THE *IN VITRO* MULTIPLICATION OF EBA POTATO CULTIVAR

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Abstract: *Solanum tuberosum cv. Eba was cultivated in vitro. The Regeneration capacity, organogenesis and multiplication of shoot tips and nodal explants have been studied on different culture media. The percent of regeneration was 60% after 2 weeks of in vitro culture. Shoot multiplication and complete organogenesis were obtained on all media containing cytokinins, but on the media supplemented with 2 mg/l Zeatine (V_6) all the parameters showed superior values in the case of regeneration from shoot tip explants in comparison with nodal explants. In vitro microtuberization was also obtained on all media, the best results being obtained on V_6 medium (in average 10-11 microtubers/explant).*

Key words: *Solanum tuberosum*, shoot tip, nodal explants, viability, multiplication, microtuberization, acclimatization.

INTRODUCTION

The in vitro regeneration, organogenesis and microtuberization of potato cultivars are strongly influenced by many factors such as the biological value of the cultivar (2), endogen uptake of plant regulators (10, 11), temperature (8), the seasonous, photoperiod (7), the mineral content of the culture medium and the exogen uptake of plant regulators (5). The Eba p. c. of potato, cultivated in vitro on media supplemented with different plant growth regulators, represents a valuable material for rapid multiplication (1). In this paper, the in vitro multiplication and microtuberization was accomplished.

MATERIAL AND METHOD

Different types of cytokinins (BA, 2iP and Zeatine) and auxins (AIB) were studied. The Basal medium was MS (9) supplemented with different concentrations of plant growth regulators as shown in Table 1. We used high concentrations of cytokinins because, the favorable effect of these compounds has been already showed (6). The best combination of plant growth regulators for preservation of potato microtubers obtained in vitro was also studied, in order to obtain valuable plant material for the germplasm collection and the protection of valuable potato cultivars.

Table 1

Culture media used for in vitro multiplication of potato cv. Eba.

Medium variant	V_0	V_1	V_2	V_3	V_4	V_5	V_6
Macroelements	MS		MS	MS	MS	MS	MS
Microelements	MS	MS	MS	MS	MS	MS	MS
FeEDTA	MS	MS	MS	MS	MS	MS	MS
Mezo-inosytol (mg/l)	100	100	100	100	100	100	100
Thiamine (mg/l)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Pyridoxine (mg/l)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Nicotinic acid (mg/l)	1.0	1.0	1.0	1.0	1.0	1.0	1.0
Sucrose (g/l)	30	30	30	30	30	30	30
Agar (g/l)	7	7	7	7	7	7	7
pH	5.5	5.5	5.5	5.5	5.5	5.5	5.5
BA	-	1.0	2.0	-	-	-	-
2iP	-	-	-	1.0	2.0	-	-
Zeatine	-	-	-	-	-	1.0	2.0
AIB	-	0.5	1.0	0.5	1.0	0.5	1.0

Plant material

The Eba p.c. cv. of potato is a well characterized cultivar originating from Holland, it is a late cultivar with a vegetation period of 130 days and a long period of seed dormancy.

The experiment was initiated at the end of February after a period of 5 days of cold treatment at 18 °C, in the dark. After this period, shoots were developed. The in vitro culture was initiated from shoot tips and nodal explants cut from shoots. The shoots were disinfected with calcium hypochlorite and then, rinsed with sterile water. The explants were placed on MS medium having ½ mineral strength, supplemented with 50 mg/l thiamine, nicotinic acid and pyridoxine, 100 mg/l mezoinosytol and 30 g/l sucrose. The Medium was solidified with 7 g/l agar. The pH was adjusted at 5.5 prior autoclavation.

Culture conditions

The explants were kept under vegetation chamber conditions: illumination with fluorescent tubes, light intensity was 1200-1400 lux, photoperiod 16 h light/8 h dark, room temperature was 17 °C.

Culture evaluation

The Culture was evaluated after 20 days, the percent of viability was studied. After 40 and 80 days, the number of shoots/explant, the length of shoots, the number of roots/explant, the length of roots, microtuber induction and the diameter of microtubers were studied. A number of 100 replicates were studied for each variant.

RESULTS AND DISCUSSION

After 20 days of in vitro culture, the percent of viability was analyzed. The results are shown in Fig.1. The percent of necrosed explants was 20%, 80% from explants were viable, 20% of them being stationary, and 60% showed regeneration response on different culture media.

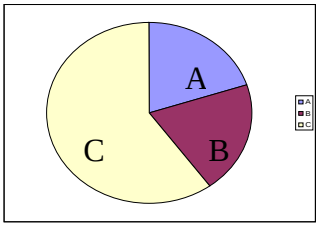

Fig. 1. The viability of explants prelevated from potato tubers cv. Eba
(A-necrosed explants, B-Viable explants without regeneration, C-Viable explants with regeneration response).

