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THE COMPARISON BETWEEN FOUR POTATO CULTIVARS MULTIPLE AXILLARY BUD MICROPROPAGATION SYSTEM EFFICIENCY

COMPARAREA EFICIENŢEI SISTEMULUI DE MICROPROPAGARE PRIN LĂSTĂRIRE AXILARĂ MULTIPLĂ LA PATRU SOIURI DE CARTOF

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The growth in vitro of several potato cultivars (Amelia, Christian, Nicoleta and Roclas) on media containing plant growth regulators has been studied with a view to accelerating micropropagation of slow-growing cultivars. Whilst 0,5 mg/l GA_3+0.1 mg/l IAA substantially increased the height of plantlets of most cultivars, the combination of 0,1 mg/l GA_3+0,1 mg/l IAA+ 1,5 mg/l BAP was more effective in increasing the number of shoots which could subsequently be cultured. Using PM medium, a specific medium designed for potato, slow-growing or recalcitrant cultivars, such as Amelia, could be multiplied more rapidly than on basic Murashige & Skoog medium. Cultivars studied showed wide variation in their response to the plant regulators, best results being obtained for the cultivar Nicoleta.

Key words: micropropagation, axillary bud, hormonal balance, culture medium

Introduction

The axillary bud micropropagation system is one of the most important methods of plants *in vitro* propagation, giving good results regarding new plants productivity in a very short time, but also a good fidelity of the genetic information heritance (Bhojwani and Razdan, 1996). This method is suitable for many crops propagation and also for potato micropropagation (Bajaj, 1992). Using, as initial biological material, fragments of leaves, stalk, roots, flower elements that involves meristem tissues beside the definitive tissues can be realized thus two ways of regeneration, one direct and one indirect. The indirect regeneration way presents a high probability of somaclonal variation thus for it is not suitable for propagation aims (Espinoza *et al.*, 1997). Axillary bud micropropagation system involves initiation of tissue culture from apical or axillary buds, favorable for micropropagation because reduces the apparition of genetic variations (Cachita-Cosma *et al.* 2004). The explants constituted of apical or axillary buds contains one or two more many leaf primordia than the meristem regions and present few advantages as, their excision is more facile, have a higher surviving rate, the multiplication cycle is shorter (few weeks) and the number of plants obtained is

higher (Cachita-Cosma *et al*, 2004; Dumont, 1993). The multiplication coefficient of this propagation system is very high. Holdgate (1982) mentioned that from one donor plant 100.000 clones could be obtained in the first year of culture, the number of exemplars growing every year. The aim of these studies was to establish the protocols for micropropagation of four important Romanian potato cultivars.

Material and Methods

The biological material used in these experiments was constituted of four economically and alimentary important Romanian potato cultivars, created at Potato and Sugar Beet Research - Development Institute from Braşov, Romania and of six potato viruses, Romanian strains provided from the viral collection of Clonally and Seed Production Center Lăzarea – Braşov, Romania. The four potato cultivars were: Amelia – a semi-late red cultivar with a good resistance to viruses, quality class B and an average yield of 80.6 t/ha; Christian – a semi-early red cultivar with a medium resistance to viruses, having an average yield of about 70.6 t/ha; Nicoleta – a semi-late yellow cultivar with a good resistance to viruses, having an average yield of about 70.4 t/ha; Roclas - a semi-early yellow cultivar a good resistance to viruses and bacterial diseases, having an average yield of about 65.9 t/ha.

The shots used as initial material for micropropagation were obtained from meristem culture. The culture media used were Murashige – Skoog (Murashige and Skoog, 1962) – MS and Potato medium (Loebenstein and Alper, 1985) – PM, which differ in chemical composition. Four different hormonal variants were used for multiple axillary bud micropropagation: L1 – 0,3mg/l gibberellic acid; L2 - 0,1mg/l gibberellic acid + 0,1mg/l alpha indole acetic acid + 1,5 mg/l kinetin; L3 - 0,1mg/l gibberellic acid + 0,1mg/l alpha indole acetic acid + 1,5 mg/l benzyl amino purine; L4 -0,1mg/l gibberellic acid + 0,1mg/l alpha indole acetic acid + 1,5 mg/l zeatin.

Results and Discussions

Shoots propagation consisted of their fragmentation in 4mm segments including one node and half of the neighboring internodes and subcultured for other four weeks on the micropropagation medium. Micropropagation depends in a great manner of the cultivar, the nutrients in the culture media and the hormonal balance (Cachita-Cosma *et al*, 2004; Danci and Danci, 2007).

A very important role, in micropropagation, 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 other genotypes. The best axillary shooting capacity was given by Nicoleta followed closely by Roclas, indifferently of the culture media and the hormonal variant used.

