THE ROLE OF NATURAL EXTRACTS IN THE *IN VITRO* CULTURE OF SOLANUM TUBEROSUM L. VARIETY

Agud Eliza*

**University of Oradea, Faculty of Environmental Protection, 26 Gen. Magheru, St., 410048 Oradea; Romania, e-mail: eliza_agud@yahoo.com*

Abstract

The apex taken from the Solanum tuberosum L variety, Desirée and Ostara, after the disinfection have been cultivated on a Mrashige - Skoog basic medium, where we added maize and pumpkin extract in concentration of 3 and 5g/l. The experiment has included the following variants of medium: V_o = NS1/2; V_1 = 1mg/l BA+0,5mg/lAIA; V_2=MS+3g/lE_p(extract of maize germs); V_3=MS+5g/lE_p; V_4=MS+3g/lE_d (extract of pumpkin germs); V_5=MS+5g/lE_d. After about 50-55 days of in vitro culture, the apexes cultivated on the extract mediums (V_2 – V_5) have shown a superior reaction. On the mediums with bigger concentration from both extracts (V_3, V_5), the percentage of regeneration at the Desirée variety reaches at 100 – 98%, and the one of tuberization about 66%. At Ostara variety we have also seen a good evolution, which was inferior to the Desirée variety, 85-90% regeneration and about 60% tuberization. We recommend the replacement of growth hormones from the culture medium (which are expensive and sometimes difficult to find), with natural extracts from maize and pumpkin germs, in concentration of 5g/l. Introduced in the MS culture medium, the natural extracts produce a superior percentage of regeneration and tuberization and diminish the time of evolution. We also recommend taking into the consideration the type of explant, of variety and the time of year when the experiment is made. In the case of the potato varieties, they both have a good reaction in vitro, and the time of the year when the experiments have been initiated was at the beginning of March. We believe that the superior results are due to the composition of the explants, but also to the fact that they have been prepared and used on the same day.

Key words: in vitro, apex, regeneration, Solanum tuberosum L, Desirée, Ostara varieties, phitohormones.

INTRODUCTION

It is well known the interest for knowing the reaction of plants and tissues detached from them, in the presence different natural extracts (*Butiuc,A., Zăpârţan, M.,* 1996) in the culture substratum (either soil or aseptic medium), either for replacing some hormonal addition from the medium (*Zăpârţan, M.,* 2001), either for reducing the time of in vitro culture and the superior organogenetic effects. The effect of those extracts in the stimulation of the caulogenesis processes is due to the supplement of hormones, of natural vitamins, of essential amino acids, and to the presence of some chemical elements, and complex organometric (Butius-Keul., A., Deliu, C., 2000), but also to some bioactive compounds easily assimilated by the plants' cell.

Some researchers say that the vegetal extracts from some fruits, vegetables and plants, contain in their composition complex substances, little or unknown at all. The most common tested are the extracts of yeast,

citric, tomato, maize germs, hydrolyzed casein, coco nut milk, etc. (*Cachiță, C.D.*, 1987). Cachiță believes that at the in vitro culture of explants, the ideal concentration of the extract is of 0,1 -1,0 g/l. – which is generally a small concentration. The author continues by suggesting that the dose can be increased according to the variety, the type of tissue cultivated in vitro and according to the purpose that we desire to reach (organogenesis, callus initiation, bulbs initiation, tuber initiation, plant regeneration from callus, etc.). This way, starting from this idea, a multitude of in vitro varieties have been experimented, and many comparative studies concerning the effect of the growth hormones over the variety and the replacement of those with different types of extracts (Zăpârțan, M., 1996c), about the replacement of hormones with natural extracts, have been written.

The results obtained in the experiments with natural extracts introduced in the mediums of in vitro culture depend on the nature of the extract, on the variety and on the type of tissue cultivated in vitro, but also on the period of the year when the experiment is initiated. We recall the effect of the extract from the maize germs in the rapid multiplication at *Dianthus spiculifolius* (*Zăpârțan, M.* 1995b) and *Leonthopodium alpinum* (*Zăpârțan, M.*, 1996a; *Butiuc, L, and Zăpârțan M*, 1996) varieties. For the regeneration of plants from callusar tissue, the coco nut milk has proved its efficiency at *Scilla indica* variety, in concentration of 15% (*Chakravarty B., Sen. S.*, 1989). The yeast extract in concentration of 0,5 g/l produces the formation of callus at *Lilium longiflorum* (*Yamagishi*, M.,1995), callus produced from floral organs (stamen filaments). The purpose of the present study is that of using the natural extracts in culture mediums of some varieties of *Solanum tuberosum* L. The variety was analyzed under the aspect of in vitro behavior of some genotypes in the presence of bioactive nanocomponents in the medium (*Baciu, A.*, 2008), under the aspect of the implications of some hormones in the tuberization process (*Agud, E* et all., 2008), and also under the aspect of the effect of the photoperiod and of the concentration of sucrose in the regeneration of some potato varieties *in vitro* (*Agud, E.*, et all. 2009).

