THE ASPECTS CONCERNING IN VITRO TUBERRING AT THE POTATOE VARIETIES

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Abstract

The study comprises a protocol concerning the in vitro neoformation of potatoe minitubers, starting from the apical tissue (apex) detached from the sprouts obtained by forcing the mature potatoe tubers (Solanum tuberosum L). A number of varieties have been experimented: Désirée, Ostara, Eba, Cristian, Muresan, Super and Caty. The percentage of in vitro rooting at some of these varieties has been of about 45%, after two months and of over 85% after three months from the culture on mediums with phitohormones. The results depend on the variety and on the nature of phitohormones. The presence of the zeatin and of the benziladenine has proved efficient, and the dose of 0.5 mg/l AIB favourable for the rooting at all varieties, stimulating the diameter of the minitubers formed in vitro. On the witness variants (V_0,V_1) the rooting is sporadic reaching at some explants at only 10%.

Key words: *in vitro*, apex, rooting*, Solanum tuberosum L,* variety

INTRODUCTION

We can see that the supplements in aseptic medium, for example different doses of sucrose (*Butiuc-Keul* et al, 1997-1998)), the treatment with different photoperiod (*Agud et* al 2009) or with nanocomposed bioactive magnetofluids (*Baciu*, 2008), in most cases have favourable effects, especially on some varieties which present a negative reaction or even are reticent at the clasic medium experimented for the *in vitro* regeneration. The role of phitohormones (*Agud* et al. 2008), the capacity of *in vitro* regeneration of the potatoe meristem (*Agud* et al 2009) have been detailed analised with results over the rooting according to the hormonal balance, the dose of cytokinine, its nature and the period the culture was initiated (*Agud*, 2009).

The purpose of the present study is the following of the effects of the hormonal balance over the in vitro rooting at a number of 7 potatoe varieties, by adding a balanced dose of cytokinine, combined with a small concentration of auxine. The *Solanum tuberosum* L variety has been long studied regarding the aspect of the cytokinines' implications in the differenciation of *in vitro* minitubers (*Butiuc* et al., 1996), regarding the effect of the culture medium in duble layer (agar and liquid), with remarcable results concerning the rooting (*Cachiţă – Cosma* and Zăpârţan, 1991). Some supplements such as the coumarin and the cicolcel (CCC) have had effect over the *in vitro* neoformation of potatoe neoplantlets, *in vitro* minirooting being stimulated in a smaller percentage (Zăpârţan, 1992), than in the presence of moderate doses of zeatine and benziladenine (Zăpârţan, 1992). These studies invocqued have followed the rooting phenomenon only at one or at least two varieties, in exchange our experiment is an extensive testing of the in vitro rooting capacity at seven varieties, of economic and benefic interest, using different hormonal balances.

Of real interest are also the studies concerning the stimulation of the in vitro minibulbs formation at some species from the family. The Liliaceae, which have presented an exceptional reaction according to the nature of the cytokinine and of the great dose of

the concentration, about 2 – 3mg/l (Zăpârțan et al 1999-2000), which has determined us to test a greater number of cytokinine and to study the potatoe varieties which present interest for us, estabilishing this way a protocol, a technology for obtainng in vitro the plant material (minitubers).

MATERIAL AND METHODS

The capacity of *in vitro* formation of minitubers, at *Solanum tuberosum L* variety: *Desirée*, *Ostara, Eba, Muresan, Super and Caty* varieties, (the first three frequently met in culture, and the followings improved at the Stupini Station for the Study of the potatoe - Braşov), has been followed on a few variants of medium, with contents of cytokinine (BA, Z, K, 2iP) in dose of 1 mg/l and auxine (ANA and AIB) in small concentration of 0.5 mg/l. The explant was formed of *apex*, detached from the sprouts obtained from the mature potatoes of the presented varieties, and forced (at temperature of over 22^{o}C and dark about 10 days). From the sprouts we have detached apexes of about 0.5 cm, which were cultivated on the basal medium according to *Murashige – Skoog* (MS)- 1972, with the following composition: macroelements, microelements and FeEDTA – MS; mezoinozitol – 100mg/l; tiamine HCl, pyridoxine HCl and nicotinic acid 1 mg/l; sucrose – 30 g/l; agar – 7 g/l; pH = 6.1. At this medium considered basal medium (MS) we have added the phitohormones combinations specified in table 1, the four cytokinines in the same dose, balanced (1,0 mg/l), and the auxines in small dose (0.5 mg/l). For comparison there were used two variants considered witness variants, formed only of the basic medium + 3 mg/l charcoal (V_o) and one with the basal components, halved macro and microelements (V_1).

