THE *IN VITRO* REGENERATIVE CAPACITY OF THE POTATO CULTIVARS Ostara, Desirée AND Eba MERISTEM

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Abstract: *The meristem of three cultivars of Solanum tuberosum L., Ostara, Desirée and Eba was cultivated in vitro in order to observe the regeneration capacity and the organogenetic evolution of the tissues on various culture media. Meristems of 0.1-0.2 mm were prelevated from forced tubers; they were then inoculated on the different medium variants. (Table 1) The regeneration percentage for the in vitro meristem varied with the cultivar: 80 - 95% for Ostara, 70 – 80% for Desirée and 50 – 67% for Eba, as compared to 18 – 20% for the control medium (without hormones).The best variants proved to be M7 and M8 with all the cultivars, the regeneration percentage being the highest: Ostara 95%, Desirée 85% and Eba 67%. The in vitro organogenesis (number of stems, their height, number of roots and their length) was visible on all variants, according to the hormonal balance. The variants containing zeatine and 2-isopentenyl adenine (M_5, M_6, M_7 şi M_8) provided the best results. The Desiree had a special evolution, with about 45 neoplantlets /explant and almost double values in comparison with the other cultivars.*

Key words: *meristem, Solanum tuberosum L., Ostara, Desirée, Eba, in vitro regeneration percentage, organogenesis, hormonal balance, genetic inheritance, „natural vegetation period”.*

INTRODUCTION

Our research on the evolution of the meristem and of other tissues that have been prelevated from *in vitro* cultivated potato cultivars comprises three phases: the first one refers to “The *in vitro* behaviour of the potato cultivar meristem”, the second one is “The initiation of *in vitro* minitubers with a view to obtaining biologic material to be used in fast multiplication of potato cultivars”(1); the third phase, an experimental one, has as its objective “The use of meristems for obtaining healthy, virus-free potato plants, in order to ensure valuable sowing material”. The *in vitro* regeneration and organogenesis of the meristem tissues are influenced by a multitude of factors among which: the biological value of the genotype that the tissue was prelevated from (2); the hormonal endogenous factors (11), temperature (8), season, photoperiod (12), minerals in the medium, and also the phyto-hormones (auxins and cytokinins)(5). The main purpose of the research was to ensure the existence of valuable material, which, as far as organogenesis and evolution are concerned, could allow the initiation of new experiments and the success of the next phases on the agenda. The cultivar has a significant impact upon the multiplication rate in any species, together with the contribution of the hormonal balance of the medium. (9)

For fast multiplication of some potato cultivars, the Murashige-Skoog-1962 basic medium was used (10) Also, the stimulating effect of small concentrations of growth

hormones was demonstrated, as well as the repressive effect of big concentrations, for the *in vitro* culture of some genotypes of *Solanum tuberosum* L. (6).Conservation through *in vitro* multiplication of some potato cultivars has been also used lately for creating an *in vitro* collection and protecting the potato lines.(7)

MATERIAL AND METHOD

The main direction of our research was to obtain *in vitro* potato plants and, at the same time, to look at the evolutional capacity and the vigour of the meristem tissue, in relation with the cultivar and the hormonal balance of the medium. Identifying the right culture medium can help preserve a potato genotype through in vitro methods and consider it as an explant donor for initiating future experiments. The biologic material consisted of potato tubers of the previous year (autumn), forced to sprout under the conditions of the growth chamber, with a temperature of about 17^{o}C and in the dark. After 7-8 days, sprouts of 5-10 cm started to come out of the eyes. After sterilization (5-10 minutes in hypochlorite), a meristem of 0.2-0.3 mm detached itself under the binocular and was inoculated onto the media variants described in table 1.

During the experiment, we are going to analyse the role that "the natural vegetation period" of the cultivar has upon the in vitro regeneration and multiplication capacity. The meristematic tissue was cultivated on a basic medium (MB), after *Murashige-Skoog* (1962), with eight variants. In choosing the variants, the starting point was the fact that moderate quantities of cytokinins stimulate the *in vitro* micro-multiplication. (3), the sprouting and leaf formation, while auxins stimulate the formation of the root system, as well as the number and length of the roots. The variants were called M_0 to M8; the composition of the variants, the hormonal balance, the hormone nature and dose are all presented in table 1.