MATERIAL AND METHODS

The vegetal material was composed of apexes detached from the *Desirèe* and *Ostara* varieties, and cultivated on the variants specified in Table 1; some of the mediums with a composition of maize germs extract (E_p) and some with pumpkin extract (E_d). In the last experiments those varieties have proved a good capacity of in vitro regeneration and tuberization, but in a long term (*Agud, E.*, et all, 2009), and in vitro tuberization has proved successful at Desirèe variety on mediums with a height concentration of cytokinine (*Butiuc A.*, et al. 1996). Watching Table

1 we can see that the basic medium was Murashige-Skoog, 1962, used single, but with the macro elements, the microelements, FeEDTA, the vitamins and the sucrose at half a dose (V_o), then in order to have the comparison term, we have used MS with a cytokinine and an auxine (V_1). The other variants have contained only E_p 3mg/l (V_2) and 5mg/l (V_3), or E_d, 3mg/l (V_4) and 5mg/l (V_5). The observations have been made after less than 2 months of *in vitro* culture.

Table 1

Var.	**Basic medium**	**BA (mg/l)**	**AIA (mg/l)**	**Maize Extract E_p (g/l)**	**Pumpkin Extract E_d (g/l)**
V_o	MS1/2	-	-	-	-
V_1	MS	1	0.5	-	-
V_2	MS	-	-	3	-
V_3	MS	-	-	5	-
V_4	MS	-	-	-	3
V_5	MS	-	-	-	5

Table 1. The medium variants with natural extracts (MS = after Murashige- Skoog 1962)

RESULTS AND DISCUSSION

After about 50 – 60 days we have discovered a good regeneration and also a remarkable percentage of tuberization. Table 2 includes the in vitro evolution of the tissue of the two varieties and the observations concerning the behavior of the apex on mediums with natural extracts, in comparison with the witness medium (V_o) and with the hormones (V_1). The results are in addiction with the extract concentration, with the type of explant, with the variety and with the time of the year when the inoculation of the explant took place. The present experiment has been initiated at the beginning if March, in the period of the maximum faze of the Moon in growing, the effect of the Moon fazes being well known for the success of some biologic processes from plant.

At *Desirée* variety, on V_o the tuberization time was of about 70 days, and the percentage of regeneration of about 25% (only a quarter of the apexes). On V_1, the time of regeneration and tuberization is of about 60 days, the percentage of regeneration of 80% and the tuberization at about 40 – 45%. On the mediums with maize extract (V_2 and V_3), the evolution time is of 50-55 days, with 50% tuberization on medium with 3 g/l E_p, and 66% tuberization on medium 5g/l E_p, proving the best evolution of this variety on those variants. The phenomenon is well presented in fig. 1 and 2 concerning the regenerative and tuberization capacity of the Desirée variety, on

mediums with natural extracts, in comparison with the evolution on the witness medium with growth hormones.

Table 2

The variety	Var.	Regene.%	Tuberiz.%	Observations	Evaluation
Desirée	V_o	25	5	- the time for tuberization was of about 70 days and in small percentage, about 25% have regenerated, a quarter from the apexes	xx
	V_1	80	45	- the time regeneration and tuberization is of about 60 days	xxxx
	V_2	87	55	- the regeneration and the tuberization take place in about 50-55 days, with tuberization at over 50% of the apexes	xxxx
	V_3	**100**	**66**	- the time is the same, of 50-55 days, but the percentage of tuberization is of 66%. **The best variant, on maximum concentration of extract**	**xxxxx**
	V_4	60	52	- the time of tuberization is the same, of 50-55 days, with over 50%, inferior to the maize extract	xxxx
	V_5	**95**	**60**	- superior to V_4, **the concentration of pumpkin extract, of 5 g/l, is the best,** but easy under the variant on maize	**xxxxx**
Ostara	V_o	15	-	- the evolution of this variety is **inferior to the Desirèe variety,** it does not root	x
	V_1	72	31	- tuberization in 55 days, with about 31% tuberization	xxx
	V_2	77	45	- it roots less then half of the apexes, the evolution is good	xxxx
	V_3	**90**	**55**	- the maize extract, in concentration of 5g/l has proved to be **the best, with over 60% tuberization at Ostara variety**	**xxxxx**
	V_4	72	40	- the 3g/l concentration of pumpkin extract ensures 40% tuberization in the same period of time, 55 days	xxxx
	V_5	**85**	**45**	- the pumpkin extract, in concentration of 5g/l has proved to have the best evolution	**xxxxx**