After the detaching and desinfecting the tissue, the apexes were passed on the ten mediums (see table 1), and were kept at the temperature from the growth chambre. The majority of experimental varieties was the subject of analyse of a theses of doctorat with remarcable results (Agud, 2010). The varieties were followed under two aspects: the capacity of *in vitro* regeneration, the percentage of regeneration and the percentage of rooting; and the number of neoformed tubers per explant.

Table 1

The composition of aseptic mediums for the rooting of the potatoe varieties

Var.	MB	BA mg/l	2iP mg/l	K mg/l	Z mg/l	ANA mg/l	AIB mg/l	charcoal
V_o	MSC	-	-		-	-		3 g/l
V_1	MS1/2	-	-		-	-		-
V_2	MS	1.0	-		-	0.5		-
V_3	MS	1.0	-		-	-	0.5	-
V_4	MS	-	1.0		-	0.5		-
V_5	MS	-	1.0		-	-	0.5	-
V_6	MS	-	-	1.0	-	0.5	-	-
V_7	MS	-	-	1.0	-	-	0.5	-
V_8	MS	-	-	-	1.0	0.5		-
V_9	MS	-	-	-	1.0	-	0.5	-

(MB=basal medium; MS= Murashige-Skoog medium; MSC= MB + 5g/l activated charcoal;BA=benzyladenine; 2iP= izopentyladenine; Z = Zeatine; K = kinetine; AIB = betaindolyl acid; ANA= naphtyla acetic acid)

The *Solanum tuberosum L* variety was a lot studied from the point of view of the *in vitro* rooting and of the one of inducing mutations, with remarcable results, proving itself to be a variety with a high capacity of *in vitro* regeneration and multiplication, according to the hormonal balance from the medium ant to the used dose of phitohormones (Zăpârțan et al 2006). According to Pătru and Cachiță 2005, many potatoe varieties regenerate and propagate in vitro on mediums in which the sucrose is replaced by honey, with satisfying results. This experiment was initiated in an unfavourable period of the year, in the middle

of winter (January) so the effect of the hormonal balance, the nature of phitohormons and the capacity of each variety studied, could be well determined.

RESULTS AND DISCUSSION

I.The evolution of explants on mediums without phitohormons

The observations that have been made after two months of *in vitro* culture, according to the percentage of *in vitro* regeneration, to the number of regenerated neoplantlets, to the value of the formed radicular system, and the differenciation of *in vitro* minitubers, after three and four months. We left from the well known idea that the *Solanum tuberosum* L variety conserves itself on mediums with no hormons, with satisfying results. So, in table 2 we show the behaviour of the seven potatoe varieties, on mediums with no stimulating substances (V_o and V_1), with good results in what conerns the percentage of regeneration, but also the one of *in vitro* rooting.

Only after four months tubers are formed on these mediums, Désirée variety having the best reaction, especially on the charcoal medium (Vo). This reaction is presented in Fig. 1 for all the seven varieties experimented, from which we can see the superiority of the rooting percentage on charcoal medium (Vo), at all varieties.

Table 2

The evolution of the potatoe explant on mediums without growth hormons, after four months

No. crt.	Variety	% of regeneration		% of rooting		Note
		V_o	V_1	V_o	V_1	
1.	Désirée	30	20	20	10	XXX
2.	Ostara	35	10	10	5	XXX
3.	Eba	10	5	10	-	XX
4.	Cristian	9	5	3	1	X
5.	Muresan	8	5	2	-	X
6.	Super	10	10	2	3	X
7.	Caty	15	10	8	3	XX