Table 1

Medium composition for the potato meristem culture

Elements mg/l	M_o	**M_1**	**M_2**	**M_3**	**M_4**	**M_5**	**M_6**	**M_7**	**M_8**
Macroelements	MS1/2	MS	MS	MS	MS	MS	MS	MS	MS
Microelements	MS1/2	MS	MS	MS	MS	MS	MS	MS	MS
FeEDTA	MS1/2	MS	MS	MS	MS	MS	MS	MS	MS
Mezo - inozitol	50	100	100	100	100	100	100	100	100
Tiamine	0,5	1	1	1	1	1	1	1	1
Pyridoxine	0,5	1	1	1	1	1	1	1	1
Nicotinic acid	0,5	1	1	1	1	1	1	1	1
Sucrose (g/l)	25	30	30	30	30	30	30	30	30
Agar (g/l)	6	7	7	7	7	7	7	7	7
Hormones(mg/l): **Cytokinins:** K	-	1,0	1,0	-	-	-	-	-	-
BA	-	-	-	1,0	1,0	-	-	-	-
2iP	-	-	-	-	-	1,0	1,0	-	-
Z	-	-	-	-	-	-	-	1,0	1,0
Auxins: AIB	-	0,5	-	0,5	-	0,5	-	0,5	-
ANA	-	-	0,5	-	0,5	-	0,5	-	0,5

MS-medium after Murashige – Skoog(1962); MS1/2. with half macro and microelements; K - kinetine; 2iP - izo-pentyl adenine; Z – zeatine; AIB – indolilbutyric acid; ANA –naphtylacetic acid

The potato cultivars Ostara, Desirée şi Eba are genetically consolidated, with well-defined characteristics. Ostara has a shorter vegetation period, of about 80 days, originates from Holland, it is an early cultivar with good growth dynamics and a short dormant period. Desiree is a semi-late cultivar, with medium dormancy, Dutch origins and a vegetation period of about 120 days. Eba is a late cultivar of the same origin, with a vegetation period of 130 days and long dormancy. A number of 50 tubes/ variant were inoculated. The tubes containing meristems were kept in growth chambers, with 16/24 hours of light, with an intensity of 1200 – 1400 lux and a temperature of about 17^{o}C.Observations were collected in two phases: the first one, after three weeks, to analyse the regeneration percentage according to cultivar and variant, and the second one after five weeks, to observe the evolution of the potato cultivars.

RESULTS AND DISCUSSION

Table 2

Average values of the different parameters observed in the *in vitro* cultivated potato cultivars

Cultivar	Var.	Bonification	Regeneration percentage (%)	No. of stems	Length (cm)	No. of roots	Root length (cm)
Ostara	M_o	x	24	1	2.5	1	0.5
	M_1	xxx	89	3	1.0	3	1.2
	M_2	xxx	78	5	1.2	1	0.2
	M_3	xxxx	88	8	3.0	6	4.0
	M_4	xxxx	85	10	**3.7**	3	3.0
	M_5	**xxxxx**	92	14	3.0	**9**	4.0
	M_6	**xxxxx**	90	13	2.0	4	3.2
	M_7	**xxxxx**	**95**	**21**	1.0	10	1.9
	M_8	**xxxxx**	**95**	19	2.0	8	2.0
Desirée	M_o	x	18	2	2.4	2	0.3
	M_1	xxx	70	6	3.0	4	1.0
	M_2	xxx	70	4	2.5	8	1.2
	M_3	xxxx	82	14	2.5	8	0.9
	M_4	xxx	**79**	18	2.8	10	0.8
	M_5	xxxx	**85**	30	3.0	10	1.1
	M_6	xxxx	79	32	3.0	10	1.0
	M_7	xxxx	**85**	**33**	**4.1**	14	0.8
	M_8	**xxxxx**	**85**	**35**	**4.5**	12	0.6
Eba	M_o	x	20	1	2.0	1	0.3
	M_1	xx	52	2	2.1	3	0.5
	M_2	xx	50	2	2.0	3	0.7
	M_3	xxx	60	4	2.5	5	1.2
	M_4	xxx	65	4	2.5	7	1.0
	M_5	xxx	66	6	3.0	9	0.9
	M_6	xxx	64	6	3.5	7	1.0
	M_7	xxx	**70**	10	**3.2**	10	0.8
	M_8	xxx	**67**	12	**3.4**	8	0.9

