

## FACTORS WITH DIFFERENTIATED IMPLICATION IN THE IN VITRO MINITUBERIZATION AT SOME POTATO VARIETIES (*Solanum tuberosum* L)

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### Abstract

The factors involved in the *in vitro* culture of the *Solanum tuberosum* L variety analyzed in this study are: potato variety, the nature of the explant, the season in which the experiment was initiated, the photoperiod, the dose of additional sugar and the hormone balance. On the Murashige-Skoog-1972(MS) basal medium there were cultivated four potato varieties with good and medium productive value, foreign (*Désirée* and *Ostara*) and native (*Super* and *Mureșan*), using plants that were formed from the apex, meristem (of 2-4mm) and nodus.

The experiment was conducted at different periods of the year (the 15<sup>th</sup> of March, the 28<sup>th</sup> of June, the 25<sup>th</sup> of September and the 8<sup>th</sup> of January) on MS medium with the following variants: without hormones ( $C_0=MS$ ) and with a content of benzyl adenine  $C_1, C_2$  and  $C_3$  (with 1, 2 and 3mg/l+0,5ANA) and of zeatin  $C_4, C_5$  and  $C_6$  (in the same composition and concentration). The applied photoperiod was of 16, and 10 hours light of 24 hours, and the dose of supplemented sucrose of 6, 8 and 10g/l. After three months of *in vitro* culture and two series of experiments (2 years) there were analyzed the regenerative capacity of the tissues and the *in vitro* differentiation of the tubers. If at the witness sample ( $C_0$ ) the regeneration percentage is low and the rooting does not take place, at the variants with a moderate dose of cytokinin (2mg/l BA and Z), the regeneration and the rooting exceed 55%, and even 67%. The highest percentage of tubers is obtained in the presence of the zeatin (2mg/l) from the potato apex, in spring (the 15<sup>th</sup> of March), a different percentage, according to the variety (*Désirée* – 55%, *Ostara*– 67%, *Super* – 18% and *Mureșan* – 19-20%). The apex of the *Désirée* and *Ostara* varieties is rooting in the highest percentage on a medium with 2mg/l zeatin (Z), maximum photoperiod and a surplus of 10g/l sucrose; the other two varieties, *Super* and *Mureșan*, have a lower rooting percentage in the same culture conditions.

We conclude that the dose of 2mg/l zeatin (Z) and benzyl adenine (BA) has the best effect in rooting, and among the explants, the apex has proved the highest regeneration capacity, at normal photoperiod and a surplus of 10g/l sucrose, in the conditions in which the experiment was initiated in the spring (the 15<sup>th</sup> of March). Being a complex experiment, it proves the exact behavior *in vitro* of the type of explant, the best variety, the most advantageous hormone balance, the dose of sucrose with a stimulatory effect and the favorable photoperiod.

**Key words:** the nature of the explant (apex, meristem, nodus), mini-rooting, *Solanum tuberosum* L, varieties (*Désirée*, *Ostara*, *Mureșan* and *Super*), photoperiod, season, hormone balance.

### INTRODUCTION

Our long time concerns related to the *in vitro* behavior of some varieties of the *Solanum tuberosum* L specie (either foreign or native), lie in the importance of this specie for human nutrition, an aspect for which some improvement problems, the induction of mutations in potato and of *in vitro* reaction of some varieties has represented the theme of a PhD thesis (Agud, 2008).

The present study follows the reaction of the *in vitro* culture of some foreign potato varieties (*Désirée* and *Ostara*) compared with the native ones (*Super* and *Mureșan*), establishing the best variety, the balanced hormonal balance, the type of explant, the dose of sucrose and the ideal photoperiod, in order to ensure the *in vitro* rooting of these varieties. Previous research revealed the possibility of *in vitro* potato rooting provided that stakeholders are balanced (Patru, Cachita., 2005). Throughout the experiments undertaken by us (Refs. 1 – 8) the effect of some factors on the potato cultivated *in vitro* could be seen, but the detailed establishment of the *in vitro* rooting protocol related to the involvement of the factors responsible of this phenomenon, was not followed up until now. The response of the potato varieties in the presence of some phytohormones (their nature and concentration) has been watched in order to ensure the *in vitro* rooting (Agud et al., 2008, 2009; Butiuc- Keul et al.,1997), and also the effect of some additional, of the dose of sucrose, correlated with a certain photoperiod (Agud et al., 2010), the evolution of different explants (Agud, 2011), their reaction according to the variety and to the culture medium.