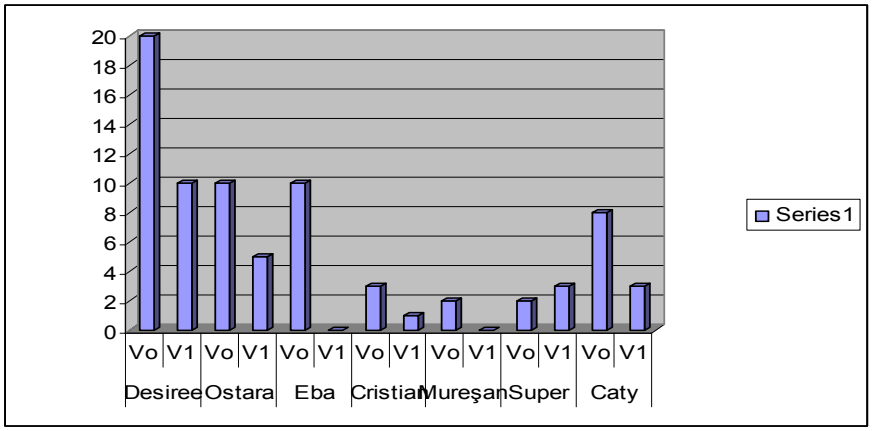

Fig. 1 The rooting percentage at the potatoe varieties on mediums without hormons (Atfer four months)

II. The evolution of explants on mediums with different hormonal balances

Table 3 shows the rooting percentage at all the seven varieties on the variants of medium with supplement of phitohormons (V_2, V_3, V_4, V_5, V_6, V_7, V_8 and V_9). Looking at

the table we can see the great percentage of rooting at Désirée variety, followed up by Ostara and Eba on all mediums, but especially on the ones with zeatine. The rooting takes place at the others varieties, but in a smaller percentage.

Table 3

The percentage of rooting at the seven potatoe varieties on mediums with phitohormons (after three months)

Variety/ variant	**V_2 %**	**V_3 %**	**V_4 %**	**V_5 %**	**V_6 %**	**V_7 %**	**V_8 %**	**V_9 %**	**The best variants**
Désirée	57	65	50	52	30	40	**95**	**98**	V_8, V_9
Ostara	50	**52**	48	89	20	30	75	**90**	V_9, V_5
Eba	35	**45**	30	**67**	20	20	60	75	V_9, V_5
Cristian	20	**32**	12	**28**	10	10	20	25	V_3 , V_5
Muresan	18	**29**	17	19	6	9	25	**28**	V_9 ,V_3
Super	18	20	20	20	10	12	**35**	**40**	V_8, V_9
Caty	40	42	35	**56**	25	26	45	**60**	V_9, V_5

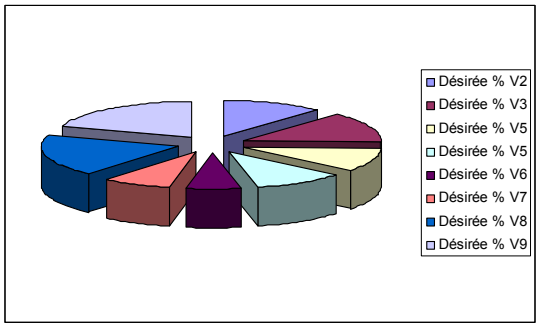

Fig. 2. *In vitro* rooting at Désirée potatoe variety

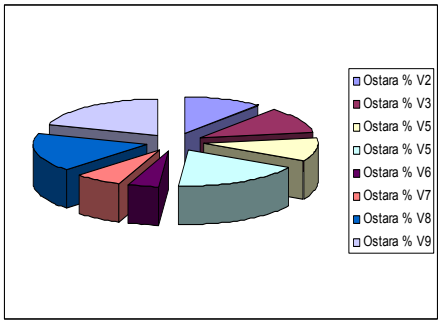

Fig. 3 *In vitro* rooting at Ostara potatoe variety

Following the variety's reaction presented on graphic (fig. 2), we see the superiority of the variants wich contain zeatine (V_8 şi V_9), but we can also see that the percentage is good for the other variants as well, even superior to the other varieties.

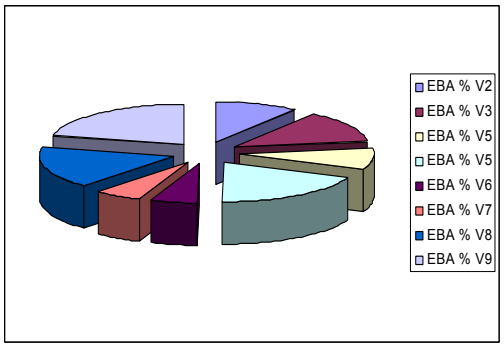

Fig. 4 *In vitro* rooting at Eba potatoe variety

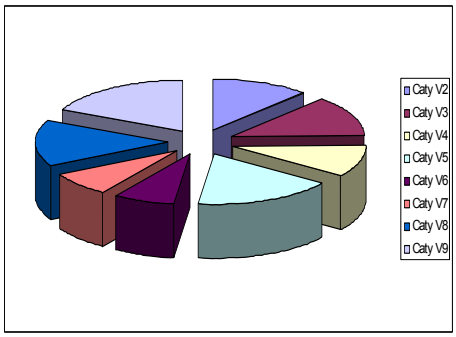

Fig. 5 *In vitro* rooting at Caty potatoe variety

In vitro reaction of Ostara and Eba varieties on the variants with phitohormons is similare, the presence of the zeatine stimulates the rooting in this case also, but regarding fig. 3 and fig. 4 we can see that on V_5 with 2iP + AIB, both varieties have a good rooting