M_o = MS1/2; **M_1** = MS + 1 mg/l K + 0.5 AIB; **M_2** = MS + 1 mg/l K + 0.5 mg/l ANA; **M_3** = MB + 1 mg/l BA + 0.5 mg/l AIB; **M_4** = MB + 1 mg/l BA + 0.5 mg/l ANA; **M_5** = MB + 1 mg/l 2iP + 0.5 mg/l AIB; **M_6** = MB + 1mg/2iP + 0.5 ANA ; **M_7** = MB + 1mg/Z + 0.5 mg/l AIB; **M_8** = MB + 1mg/Z + 0.5 mg/l ANA; Bonification: xx = reduced regeneration; xxx = satisfactory regeneration; xxxx = good regeneration; xxxxx = very good regeneration

The percentage of meristem *in vitro* regeneration for the three potato cultivars was measured after about 20 days (fig.1). After another three weeks (about 35 days) the following measurements were made: number of regenerated stems/ variant, their length (cm), the evolution of the root system (number of roots and their length), with a bonification for each variant and cultivar according to the parameters in view (table 2).

The percentage of meristem regeneration reached the maximum value with the Ostara cultivar (80-95%), mostly due to"the natural vegetation period", to the good growth dynamics of the cultivar, but also to the cytokinins in the growth medium. As shown in table 2, zeatine favoured the highest regeneration percentage (around 95%). For Desiree, the percentages ranged between 70-85%, with the highest ones obtained on the media containing 2iP and Z. Figure 1 shows that the Eba cultivar regenerated the least, with percentages under 70%, which can be due to its natural characteristics as a late cultivar, with a long vegetation period.

Organogenesis and the regeneration percentage depend on the size of the explant as well. Generally, if the meristem culture is not meant to produce virus-free plants, the meristem can be bigger than 2 mm. Small pieces of tissue are strongly traumatized and their regenerative capacity can be seriously affected.

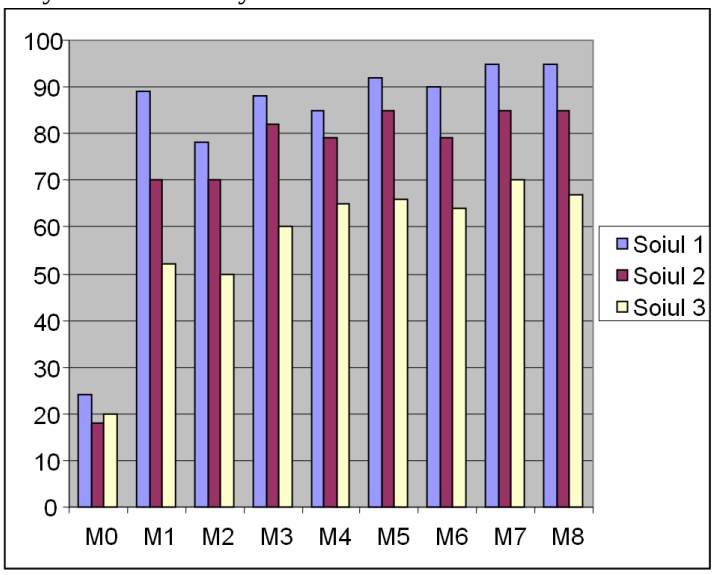

Fig. 1 The regeneration percentage of the *in vitro* cultivated potato cultivars
Legend: Cultivar 1= Ostara; Cultivar 2 = Desiree; Cultivar = Eba

The aspects related to organogenesis were analysed after five weeks (table 2). *The average number of stems* obtained from the potato meristem revealed different organogenetic capacities of the three cultivars, according to their natural characteristics and the medium composition. This parameter reached maximum values in the Desiree cultivar, especially on media containing Z and 2iP (variants M5, M_6, M_7 şi M_8), with 30-35 neo-plantlets/ explant. Fig. 2 shows the evolution of the number of stems for the three cultivars and the previously mentioned medium variants, bringing out the good evolution of the Desiree cultivar.

