RESULTS REGARDING „*IN VITRO*" MICROPROPAGATION OF JERUSALEM ARTICHOKE (*HELIANTHUS TUBEROSUS* L.)

REZULTATE PRIVIND MICROPROPAGAREA „*IN VITRO*" LA TOPINAMBURUL LEGUMICOL (*HELIANTHUS TUBEROSUS* L.)

DASCĂLU T.[1], GUTIERREZ Patricia[2], MUNTEANU N.[1]
e-mail: dascalu_teofil@yahoo.com

Abstract. *In vitro, clonal micro propagation of the Jerusalem artichoke, (Helianthus tuberosus L.) „Violleto" cultivar was realized by isolating the explants right from the level of the young sprouts, that have been formed on the tuber, after they have been forced to grow kept in dark conditions for three days. The inoculation of the explants was realized on 12 types of MS medium; differentiate among themselves by the concentration of cytokines, gibberellins, auxines, and the presence of active carbon. Measurements and plant determinations have been taken during the experiment, keeping track of the initial and final number of sprouts, the height of the plants as well as some morphological aspects: color and length of leaves, length of the internodes as well as the appearance of the senescent phenomenon. The results have relieved the superiority of the version having P6 medium of culture, which is recommended in the technology of "in vitro" micro propagation.*
Key words: Jerusalem artichoke, culture medium, micro propagation.

Rezumat. *Micropropagarea clonală „in vitro" a topinamburului legumicol, cultivarul „Violleto" (Helianthus tuberosus L.) s-a realizat prin izolarea explantelor de la nivelul lăstarilor tineri, formați pe tuberculi, după ce aceştia au stat o perioadă de trei zile la forțat în condiții de întuneric. Inocularea explantelor s-a realizat pe 12 tipuri de medii de cultură, de tip MS, diferențiate prin concentrația în citokinine, gibereline, auxine și prezența carbonului activ. Pe timpul experimentării au fost efectuate măsurători și determinări asupra plantelor, urmărindu-se numărul de lăstari inițiali și finali, înălțimea plantelor și unele aspecte morfologice: culoarea și lungimea frunzelor, lungimea internodiilor precum și apariția fenomenului de senescență. Rezultatele au pus în evidență superioritatea variantei cu mediul de cultură P6, care se recomandă în tehnologia de micropropagare „in vitro".*
Cuvinte cheie*:* topinambur , mediu de cultură, micropropagarea .

INTRODUCTION

When talking about cultivating plants there is a direct association with growing plants in the field, green house, green pots, etc. and it is divided in different disciplines such as: agriculture, horticulture, tropical agriculture, forestry etc. In 1904, Häning developed a new method for growing plants called embryo culture. He isolated immature embryo „in vitro" and he obtained viable plantlets in the case

[1] University of Agricultural Sciences and Veterinary Medicine, Iași, Romania
[2] Tuscia University ,Viterbo, Italy

of some species of cruciferous plants. Starting with 1920, many types of „in vitro" types became known: planting orchid seeds „*in vitro*", cultivation of callus, cultivation of plant organs etc. After 1945 all these culture methods mentioned above have been grouped under one collective term: plant tissue culture.

It is known that, plants can be bred in two ways: vegetative (asexual, also called cloning) and sexual; (using seeds), but both types of breeding, in some circumstances can be impossible to realize.

If the sexual breeding method doesn't give any results or is impossible to realize, in the case of the species or cultivars which do not form seeds or form too little seeds or in case they form seeds but they are unable to germinate, then vegetative breeding becomes the only solution.

Another case where generative breeding is not satisfactory appears in the case where the number of heterogeneous descendents is very big.

In „*in vivo*" conditions, vegetative breeding (realized with stem cuttings, splitting of the plant, stools, underground organs, grafting etc.) , had a tremendous role for a big period of time for many species and especially for fruit trees, vineyard, ornamental species, leguminous species, species with flowers and many others. Vegetative breeding is also very important in breeding techniques to keep the characters of the genitor line, in the gene banks or to obtain mutant forms and maintain them.

Classic vegetative breeding methods are not sufficient since most of the times they are either too difficult to realize, they take a long time to obtain good planting material, or are absolutely impossible to use for some species.