In vitro evolution of shoot tip explants

The results obtained after 80 days of culture are shown in Table. 2 and Fig. 2. As it could be seen, the percent of regeneration reach the maximal value on V_4, V_5 and V_6 variants that contain high concentration of cytokinins. On these media, the percent of regeneration was 79-85%.

Table 2

The evolution of shoot tip explants of potato cv Eba, after 80 days of in vitro culture
(xx-weak regeneration, xxx-satisfactory regeneration, xxxx-good regeneration, xxxxx-very good regeneration)

Variant	% of regeneration	No. of shoots/explant	Length of shoots (cm)	No of roots/explant	Length of roots (cm)	No. of microtubers	Diameter of microtubers (mm)	Bonification
V_0	35	1	2.0	1	0.3	-	-	xx
V_1	70	4	2.0	5	1.2	1	1.5	xxx
V_2	75	4	2.5	7	1.0	4	4.0	xxxx
V_3	70	6	3.0	9	0.9	5	2.0	xxxx
V_4	79	6	3.5	7	1.0	6	3.5	xxxxx
V_5	85	15	3.2	10	0.8	6	3.5	xxxxx
V_6	80	22	3.4	8	0.9	11	4.0	xxxxx

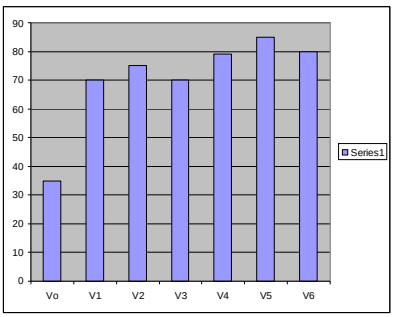

Fig.2. The percent of regeneration of shoot tip shoots explant of potato cv. Eba, after 80 days.

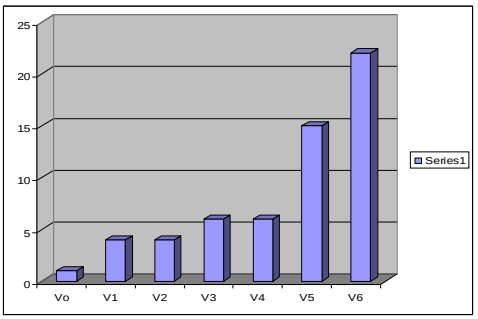

Fig. 3. The number of shoots regenerated from tip explants of potato, cv. Eba

The number of shoots after 80 days was very high on MS medium supplemented with 2iP (6 shoots/explant) and Zeatine (15-22 shoots/explant) (Fig.3). The length of regenerated shoots is very similar on all media studied, they were 2.0-3.0 cm length. Root induction was successfully obtained on al media, but on MS media supplemented with 2iP, Zeatine and IBA, the high number of root/explant were induced (in average 5-10 root/explant were induced). The length

of the roots was 0.9-1.0 cm; there were no significant differences between medium variants. On control variant the length of roots was only 0.3-0.4 cm.

In vitro tuberization was achieved on all media containing cytokinins, but there are differences between variants, regarding the number of microtubers/explant. The high number of microtubers was obtained on MS medium supplemented with high concentration of Zeatine (V_6), in average 11 microtubers have been obtained, having 4.0 mm in diameter (Fig. 4).

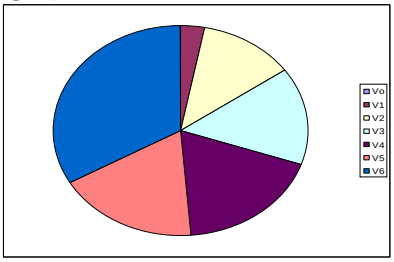

Fig. 4. Microtubers induction from shoot tip explants of potato, cv Eba.

In vitro evolution of nodal explants

In vitro organogenesis and the percent of regeneration depend on the type of explant and the composition of culture medium as well. In Table 3 are shown the results regarding the multiplication and tuberization of nodal explants of potato, cv Eba.

Table 3

The evolution of nodal explants of potato cv Eba, after 80 days of in vitro culture

(xx-weak regeneration, xxx-satisfactory regeneration, xxxx-good regeneration, xxxxx-very good regeneration).