(89 % respectiv 67%). A very similar reaction with these both varieties has Caty variety too, on the same variants, with zeatine and 2iP (on V_9 -60%, and on V_5 -56%).

In vitro rooting at the other variants: Cristian, Muresan and Super is inferior, reaching only at about 32 – 40%, according to the variant and variety, seeing in their case too the best percentage on the mediums with zeatine (V_8 şi V_9), but inferior to the presented varieties above. Also, the benzyladenine and 2 izopentyladenine in combination with idolil butiric acid, the variants V_3 and V_5, in MS medium, at these three varieties leeds to the formation of a superior percentage of potatoe tubers *in vitro* (fig. 6).

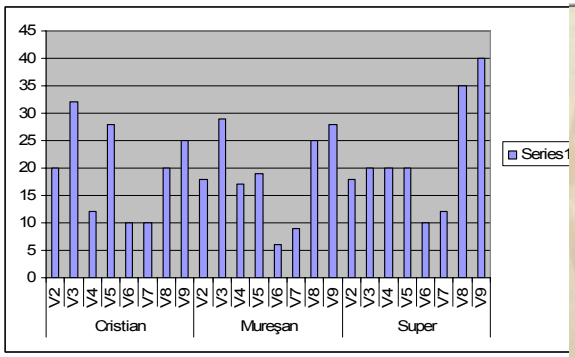

Fig. 6 *In vitro* rooting at Cristian, Muresan and Super potatoe varieties

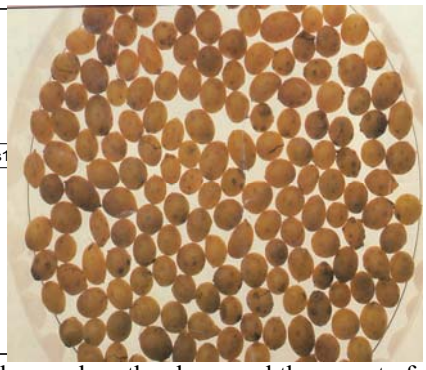

Fig. 7 The number, the shape and the aspect of the *in vitro* obtained minitubers from a potatoe apex on Murashige - Skoog medium, with a supplement of phitohormons

The rooting process has been followed after three months of *in vitro* culture, the phenomenon takes place on all variants, but the average number of tubers/apex is different according to the variety. The minitubers have been formed on all variants which contained cytokinine, differences being noticed only according to the nature of this phitohormon. The zeatine has proved to stimulate the formation of the greatest number of minitubers/explant. Figure 7 shows the aspect of the *in vitro* obtained minitubers after acclimatization, and fig 8, the aspect of *in vitro* minitubers and the place of differenciation (either on stem, or at the level of the medium).

Fig. 8 The obtained minitubers on the *in vitro* grown potatoe variety (on stems, but also at the level of the medium)

CONCLUSIONS

- MS medium without hormons stimulates *in vitro* regeneration of the experimented potatoe varieties, even the rooting in a small percentage, only after 4 months.

- The cytokinines from the culture mediums favour rooting of about five times. Among those, the zeatine determines the formation of the greatest number of tubers, between 30% till 98%, depending on the variety.

- The potatoe varieties behave very differently *in vitro*, Désirée, Ostara and Eba presenting the best capacity of *in vitro* rooting. At the others, the rooting is only at half, as a percentage.

- We recommend the suppliment in Murashige – Skoog medium of cytokinines for stimulating *in vitro* formation of tubers, the percentage of formed tubers being determined by the nature of the cytokinine and by the variety.

In conclusion, the dose of 1mg/l cytokinine and 0.5 aucine has the best effect on *in vitro* rooting, the zeatine and the indolil butiric acid proving to be the best combination. We recommend obtaining *in vitro* minitubers from the potatoe apex in order to ensure a plus of plant material, of superior quality.

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