Prisăcaru, 2010; Prisăcaru et al, 2011). The interest in the commercial aspect of the ones in the industry of food supplements led to the apparition on the Romanian market of numerous unpatented "forms of food

supplements", that are not subjected to any type of control to certify the content of their active principles, their purity, or the simplest information of pharmacokinetics and pharmacodynamics etc.

Under the name of "food supplements" we often discover mixtures of vegetal products, sometimes, much too many to be capable of fulfilling the role of pharmacodymanic synergism and which can represent not only objects of charlatanry but also a risk to human health. These phytopreparates are pointed out with advertisements that underline the effects of some universal panacea accompanied by numerous financial advantages: bonuses, free products, the possibility of getting a subscription to a smaller price etc. One of the products in this category is ASEA, a stable mixture of redox signaling molecules created and commercialized in USA from 2009. It is obtained from clean water after a 3 phases process: dissolution of a certain quantity of pure sodium chloride (NaCl) in water (I), subjecting the resulting solution to a special electrolysis process that includes breaking the bonds between the atoms of chloride, sodium, hydrogen and oxygen and transforming them in free, floating atoms (II) and their reorganization so to create stable molecules with redox signal (III) (http://asea.myvoffice.com).

MATERIAL AND METHOD

The present experiment (Table 1) tries to compare the antioxidant potential of this stable mixture (ASEA) with the antiradicalar capacity of some phytopreparates as Pycnogenol (maritime pine bark extract), a phytopreparate with an ORAC index lower than that of ASEA, but with recognized efficacy, well tested in the European therapy (Peng et al., 2010; Farid et al., 2004; Sime and Reeve, 2004). The experiment was conducted on 4 groups of white Wistar rats and lasted 6 weeks. The first group that included 5 rodents represented the reference group; they were accommodated and fed in standard conditions. The five rats from the second group represented the control group and they were subjected to a sub-acute intoxication with acrylamide (*dose pro die* of X drops). The third group was administered, apart from the daily acrylamide dose a number of X drops of hydro alcoholic solution of Pycnogenol (standardized solution with 85% of procyanidins).

Table 1

Experimental model

Groups	***Acrylamide Water solution 1.5%***	**Pycnogenol hydro alcoholic solution (85 % procyanidin) *pro die* dose**	**ASEA *pro die* dose**
Reference group	-	-	-
Control group 1	X *guttes*	-	-
Control group 2	X *guttes*	X *guttes*	-
Trial group 1	X *guttes*	-	X *guttes*

The last group (considered the trial group that provides information on the antioxidant potential of the tested product) was treated with the toxic dose of acrylamide and X drops of ASEA solution.

At the end of the experiment, in order to assess and compare the antitoxic potential of the ASEA solution on the blood collected from the animals included in the trial it was determined the catalase activity (CAT), the activity of superoxide dismutase (SOD), of glutathione peroxidase and the serum concentration of sulfhydryl groups.

RESULTS AND DISCUSSIONS

The results obtained after quantifying the activity of serum catalase and after statistically processing the information, are presented in table 2 and fig. 1. From the analysis of this data results a significant growth of its activity at the group of rats treated exclusively with acrylamide solution (645.921 U/mL), compared to that of the reference group (612.515 U/mL), which suggests the consumption of the enzyme after the attack of the free radicals of glycidamide, the acrylamide attack type, followed by the stimulation of enzyme synthesis with an abrupt growth. The figures illustrating catalase activity from the serum of the group protected with hydro alcoholic solution of *Pycnogenol* is equal to 629.990U/mL, the figure being equally distant between the value of the reference group (612.515 U/mL) and the one of the group treated exclusively with the SO producing toxic substance (645.921 U/mL) which suggests the antiradicalar effect of the procyanidins deriving from maritime pine bark. The analysis of catalase activity from the serum of the animals treated with antioxidant saline solution, ASEA, points out a value similar to the one of the group treated with Pycnogenol, but lower than that of the reference group (623.166 U/mL). The study of the second oxidative stress indicator, superoxide dismutase, indicates a sinuous trajectory of enzymatic activity, which decreases significantly from the reference group (683.132 U/mL) to the control group 1 (568.274 U/mL), after which it grows at the groups protected with ASEA (662.33 U/mL) and especially at the one protected with Pycnogenol (705.25 U/mL).

A similar evolution, almost identical, is also recorded by the third parameter glutathione peroxidase, an enzyme with an activity value equal to 121.112 μmol/min/mL; the activity decreases significantly to 89.492 μmol/min/mL for the group intoxicated with acrylic amide and then returns to values similar to those of the reference group. The highest intensity of the enzyme's activity, glutathione peroxidase, is reached in the serum of the animals that benefited from the administration of ASEA, which can suggest its significant antioxidant role.

