STUDIES REGARDING THE ELABORATION OF AN OPTIMUM MICROPROPAGATION PROTOCOL FOR A POTATO CULTIVAR RECALCITRANT TO „IN VITRO" CULTURE

STUDII PRIVIND ELABORAREA UNUI PROTOCOL OPTIM DE MICROPROPAGAREA A UNUI SOI DE CARTOF RECALCITRANT LA CULTURA „IN VITRO"

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Abstract: *The potato cultivar studied in this paper presents a special economically and alimentary importance, but is recalcitrant to the "in vitro" culture techniques. This fact makes more difficult the obtaining of healthy potato seed material, free of viruses, in a shorter time. Thus it was established an optimum micropropagation protocol for this cultivar, using two different culture media and different hormonal variants.*

Rezumat: *Soiul de cartof luat în studiu în această lucrare prezintă o importanță deosebită din punct de vedere economic şi alimentar, dar este recalcitrant la cultura "in vitro" ceea ce îngreunează obținerea de material semincer sănătos, liber de viroze, într-un timp scurt. Astfel, s-a stabilit un protocol optim de micropropagare pentru acest cultivar, folosindu-se două medii de cultură şi diferite variante hormonale.*

Key words**:** *potato, micropropagation, culture media, hormonal variants*
Cuvinte cheie: *cartof, micropropagare, medii de cultură, variante hormonale*

INTRODUCTION

Romania is the third potato tiller in Europe with 18 million hectares cultured every year and 15.5 t/ha annual production (Nedelea, 1983). The necessity of obtaining healthier seed material determined the breeders and seed material producers to make the attention for using the "in vitro" techniques in this aim. Although many species have been studied and many micropropagation protocols have been established till now, there have been found several genotypes recalcitrant to the artificial culture. This fact leaded to the necessity of establishing new micropropagation protocols for almost all the genotypes studied (Badea *et al.*, 2001).

Potato micropropagation started few decades ago, but continues today because of new cultivars appearance and the increasing request of seed material on the market. Potato micropropagation may be done using the multiple axillary shooting or single node microcuttings culture using both solid and liquid media and different hormonal balances (Badea *et al*, 2001, Cachiță *et al*, 1997). For virus free obtaining material the initial explant should be constituted of meristems of the smallest dimensions (Bâra 1987, Loebenstein *et al*, 2001) and sometimes even thermo or chemotherapy (Loebenstein *et al*, 2001).

MATERIAL AND METHOD

The biological material used in these experiments was constituted of two economically and alimentary important Romanian potato cultivars, created at Potato and Sugar Beet Research - Development Institute from Braşov, Romania.

The cultivar Amelia is the recalcitrant potato cultivar, a semi-late red cultivar with a good resistance to *Potato virus Y* and *Potato leaf-roll virus*, quality class B and an average yield of 80.6 t/ha and the cultivar Nicoleta, constituted the control and it is a semi-late yellow

cultivar with a good resistance to *Potato leaf-roll virus*, a very good resistance to *Potato virus* Y^C and *Potato virus* Y^O, but a medium sensitivity to the necrotic form of *Potato virus* Y^N, having an average yield of about 70.4 t/ha (Chiru *et al*, 1993).

Meristems of different sizes (meristematic dome without foliar primordia – DM; meristematic dome with one leaf primordia – DM+1; meristematic dome with two leaves primordia – DM+2; meristematic dome with four leaves primordia – DM+4) excised from sprouted potato tubers constituted the initial material for establishing regeneration and micropropagation of new plants obtaining protocols. The culture media used were MS - (Murashige and Skoog, 1962) and PM - (Loebenstein and Alper, 1985) that differ in chemical composition. Five different hormonal variants (table 1) were used to regenerate shots from meristem culture and other four hormonal variants (table 1) for micropropagation.

Table 1

The hormonal variants used in shoot regeneration and micropropagation of the recalcitrant potato cultivar and the control for both culture media MS and PM

Tissue culture aim	Hormonal variants	Growth regulators concentration (mg/l)					
		AIA	AIB	GA_3	KIN	BAP	ZEA
Shoots regeneration	V1	0.1	1	0.3	0.5	-	-
	V2	1	1	0.3	0.05	-	-
	V3	-	1	0.3	-	1	-
	V4	0.1	-	0.3	-	-	0.5
Micropropagation	V5	0.1	-	0.1	1.5	-	-
	V6	0.1	-	0.1	-	1.5	-
	V7	0.1	-	0.1	-	-	1.5
	V8	0.1	-	0.3	-	-	-

The vessels with the cultures were incubated in the growth rooms under controlled conditions at photo flux density of 240 μmol m^{-2} s^{-1} (16/8 h day/night period) at relative humidity of 55-60% and 25 ± 2˚C temperature. The regenerated shoots from meristems were transferred on the micropropagation media in order to establish the best hormonal variant for the recalcitrant cultivar. Shoots rooting was determined on three hormonal variants on the same two culture media as follow: one variant without any hormonal addition, one variant with AIA (1 mg/l) and AIB (1 mg/l) and one variant ANA (2 mg/l).

The statistic was calculated using the Microsoft Office Excel 2003 program.

RESULTS AND DISCUSSION

Regeneration from meristems' capacity depends in a great manner of the explant size, the cultivar, the nutrients in the culture media and the hormonal balance.

Thus for, significant differences existed between the results obtained from meristems constituted only of meristematic dome without leaf primordia and the explants constituted of meristematic dome plus four leaves primordia. The lower regeneration percentage was obtained, for both cultivars and all culture media variants, when the explants had the smallest size. The best results were noticed when the meristem had four leaves primordia, the regeneration capacity increased direct proportionally with the meristem size (Bâra 1987, Badea *et* al, 2001, Loebenstein *et al*, 2001).