Variant	% of regeneration	No. of shoots/explant	Length of shoots (cm)	No of roots/explant	Length of roots (cm)	No. of microtubers	Diameter of microtubers (mm)	Bonification
V_0	30	2	1.5	2	0.3	-	-	xx
V_1	60	5	2.0	5	1.1	1	1.0	xxx
V_2	65	6	2.0	7	0.7	2	1.2	xxxx
V_3	69	10	3.0	5	1.2	4	2.8	xxxx
V_4	70	12	3.5	8	0.9	5	3.0	xxxxx
V_5	70	18	3.0	9	1.0	6	4.0	xxxxx
V_6	75	18	2.8	8	0.8	9	4.0	xxxxx

The percent of regeneration is 60-75% and is inferior to shoot tip explants. This parameter is influenced by the composition of culture medium, high concentration of Zeatine and 2iP ensurimg the satisfactory regeneration (Fig. 5). After 80 days, organogenesis from nodal explants was observed (Table 3). Depending on the type and concentration of cytokinins, the organogenetic capacity of nodal explants was different. The maximal number of shoots/explant was obtained on media V_3, V_4, V_5, V_6, where 18 shoots/explant been obtained on media supplemented with Zeatine, and 10-12 shoots/explant on media supplemented with 2iP (Fig. 6). As it could be seen, the values are lower then those obtained from shoot tip explants. Zeatine stimulates the ramification of neoplantlets (in average 18 shoots/explant) but on the other culture media containing other cytokinins the

number of shoots is lower (Fig. 6). It is known that the high concentration of cytokinins stimulates the multiplication and in case of potato explants, in vitro tuberization is also stimulated (3).

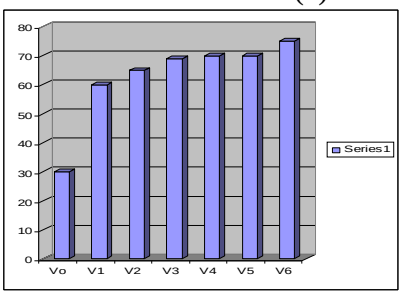

Fig. 5. The percent of regeneration of nodal from of potato cv. Eba, after 80 days

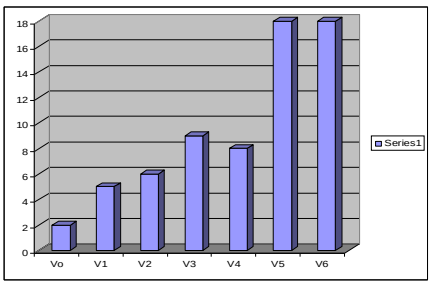

Fig. 6. The number of shoots regenerated nodal explants of potato, cv Eba

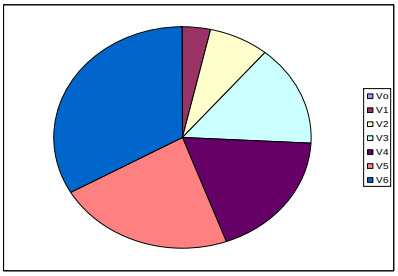

Fig. 7 Microtubers induction from nodal explants of potato, cv Eba.

The success of in vitro multiplication is thus ensured by the presence of cytokinins, their concentration having effect in correlation with the type of cultivar and the endogen ous uptake of plant growth regulators. The length of shoots is a very important parameter for in vitro multiplication, because the shoots are a valuable material for cuttings and in vitro multiplication. On control medium (V_0) the length of shoots was in average 1.5 cm, and on the variants containing plant hormones, the length of shoot was in average 2.0-3.5 cm.

Root induction from nodal explant was obtained on all media studied. On media supplemented with high concentrations of cytokinins and IBA, the number of roots/explant was in average 2-9 roots, having 0.8-1.2 cm length.

In vitro microtuberization from nodal explants is shown in Fig. 7. As it could be observed, the number of microtubers obtained from nodal explants is lower than the number of microtubers obtained from shoot tip explants. The highest number of microtubers/explant was obtained also on media supplemented with Zeatine (V_5 and V_6).

CONCLUSIONS

The evolution of shoot tip explants of potato is superior to nodal explants, in average 22 shoots/explant were obtained, having 3 cm length, and 8-10 roots/explant were induced, of about 1 cm length.

On MS medium supplemented with high concentration of Zeatine (V_6), the highest number of microtubers/explants was induced (10-11 microtubers). The evolution of nodal explants of potato cv Eba is good, but inferior to shoots tip explants. The best result has been obtained on MS medium supplemented with Zeatine. The highest number of microtubers/nodal explant was obtained on V_6 medium (in average 9 microtubers of 4.0 mm diameter). Among cytokinins, the Zeatine in high concentration (2.0 mg/l) ensures the best in vitro multiplication and microtuberization of the potato cv Eba. According to our results we recommend the Zeatine in high concentration (2.0 mg/l) in combination with moderate concentration of IBA (0.5 mg/l) for the in vitro multiplication and microtuberization of Eba potato cultivar. We also recommend inducing the in vitro culture at the end of February.

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