Table 2

Evolution of catalase and serum superoxide dismutase activity

Groups	CAT [U/mL]	SOD [U/mL]
Reference group	612.515	683.132
Control group 1	645.921	568.274
Control group 2	629.990	705.25
Trial group 1	623.166	662.33

Table 3

Oscillation of serum glutathione peroxidase and thiolic groups

Groups	G-Px [μmol/min/mL]	Free thiolic groups [μmol/ mL]
Reference group	121.112	303.262
Control group 1	89. 492	219.621
Control group 2	110.662	319.439
Trial group 1	188.131	321. 002

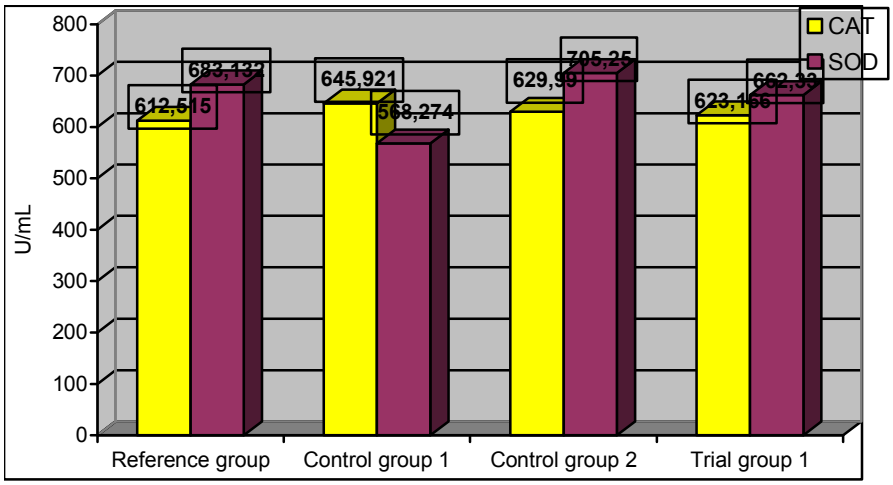


Fig. 1 - Variation of catalase and serum superoxide dismutase activity

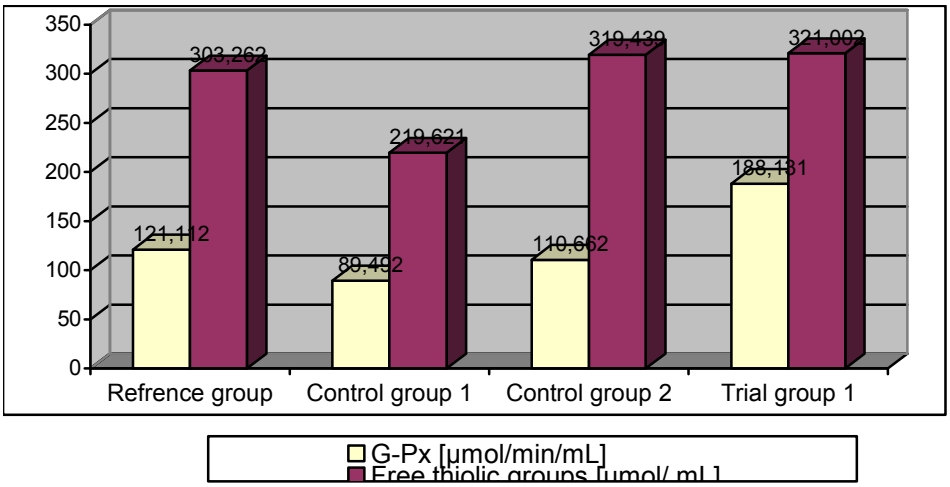


Fig. 2 - The evolution of serum glutathione peroxidase and free thiolic groups

The study of the results of the analysis of the free thiolic groups, as it is presented in table 3 and figure 2 confirm the role of the procyanidins from the marine pine bark, as a valuable antioxidant and detoxifying element. If the concentration of thiolic groups is of 303.262 µmol/ mL for the reference groups, for the group subjected to the action of the FR from the organism of the animals included in the control group 1 it decreases in an obvious manner at 219.621 µmol/ mL, while the value of mercapto groups increases significantly in the serum of the animals included in the groups treated with ASEA (319.439 µmol/ mL) and especially in the serum of the animals protected with Pycnogenol (321.002 µmol/ mL).