Since lately, the technique of „in vitro" breeding has become less expensive; trials are made to introduce this technology within commercial labs. At this moment vegetative breeding is possible using „*in vitro*" cultures for some species which have a difficult „*in vivo*" breeding.

There are many methods used for vegetative breeding in „*in vitro*" conditions: „*single node*" method, „*axillary branching*" method, adventives organ regeneration, adventives root forming, adventives stem forming, obtaining callus, somatic embryo-genesis, plant regeneration from a single cell and obtaining artificial seed.

In this paper the results of an experiment which had as aim finding the most suitable culture medium and micro multiplication of the Jerusalem artichoke, are described.

The growing and development „*in vitro*" is determined by a number of four groups of complex factors:

1. The gene pool
2. Chemical compounds: macro and micro elements, carbohydrates, water
3. Factors which affect plant physiology: light, temperature, pH, O_2, CO_2
4. Organic substances: growth regulators, vitamins etc

Explants in „*in vitro*" conditions needs more chemical compounds for growth and development, exemplified in table 1. It can be inferred from the table

that compounds are necessary in „*in vivo*" conditions as well (water, macro and micro-elements).

Organic substances and unidentified mixtures are necessary only in „*in vitro*" conditions, in other words „*in vitro*" plats are heterotrophic.

Table 1

Requirements for *in vitro* cultures for nutritious elements and hormones

Water		
Organic substances	Macro- and Micro-elements	
Carbohydrates Amino acids Vitamins Auxins Cytokinins Gibberellins Abscisic acid Ethylene	N Fe Co P Zn NI K B Al Ca Mn Mo Mg Cu I S	pH
Unidentified organic mixtures	Mold extract Coconut milk Plant extracts Casein Hydrolysate Pepton and trypton	

MATERIAL AND METHOD

In order to achieve our goal we used De Fossard's suggestion (1976) using a basic medium, with a changed concentration of the components, vital for enabling "*in vitro*" growth conditions. The culture medium that has been used, based on macro and micro elements, was Murashige-Skoog (MS) .

The experience was polyfactorial, type 6x3x3x2 where:

- A factor: cytokine's concentration BAP: 0 mg/l, 0,1mg/l, 0,25 mg/l, 0,5mg/l, 1 mg/l
- B factor: gibberellins concentration: GA3: 0 mg/l, 4 mg/l, 5 mg/l
- C factor: auxines concentration: NAA- 0 mg/l, 0,1 mg/l, 0,05 mg/l
- D factor: active carbon presence.

During the experiment we worked with „Violetto" cultivar of Jerusalem artichoke. From the 102 possible mediums, we have finally used 12 culture media (table 2).

Table 2

Culture mediums used

mg/l	P0	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
NAA	0	0,05	0,05	0,05	0,05	0,05	0,1	0,05	0,05	0,05	0,05	0,1	0,1
BAP	0	0,1	0,1	0,25	0,25	0,4	0,4	0,5	0,5	0,5	0,5	1	1
GA3	0	4	5	4	5	4	4	5	5	0	0	0	0
C. A	0	0	0	0	0	0	0	1%	0	1%	0	1%	0

Tubers have been used as biological work material, thus increasing infection risk which made it necessary for the material to be sterile. This is why any remains of dirt or dead tissue have been removed off the tubercles, which have then been washed with clean water in order to remove any possible sources of contamination.

Only after these steps had been followed the sterilization took place. The tubers were immersed in 70% concentration of alcohol liquid for a few seconds in order to eliminate air bubbles and next they were introduced in NaCl 1% solution for 25 minutes. Next, the tubers had been immersed 3 times in distilled water for 2, 5 and respectively 15 minutes in order to remove any traces of hypochlorite off the tubers.

Once the sterilization procedure has been finished it has been proceeded to cut the tubercles into segments (slices) so as every section should contain 2-3 buds. This phase took place in sterile conditions inside the lab provided with laminar air flux.

The isolation of the explants was made at the level of the young sprouts, formed on the tubercle, after they have been forced (in darkness conditions) for a three day period, in the culture chamber, being treated with GA3 and BAP solution.

Observations and plant determinations have been taken during the experiment, keeping track of the initial and final number of sprouts, the height of the plants as well as some morphological aspects: color and length of leaves, length of the internodes as well as the appearance of the senescent phenomenon (premature aging).