The phenomenon of *in vitro* mini-rooting was followed not only at the *Solanum tuberosum* L specie, but also at some bulb flower species<sup>21</sup>. Potato apex proved good *in vitro* regeneration capacity at some other plant species too (herbs, ornamental plants, etc.) (Zăpârțan et al.,1991; Zăpârțan, 1992 ), and using a balanced hormonal balance proved its efficiency at the *in vitro* conservation of some rare, endemic and vulnerable plants within the country's flora. Some studies have established another type of *in vitro* behavior of the potato genotypes (Laslo et al., 2011), different according to the variety, nature and concentration of growth substances (Murashige and Skoog 1962,). Noteworthy are the attempts to replace sucrose from the medium with other types of sugars (fructose, honey) with good results, but still unapplied on a large scale in the plant biotechnologies. Due to the economical value of potato, our research was often directed towards the establishment of some technologies of advantageous and economical multiplication, using small doses of phytohormones, (Laslo et al., 2011) culture mediums without hormones, or with some additional natural extracts on the mediums (Cachita-Cosma, Zapartan., 1991).

## **MATERIAL AND METHOD**

There were tested four potato varieties (*Désirée*, *Ostara*, *Super* and *Mureșan*) with the approximately the same productive value, from which three types of explants were detached, meristem (M), apex (A) and nodus (N) which were cultivated on a basal medium according to Murashige-Skoog, 1962, conceiving six medium variants plus the witness sample

(C<sub>0</sub>=MS) and the C<sub>1</sub>– C<sub>6</sub> variants with three doses of sucrose (6, 8 and 10g/l), photoperiodic regime of 16 and respectively 10 hours light of 24 hours and the hormonal balance (citokinin and auxine) presented in table 1. The hormonal balance points out the using of two citokinines (BA=benzilaminopurine and Z=zeatin) in three doses (1, 2 and 3mg/l) and a single auxine (ANA= naphthyl acetic acid) in a single concentration (0,5 mg/l), with the presented dose of sucrose and photoperiod (table 1).

Table 1

The composition of the *in vitro* culture mediums and the treatment applied to the potato varieties

Var.	Basal medium	Citokinine (mg/l)		Auxine (mg/l)	Photoperiod (hours) light/dark)	Sugar dose (g/l)
		BA	Z	ANA		
C <sub>0</sub>	MS	-	-	-	16/24; 10/24	6; 8; 10.
C <sub>1</sub>	MS	1	-	0,5	16/24; 10/24;	6; 8; 10.
C <sub>2</sub>	MS	2	-	0,5	16/24; 10/24;	6; 8; 10.
C <sub>3</sub>	MS	3	-	0,5	16/24; 10/24;	6; 8; 10.
C <sub>4</sub>	MS	-	1	0,5	16/24; 10/24;	6; 8; 10.
C <sub>5</sub>	MS	-	2	0,5	16/24; 10/24;	6; 8; 10.
C <sub>6</sub>	MS	-	3	0,5	16/24; 10/24;	6; 8; 10.

(MS = Murashige-Skoog 1972; BA =benzyl adenine; Z=zeatin; ANA=  $\alpha$  – naphthyl acetic acid; photoperiod: 8 hours light out of 24; initially 6 days of darkness)

The experiments were initiated within two consecutive years, in two year periods, in March and in June. The value differences of the parameters watched within the two years (*in vitro* regeneration and rooting of the potato varieties), within the same periods and culture conditions, being insignificant, has resorted in calculating the average of the parameters of the best year, percentages presented in tables 2 and 3.