CONCLUSIONS

1. The analysis of catalase activity from the serum of the animals treated with antioxidant saline solution, ASEA, indicates a value similar to the one of the groups treated with Pycnogenol and lower than that of the reference group;

2. The study of superoxide dismutase, indicates a sinuous trajectory of enzymatic activity, which decreases significantly from the reference group to the control group 1, after which it grows at the groups protected with ASEA and especially at the one protected with Pycnogenol;

3. The highest intensity of the enzyme's activity, glutathione peroxidase, is reached in the serum of the animals that benefited from the administration of ASEA, which can suggest its significant antioxidant role.

4. The concentration of mercapto groups increases significantly in the serum of the animals included in the group treated with ASEA (319.439 μmol/ mL) and especially in the serum of the animals protected with Pycnogenol (321.002 μmol/ mL).

5. The stabilized mixture with redox signaling molecules present antiradicalar action but according to the parameters studied, it is inferior to the antioxidant action of Pycnogenol.

REFERENCES

1. **Burlacu Anca-Irina, Prisăcaru Cornelia, 2006** – *The Diminution of Sterigmatocystin by the Antiradicalic Action of some Vegetal Flavonoid Containing Products*, 29th Balkan Medical Week, 28-30 September, Gloden Sands, Varna;
2. **Farid, R., Mirteizi, A., Mirheidari, M., Rezaieyazdi, S., Mansouritorghabeh, H., Zibadi, S., Watson, RR., 2004** - *Pycnogenol as an adjunct in the management of childhood astma*, Asthma 41, pp.825-832;
3. **Halliwell B., Gutterridge J. M. C., 1989** – *Free Radicals in Biology and Medicine, Clarendon, Oxford*, p.116;
4. **Olinescu R., Greabu M., 1990** – *Mecanisme de apărare ale organismului împotriva poluării chimice*, Ed. Tehnică, Bucureşti;
5. **Peng, Q., Wei and Lau B.H.S., 2010** - *Pycnogenol inhibits tumor necrosis factor-lafa-induced nuclear factor Kappa B activation and adhesion molecule expression in human vascular endothelial cells*, Cell. Mol. Life Sci., 57, pp. 834-841;
6. **Percival M., 1998** – *Antioxidants, Clinical Nutrition Insights*, NUT031 1/96, Rev. 10/98;
7. **Prisăcaru Cornelia, Burlacu Anca-Irina, 2009** – *Evaluation of the Antitoxic Effect of Phthalides from Apium graveolens in Acrylamide Intoxication I. Evolution of the Hepatic Cytolysis and Proteosynthetic Parameters in Acrylamide Intoxication on Background of Phthalide Protection*, Rev. Notulae Botanicae Hort. Agrobot., vol. 37 (2), pp.129-133;
8. **Prisăcaru Cornelia, 2010** – *Monitoring the Antitoxic Effect of some Glutathione Rich Vegetal Products*, Lucrări ştiinţifice USAMV, secţia Medicină Veterinară, Iaşi, 2010, Vol 53(12), pp.484-489;
9. **Prisăcaru Cornelia, Prisăcaru Anca-Irina, Rotaru Liliana, 2011** – *Evaluation of the Antitoxic Potential of some Quercetol Containing Vegetal Products*, Lucrări ştiinţifice USAMV Iasi, seria Horticultură, vol 54 (1), pp.103-108;
10. **Sime, S., Reeve, VE., 2004** - *Protection from inflammation, immunosuppression and carcinogenesis induced by UV radiation in mice by topical Pycnogenol*, Photochem Photobiol, 79, pp.193-198;
11. http://asea.myvoffice.com

Consilier editorial: **Vasile VÎNTU**

Tehnoredactori: **Liliana ROTARU**
Liliana Elena CHELARIU

Corectori: **Lucia DRAGHIA**
Liliana ROTARU

Bun de tipar: **01.07.2014**
Apărut: **2014**
Format: **61x86/16**
Editura: **„Ion Ionescu de la Brad" Iaşi**
Aleea M. Sadoveanu, 3
Tel.: 0232-218300
e-mail: editura@uaiasi.ro

ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

PRINTED IN ROMANIA

Editorial Consultant: **Vasile VÎNTU**

Technical Editors: **Liliana ROTARU**
Liliana Elena CHELARIU

Readers: **Lucia DRAGHIA**
Liliana ROTARU

Imprimatur: **01.07.2014**
Published: **2014**
Format: **61x86/16**
Publishing House: **„Ion Ionescu de la Brad" Iaşi**
Aleea M. Sadoveanu, 3
Tel.: 0232-218300
e-mail: editura@uaiasi.ro

ISSN–L=1454-7376
(Print)-ISSN 1454-7376
(Online)=ISSN 2069-8275
(CD-ROM) = ISSN 2069 – 847X

PRINTED IN ROMANIA