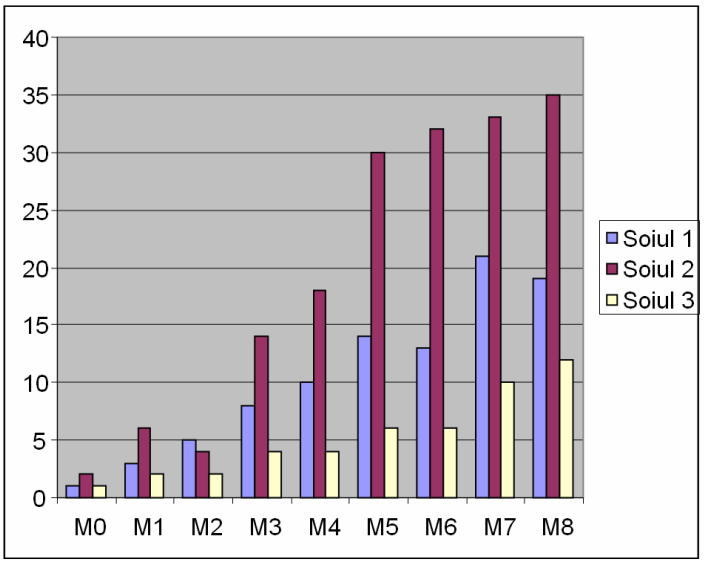

Fig. 2 Number of *in vitro* regenerated stems from the potato cultivar meristem
Legend: Cultivar 1= Ostara; Cultivar 2 = Desiree; Cultivar = Eba

It has been noticed that the Ostara cultivar generates a fairly high number of sprouts/variant, with insignificant differences produced by the cytokinins in the growth media. The zeatine and 2iP in the medium produce 20, respectively 14 neo-plantlets/ explant. The number of stems on media with BA and K is lower: 10, respectively 8 neo-plantlets/explant.. In the Eba cultivar, the values are lower (fig. 2). Zeatine stimulates the ramification of the neo-plantlets in this cultivar, too (about 10-12 stems/explant), but the other media, containing cytokinins, produced lower numbers. In our opinion, the cytokinin dose in the growth medium should be increased for this cultivar, in order to obtain a higher number of plants. It is well-known the fact that high doses of cytokinins favour neo-formation of plantlets in almost all species, and in potato, it also favour the *in vitro* obtaining of mini-tubers (3).

Successful *in vitro* multiplication depends on the presence of cytokinins in the medium, the effect of their concentration being correlated with the nature of the cultivar and the endogenous contribution of growth substances from the plant tissues. The mean of *the stem length* is an important parameter for potato multiplication, the stem being valuable material as donor for mini-cuttings, which can increase the *in vitro* multiplication rate. The differences in the case of this parameter are not significant. However, on the zeatine media, the stems of the Desiree cultivar can grow up to 4.0-4.5cm high, the Eba ones-up to 3.2-3.4 cm, and the Ostara ones merely up to 1.0-2.0 cm.(fig.3) As for the control variants (M_0), the height of the sprouts on some explants can go over 2.0 cm. It is well-known the fact that even on hormone-free media, with only half of the micro and microelement quantities, the stems of the *in vitro* plants can grow quite high (the lack of hormones favours the formation of long internodes). On the variants containing hormones, the stems grow longer than the mean of 3-4 cm/explant

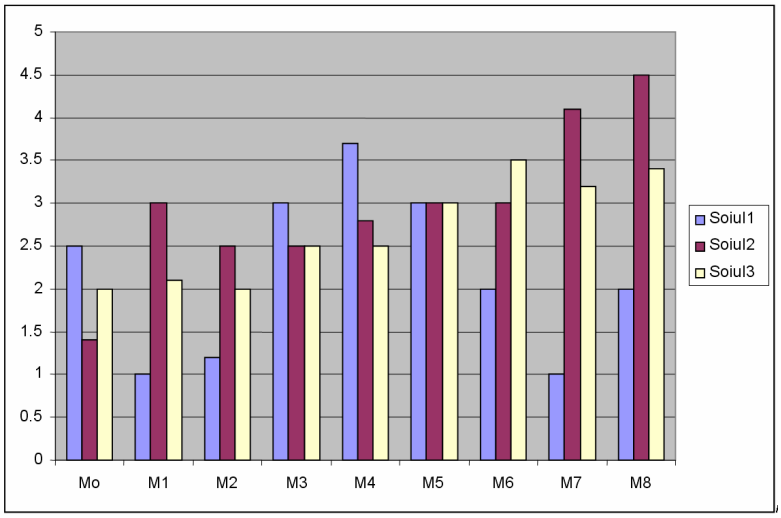

Fig. 3 The length of the *in vitro* regenerated stems for the three potato cultivars
Legend: Cultivar 1= Ostara; Cultivar 2 = Desiree; Cultivar = Eba