RESULTS AND DISCUSSIONS

The results regarding the main determinations regarding the evolution of the biologic material are shown in tables 3 and 4.

Table 3

The distribution of the plants height according to the sublayer of culture for "Violleto" cultivar

Hight	Culture Mediums												
	P0	P1	P2	P3	P4	P5	P6	P7	P8	P9	P10	P11	P12
< 5mm	0	83	78	83	75	50	46	0	46	27	50	0	0
5-10 mm	100	17	22	17	25	50	54	17	54	27	50	28	100
11-20 mm	0	0	0	0	0	0	0	83	0	35	0	17	0
21-40 mm	0	0	0	0	0	0	0	0	0	11	0	48	0
> 40 mm	0	0	0	0	0	0	0	0	0	0	0	7	0

It can be determined that for the Violleto clone the height of the shoots, in „*in vitro*" conditions, varied according to the culture medium from 5 mm up to 40

mm. it can be noticed in fig. 1 that when using P7 culture medium the height of the plants in high proportion varied between 11-20 mm values, having a very good multiplication report (number of final sprouts / number of initial sprouts) of 2,5, this way the conditions necessary for the internodes are being met, conditions necessary to obtaining micro tubers in a further phase of the experiment.

In case there is need for rapid multiplication of the „Violleto" clone the P6 culture medium will be used, having a multiplication report of 4,17, and which provides a reasonable height in order to enable a subculture in optimal conditions.

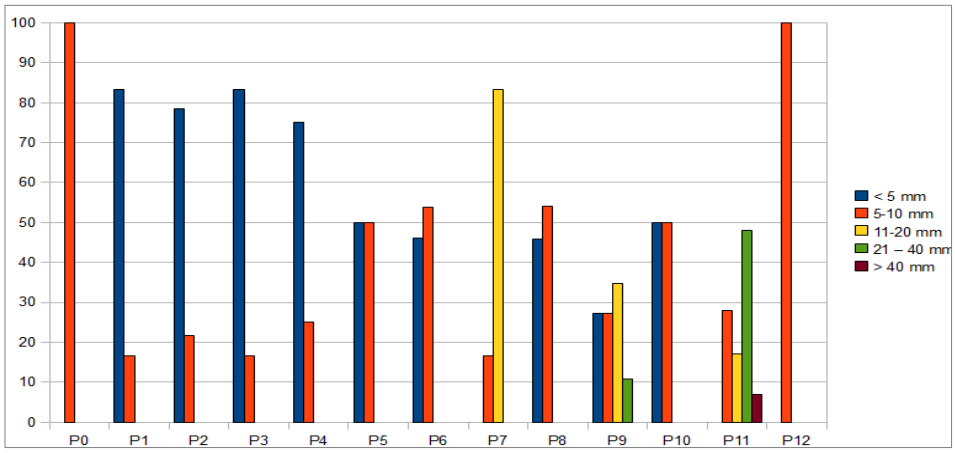

Fig. 1 - Graphical representation for the distribution of plants' height according to the sublayer of culture for "Violleto" cultivar

Table 4

Phenotype characteristics of the plants in "*in vitro*" conditions for the Violleto cultivar

Culture mediums used	Characteristics			
	Leaf color	Leaf size	Internodes size	Senescence
P0	Green/ yellow	close	Short	yes
P1	Yellow / white	Small	Short	yes
P2	Yellow / white	Small	Short	yes
P3	Yellow / white	Small	Short	yes
P4	Yellow / white	Small oval	Short	yes
P5	Light green	Small oval	Short	Yes/ no
P6	Light green	Small oval	Short	yes
P7	Dark green	Medium	Medium	no
P8	Light green	Small	Short	yes
P9	Dark green	Medium long	Long	no
P10	Dark green	Medium oval	Medium	no
P11	Green	Medium	Medium	no
P12	Green	Close	Short	no

CONCLUSIONS

1. Successful micro propagation is possible by using two culture media: one to provide the subculture (multiplication) and the second (which contains active coal) provides growth and height so that the length of the internodes is sufficient for obtaining micro tubercles.

2. Using culture media in „*in vitro*" culture for species *H. tuberosus* L. can lead to the appearance of the senescence phenomenon (premature aging).

3. After the experiment there have been identified culture mediums appropriate for producing internodes segments to be used further on a future experiment for obtaining micro tubers.

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