Table 2. The results obtained at the culture of the potato apex on mediums with natural extracts (after less than 2 months)

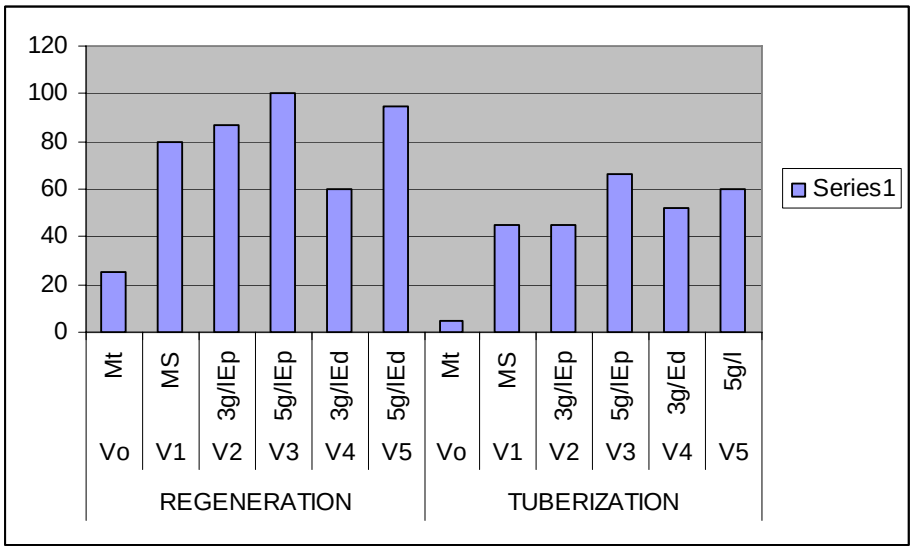

Fig. 1. The evolution of the Desirée potato variety on mediums $V_o - V_5$

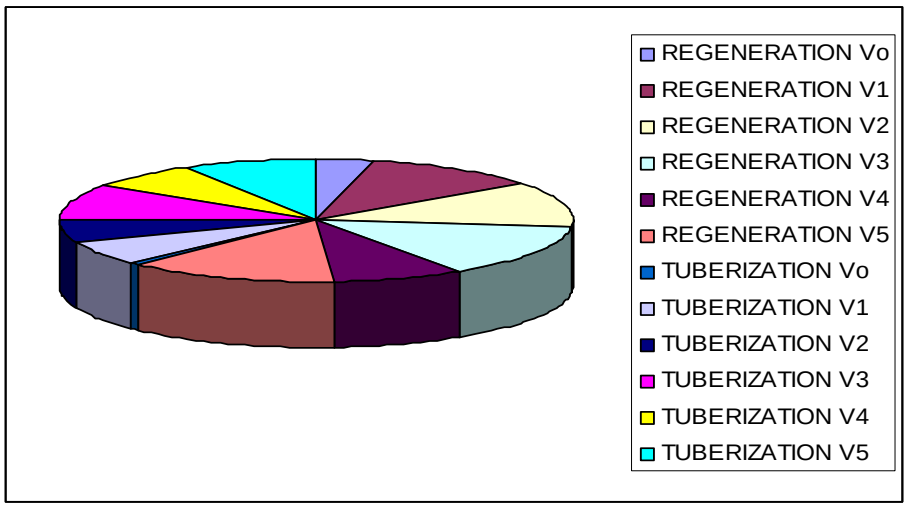

Fig. 2. The regenerative and tuberization capacity of Desirée variety on mediums with natural extracts

Ostara potato variety, cultivated *in vitro* presents a good evolution, which is inferior to Desirée variety, on the control variants and also on the ones with natural extracts. In this case also, the maize extract, in concentration of 5g/l, has given the best results, about 90% regeneration and 60% from the explants tuberization, the pumpkin extract in the same concentration gives good results (V_5), but easily inferior to the ones on the maize extract. Figures 3 and 4 show the evolution of the potato apex of Ostara variety on mediums with natural extracts, in comparison with the mediums without hormones or supplement of hormones.