## RESULTS AND DISCUSSION

The explants inoculated on the presented variants were analyzed concerning the **percentage of the *in vitro* regeneration**, after 40 days of culture, following the general aspect of the potato neoplantlets and their organization (the number of plantlets and the form of the root system). After 80 days from the incubation of the explants, their *in vitro* mini-rooting was watched (the percentage of explants on variant which induced the formation of tubers), according to the variety, the nature of the explant, the hormonal balance and the photoperiod. The regeneration percentage of the potato varieties on the experimented mediums (after 40 days) is presented in table 2.

Table 2 includes percentage values concerning *in vitro* regeneration at *Désirée* and *Ostara* foreign varieties, on the seven variants. Among these varieties, *Désirée* proved to have the best reaction at the *in vitro* culture,

under all aspects. On the variants with moderate dose of cytokinine (2mg/l BA and Z) and 10g/l sucrose, the regeneration percentage was even of 100% in the incubation (initiation), month of the culture – in March.

Table 2

Regeneration percentage of the potato explants according to the variety, the hormonal balance and season (March), after 40 days

Var.	Désirée(%)			Ostara(%)			Super(%)			Mureşan(%)			Bonus (March)
	M.	A.	N.	M.	A.	N.	M.	A.	N.	M.	A.	N.	
C <sub>0</sub>	8	14	7	8	10	8	8	12	8	5	8	4	x
C <sub>1</sub>	50	70	52	30	60	33	48	62	42	40	50	40	xxxx
C <sub>2</sub>	75	100	76	45	80	54	55	90	57	40	65	42	xxxxxx
C <sub>3</sub>	32	50	30	15	30	15	20	35	20	14	18	11	xxx
C <sub>4</sub>	40	70	50	40	30	30	30	55	28	16	22	17	xxxx
C <sub>5</sub>	82	100	79	50	81	48	60	90	55	30	60	25	xxxxxx
C <sub>6</sub>	28	50	30	20	32	28	17	45	18	14	20	13	xxx

(M=meristem; A=apex; N=nodus)

Generally this variety responds positively to all variants, but the regeneration percentage is smaller on some variants (about 70% on the mediums with 1 mg/l BA and Z(C<sub>4</sub>), and 50% at the concentration of 3 mg/l BA and Z(C<sub>6</sub>). It seems that the high dose of cytokinine produces a slight inhibition or delay of the *in vitro* regeneration. The differentiated plants are completely conformed as number of sprouts and roots. The regeneration percentage of the apex detached from *Ostara* variety is good, but inferior to *Désirée* variety, of about 80% on C<sub>2</sub> and C<sub>5</sub> (see figure 1 – after 40 days). On the variant without hormones (C<sub>0</sub>) the apex of those potato varieties regenerated *in vitro*, but in a lower percentage, between 10 – 14%.

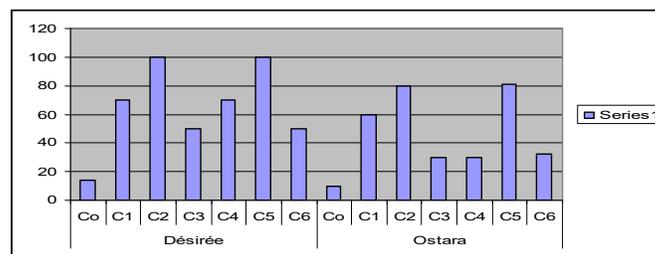


Fig. 1 Apex regeneration of *Désirée* and *Ostara* potato varieties after 40 days of *in vitro* culture (experiment in March)

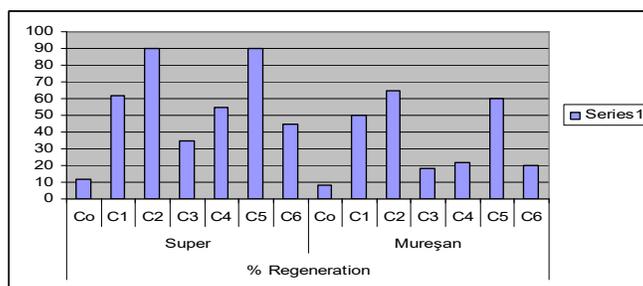


Fig. 2 Apex regeneration of the *Super* and *Mureșan* native potato varieties after 40 days of *in vitro* culture