The root system of the three cultivars differs from the point of view of the number of roots (the presence of the β indolyl butyric acid in the medium stimulates the formation of the highest number of roots). The naphthyl acetic acid also stimulates root formation, but at a lower rate. Desiree proves again to be the most receptive cultivar to the in vitro culture, with an impressive number of roots /explant (fig. 4), mainly for the variants containing AIB + Z, 2iP and BA (M_7, M_8, M_4, M_5, M_6). The next-ranged cultivar, from the point of view of the evolution of the root-system, is Ostara, with maximum results on the media containing AIB (M_7, M_5). The Eba cultivar gives lower values, but almost uniform ones for the variants containing auxins and cytokinins (BA, 2iP, and Z), about. 5 – 10 roots/explant.

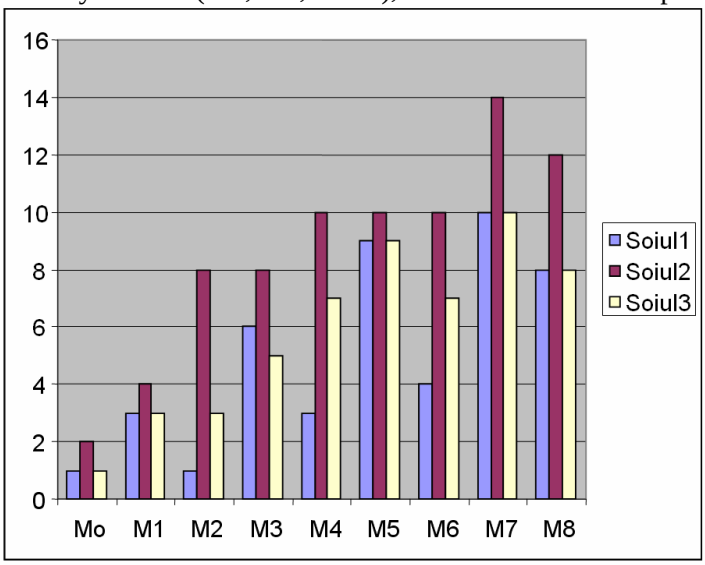

Fig. 4 The number of roots /explant for the three *in vitro* cultivated potato cultivars
Legend: Cultivar 1= Ostara; Cultivar 2 = Desiree; Cultivar = Eba

Root length values are shown in table 2. The Ostara cultivar gives medium-long roots, with values around 3-4 cm, whereas the roots of the other cultivars are shorter, with values between 0.3- 1.2 cm (irrespective of the nature of the auxin or of the cultivar). In the case of the controls, the length is between 0.3-0.5 cm, without differences between the cultivars.

CONCLUSIONS

- *the percentage of in vitro regeneration* of the potato cultivars was very good on the variants containing hormones. The percentages were between 78-95% for Ostara, between 70 – 85% for Desiree, whereas, in the case of Eba, only half of the explants regenerated- 50-66%.The regeneration percentages for the controls were as follows: Ostara- 24%, Desirée- 20% and Eba -18%;
- *the variants containing 2iP and Z* (M_5, M_6, M_7 and M_8) stimulated the regeneration percentage the most, the only differences being among cultivars;
- *organogenesis* reaches its climax after about 35 days (five weeks), the Desiree cultivar being the most receptive in this respect. The control variants only give 1-2 neo-plantlets/explant;
- the presence of zeatine and 2iP generated the highest number of neo-plantlets/explant, with the only differences being cultivar-generated: Desirée- 33-53 neo-plantlets/ explant, Ostara- 19-21 neoplantlets/ explant, and Eba- 10 neo-plantlets/ explant;
- the root system is stimulated by the presence of the the β indolyl butyric acid (AIB). In association with Z and 2iP, the AIB generated the highest number of roots/explant: about 14 roots/explant, 0.8 cm in length in the Desirée cultivar, 8-10 roots/explant, 0.8 – 1.0 cm long in Ostara, and 7- 10 roots/explant of about 0.8 cm in length, in the Eba cultivar. The mean obtained in the case of the controls was quite low, about 1-2 roots/explant, around. 0.4 cm in length.

By analysing the ratio between "the natural vegetation period" and the *in vitro* evolution of the potato cultivars, the conclusion is that the *in vitro* regeneration percentage is inversely proportional to the vegetation period, which requires specific culture media, according to the genotypic reaction of the cultivars.

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