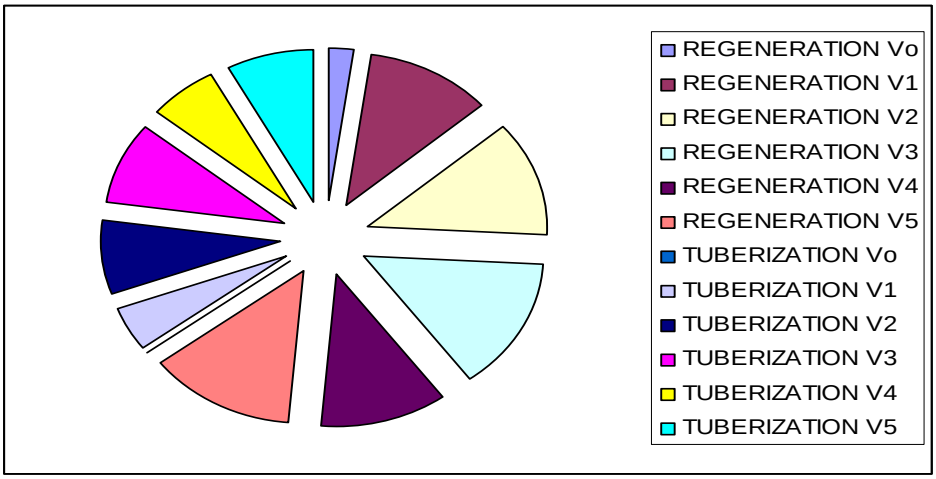

Fig. 3 The regenerative and tuberization capacity of Ostara variety on mediums with natural extracts

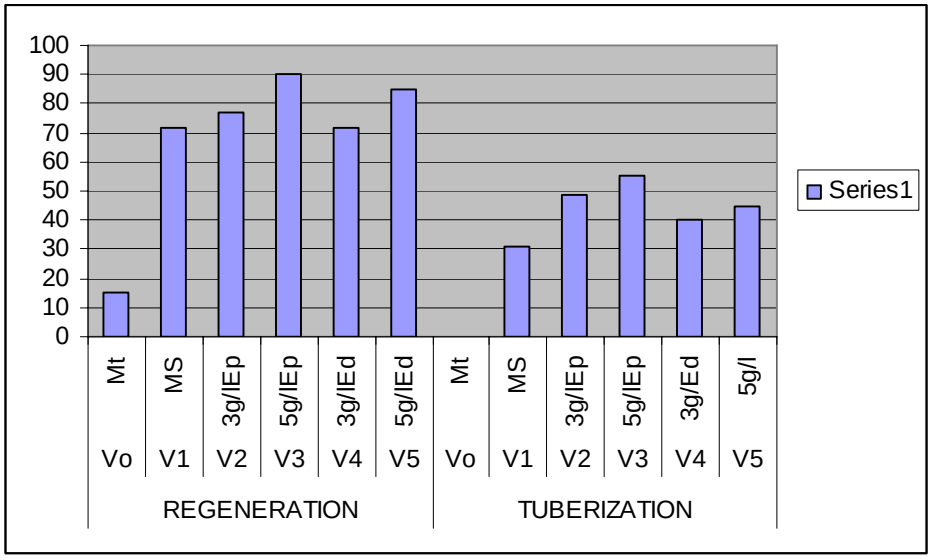

Fig. 4. The evolution of Ostara potato variety on V_o – V_5 mediums

We recommend the replacement of the growth hormones from the culture medium, which are expensive and difficult to obtain, with natural extracts from maize germs or pumpkin extracts, in concentration of 5g/l, introduced in MS medium, this way ensuring a superior percentage of regeneration and tuberization and reducing the period of evolution. We also recommend taking into consideration the type of explant, the variety and the period of the year when the experiment is being initiated.

CONCLUSIONS

1. *Desirée* potato variety has encountered the highest percentage of regeneration and tuberization on V_3 medium (MS+ sg/l E_p);
2. The pumpkin extract, at Desirée potato variety, in concentration of 5g/l, also ensures a good percentage of regeneration of about 100% and of tuberization of 66% and even over it;
3. Desirée is a potato variety which presents the best behavior on mediums with natural extracts, in concentration of 5g/l on mediums with maize extract and also on the ones with pumpkin extract;
4. The behavior at *Ostara* potato variety is good, but inferior to the one of Desirée variety, the regeneration and tuberization period being of 50-55 days also;
5. Ostara also gives the best results on medium with 5g/l E_p, tuberization about 60% from the explants, on (V_o) witness the tuberization does not take place;
6. On the extract of pumpkin germs (E_d) the explants' evolution is good, but it is easy inferior to Desirée variety, the regeneration is over 80% and the tuberization is of about 50%;
7. We recommend the replacement in the culture medium of the growth hormones with natural extracts, this way ensuring an economic in vitro culture and a superior evolution concerning the regeneration and the tuberization of the potato varieties.

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