A very important role, in regeneration capacity, is played by the genotype, knowing that there are recalcitrant genotypes to *in vitro* culture and genotypes with a good "cultivability". The lowest results were obtained for Amelia variety that regenerated the lowest number of shots on all the hormonal variants and on both culture media, its results being significantly inferior to the control genotype Nicoleta. The chemical composition of the culture

media has an important role in regeneration of shoots from meristem culture. The best results, in our case, were obtained on PM culture medium, for both cultivars on all the hormonal variants, results that differed significantly comparing with those obtained on MS culture medium.

The best regeneration percentage was obtained on the hormonal variant V2 constituted of two auxins: indole-3-acetic acid (1mg/l) and indole-3-butyric acid (1mg/l), a cytokinin: kinetin (0.05 mg/l) and gibberellins as gibberellic acid (0.3 mg/l), for both cultivars studied on both culture media (figure 1).

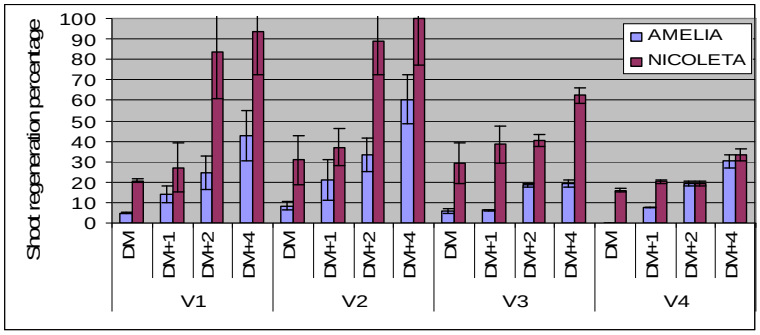

Figure 1. Regeneration capacity depending on the hormonal variant and meristem size for the recalcitrant cultivar Amelia in comparison with the control Nicoleta

The differences between the recalcitrant cultivar and the control regarding the regeneration capacity are significant. We obtained on the control cultivar Nicoleta in average of about 1.56 shoots/ meristem on the basal medium PM and on V2 as hormonal variant, when the regeneration started from explants constituted of meristematic dome plus four leaves primordia comparing with the recalcitrant cultivar Amelia, were the best result was in average of about 0.42 shoots/meristem.

The best results for the recalcitrant cultivar Amelia regarding the number of shoots regenerated by multiple axillary shoots technique was done on the hormonal variant V5 on the PM culture medium. On the MS culture medium the results were lower the higher number of shoots regenerated per Inocul being on the hormonal variant V6, but also on the variant V5, the statistical differences between these results are insignificant. Thus for Amelia the best cytokinin for shoot proliferation is kinetin comparing with the control where the best results were obtained on V6 added with benzyl amino purine, the differences from the hormonal variant V5 results are statistically very significant (figure 2).

Micropropagation depends in a great manner of the cytokine introduced in the culture media because this phytohormone determine the axillary dominance inhibiting the apical dominance thus generating shoot proliferation in detriment of the shoots size (Neamțu and Irimie, 1991). The shoots will be numerous but small. But also the micropropagation depends on the genotype and this is the explanation that for Amelia the best results were obtained on the culture media added with kinetin comparing with Nicoleta that gave the best results on the culture media added with BAP.

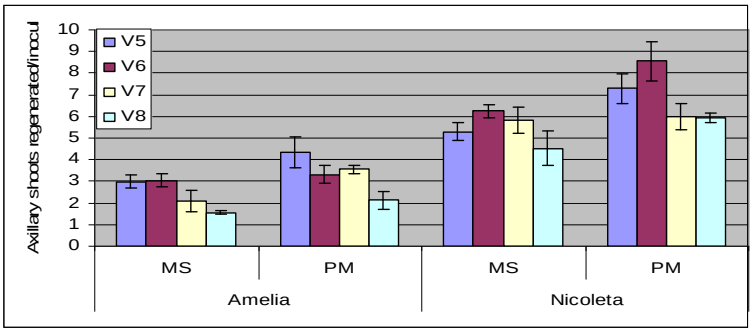

Figure 2. Micropropagation capacity for the recalcitrant cultivar Amelia
comparing with the control cultivar Nicoleta depending on the culture media and the hormonal variant

It can be also observed, from the figure 2, that in general, the best results were obtained on the culture medium PM. This culture medium contains monosodium phosphate NaH_2PO_4 a chemical substance needed of potato that proved to increase this specie *in vitro* regeneration and multiplication capacity (Loebenstein and Alper, 1985). The differences between the results obtained on these two culture media for the cultivar Amelia proved to be significantly important comparing with Nicoleta that gave similar results on both media, especially on the hormonal variant V7. Higher differences were registered on the hormonal variant V6 that proved to have for both culture media the best hormonal balance for the control cultivar Nicoleta.

CONCLUSIONS

Regeneration capacity depends direct proportionally with the size of the meristem and also on the genotype and on the hormonal variant used and less on the culture media used. The best results regarding regeneration from meristem culture for the recalcitrant cultivar Amelia proved to be given when the meristem had four foliar primordia on the hormonal variant that contains AIA and AIB in equal concentrations. Micropropagation capacity depends on the culture media, hormonal balance and genotype. For our recalcitrant cultivar the best results were obtained on the PM medium and the hormonal variant that contains the kinetin as the main phytohormone.

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