Among the native varieties (improved at the Stupini Research Station – Brasov) *Super* variety has a regeneration capacity superior (about 80%) to *Mureșan* variety (60%), in the same culture conditions (on C<sub>2</sub> and C<sub>5</sub> mediums). Figure 2 presents the regeneration percentage, the evolution of the two Romanian varieties being similar with the one of the foreign ones, slightly lower, but good. The witness evidence (C<sub>0</sub>) with MB (basal medium) only determined the apex regeneration in a low percentage on the mediums with a medium dose of cytokinin (2 mg/l), between 8 – 12%.

Table 3.

Mini-rooting capacity (%) of the potato explants according to the variety, to the season and to the hormonal balance, after 80 days

Var.	Désirée				Ostara				Super				Mureșan				Season (March, June)	
	M.	A.	N.	Sugar (g/l)	M.	A.	N.	Sugar (g/l)	M.	A.	N.	Sugar (g/l)	M.	A.	N.	Sugar (g/l)		
C <sub>0</sub>	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	March	
C <sub>1</sub>	5	14	3	10	1	4	2	10	3	10	2	10	1	3	1	10	March	
C <sub>2</sub>	21	<b>45</b>	8	10	4	<b>15</b>	3	10	10	<b>30</b>	9	10	1	<b>10</b>	2	10	March	
C <sub>3</sub>	4	18	3	10	2	9	2	10	3	10	3	10	-	8	-	10	March	
C <sub>4</sub>	8	20	5	10	2	10	3	10	2	12	2	10	3	4	3	10	March	
C <sub>5</sub>	29	<b>50</b>	18	10	12	<b>25</b>	10	10	10	<b>40</b>	12	10	3	<b>12</b>	2	10	March	
C <sub>6</sub>	-	14	8	10	-	11	7	10	-	12	3	10	-	8	-	10	March	

(M=meristem; A= apex; N=nodus)

The percentage of *in vitro* rooting, an essential aspect followed at the four potato varieties is presented comparatively in table 3. After 80 days of *in vitro* culture the explants differentiated mini-tubers with the diameter of about 2-3 mm, the percentage values depending on the hormonal balance from the medium. Potato apex proved also the highest rooting capacity on the mediums with a medium dose of cytokinin (2mg/l BA and Z) and admixture of 10g/l sucrose, culture maintained at a photoperiod of 16 hours light/24 hours and initiated in the spring (in March). Figure 3 presents the percentage of mini-tubers obtained in these conditions at the foreign potato varieties, 45% at *Désirée* on C<sub>2</sub> variant (MS+2mg/lBA+0,5mg/l ANA) and

50% on C<sub>5</sub> (MS+2mg/lZ+0,5mg/l ANA), it seems that zeatin has a better effect on rooting.

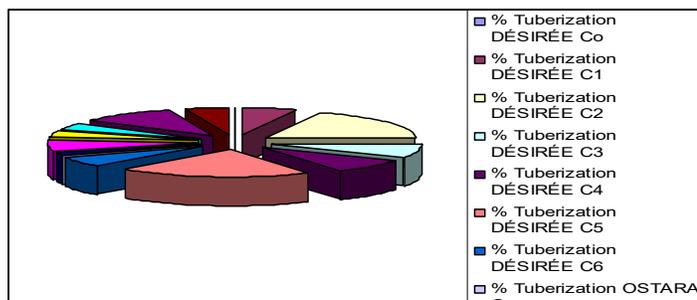


Fig. 3 Number of mini-tubers (%) formed from the potato apex at *Désirée* and *Ostara* varieties after 80 days of *in vitro* culture

Native potato varieties also form tubers after 80 days of *in vitro* culture in the spring season, but in a lower percentage. *Super* variety is rooting in the highest percentage, meaning 35% on C<sub>2</sub> variant and 40% on C<sub>5</sub>, and *Mureşan* variety only about 14 – 15%, an evolution presented in figure 4 for all the variants. The presence of zeatin in the medium (C<sub>5</sub> variant) in this case also stimulates the formation of a great number of *in vitro* tubers.

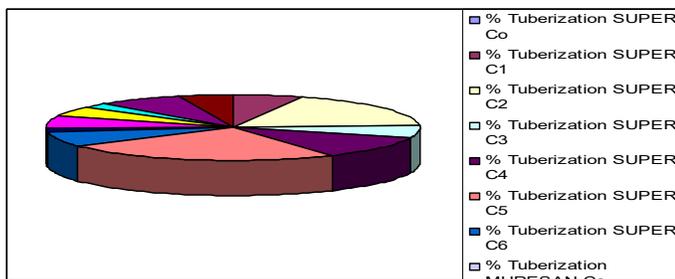


Fig. 4 *In vitro* rooting of the native potato varieties (*Super* and *Mureşan*) from apex, after 80 days of *in vitro* culture

Following figures 3 and 4 we see that on the witness evidence (C<sub>0</sub>), without hormones, but in the same conditions of culture, no explant differentiated any mini-tubers. The high dose of cytokinin from the medium, 3mg/l (C<sub>3</sub> and C<sub>6</sub> variants), leads to the formation of a low percentage of *in vitro* tubers of 14-11% at *Désirée* and *Ostara*, and of 12-8% at *Super* and *Mureşan* native varieties, so they present a slight inhibition reaction. Also, the rooting percentage is influenced by the period of the year in which the initiation of the *in vitro* culture is made, the spring season having a positive influence in comparison with the summer one.

The mini-tubers differentiated *in vitro* were perfectly acclimatized at the *ex vitro* culture, without essential loss, acclimatization capacity depending on their size (the greater their diameter, the more successful the acclimatization), this is why the process of taking the tubers out of the bottles was performed after about three months (Photo 1). The mini-tubers were formed on all variants containing cytokinin, with differences according to the nature of this phytohormone. Zeatin proved to stimulate the formation of the greatest number of mini-tubers/explant, Photo 1 presents the aspect of the mini-tubers obtained *in vitro* after the acclimatization.

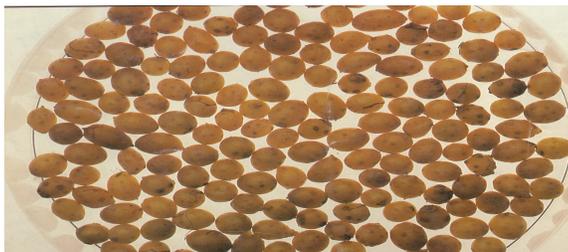


Photo. 1. Mini-tubers obtained *in vitro* from potato apex (*Super* variety) on Murashige - Skoog medium, with addition of phytohormone

## CONCLUSIONS

Potato apex manifests a good and very good *in vitro* regenerative capacity, up to 100% at *Désirée* variety, and about 90% at *Super* Romanian variety, on the mediums with a moderate dose of cytokinin (BA and Z 2mg/l), in a culture initiated in spring (in March), with 10g/l additional sucrose, and a photoperiod of 16 hours light. On the control medium ( $C_0$ ), the regeneration percentage of the potato apex takes place, but with an inferior percentage, of only 8-14%. The other experimented tissues (the meristem and the nodus) have a good *in vitro* regeneration, but in a percentage which is inferior to the apex, and only in the spring season (in March).

*In vitro* rooting capacity of the potato apex is determined in a moderate dose by phytohormones ( $C_2$  and  $C_5$  variants), reaching up to 45-50% at *Désirée*, and respectively up to 30 % at *Ostara* (with 10g/l additional sucrose and a photoperiod of 16 hours light). In the same conditions of culture, the apex detached from the Romanian potato varieties, manifest a rooting percentage of 35-41% at *Super* variety, and of 14-15% at *Mureșan* variety, so these ones could successfully replace the foreign varieties. On the control medium ( $C_0$ ), rooting was not detected.

For the varieties improved within the country it is important to see their *in vitro* rooting capacity in order to obtain a properly seedling material and also for the establishment of the value of those native varieties in comparison with the foreign ones, and finally, their extension in culture.

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