The highest regenerated shoots number, of about 7,46 shoots/inoculum, was obtained for the cultivar Nicoleta, using axillary bud propagation technique, on the basal medium PM and on the hormonal variant L3, constituted of indole-3-acetic acid (0,1mg/l), gibberellic acid (0,1mg/l) and 6-benzylaminopurine (1,5mg/l). The lowest results were obtained from the cultivar Amelia, for all the hormonal variants used, that proved to be a "recalcitrant" genotype for *in vitro* culture.

Table 1

Basic culture medium, hormonal balance and genotype influence on axillary buds micropropagation

Basal medium	Hormonal variants	AMELIA	CHRISTIAN	NICOLETA	ROCLAS
MS	L1	1.16	1.48	1.48	1.36
	L2	2.58	4.67	5.92	5.88
	L3	3.37	4.98	6.24	6.37
	L4	2.08	4.72	5.82	5.74
PM	L1	1.54	1.88	1.74	1.67
	L2	2.76	3.67	6.56	6.98
	L3	3.88	7.08	8.66	7.23
	L4	2.14	2.98	4.94	4.92

The best basal medium, for all four potato cultivars studied, proved to be PM that contains phosphorus and glycine supplementary comparing with MS, their importance in formation, growth and development of potato shoots being relevant in this case (Loebenstein and Alper, 1985).

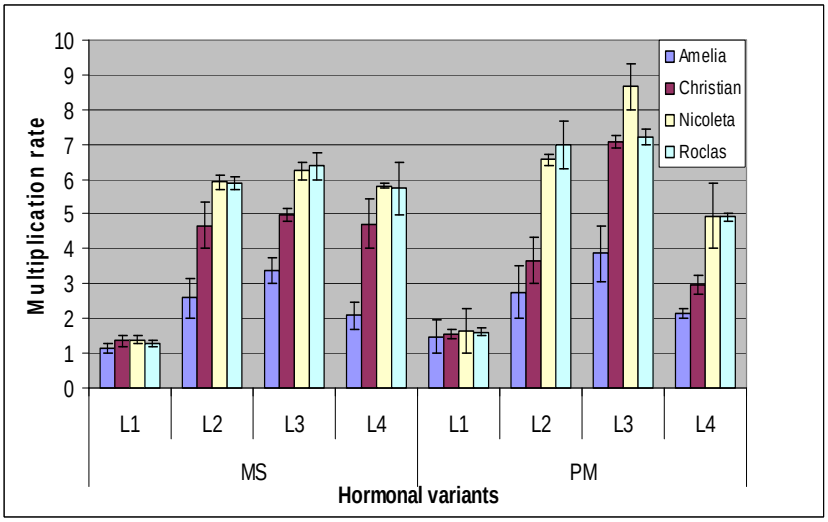

Figure 1 **Graphic representation of multiplication of axillary shoots and simple-node propagation capacity**

Comparing the hormonal variants used for shoots generation, can be observed, that, depending on the cultivar studied, very significant differences exist inside each cultivar, between the hormonal variants studied (table 1). The lowest number of shoots was generated on the L1 hormonal variant that presented very significant negative differences comparing with the other hormonal variants.

Best results, for all four cultivars have been given on the hormonal variant L3 that is constituted of 0,1mg/l gibberellic acid + 0,1mg/l alpha indole acetic acid + 1.5 mg/l benzyl amino purine. Good results were obtained on the variants L2 and L4 that contain as principal hormone one cytokinin, kinetin and zeatina respectively.

Also, the best hormonal balance for shoot proliferation was provided by the hormonal variant L3, for all the cultivars studied, because 6-benzylaminopurine, an important cytokinin, inhibited apical dominance and stimulated lateral dominance determining axillary shoots formation (Neamțu and Irimie, 1991).

Very good results were obtained as well on the hormonal variant L2 that contains kinetin instead of 6-benzylaminopurine, in the same concentration of 1.5 mg/l, being the best hormonal variant for Roclas (Fig. 1).

Comparing the results obtained, regarding micropropagation capacity for the four cultivars studied, on can observe in the table 2 that significant differences between all the cultivars were registered. These results prove the influence exerted by the genotype on the *in vitro* "culturability" capacity (Cachiță-Cosma *et* al., 2004) and most over micropropagation capacity in the artificial culture conditions under exogenous phytohormones effect (Ranalli, 1997).

Data shown in table 2 emphasize that the cultivar Nicoleta presents significant positive differences comparing with the other cultivars, being the cultivar with the best micropropagation capacity, closed followed by the cultivar Roclas.

Table 2

Differences significance between potato micropropagation results obtained for all four cultivars studied

Cultivar	**Average** (no shoots/inoculum)	**Difference toward the contro**			
		Amelia	**Christian**	**Nicoleta**	**Roclas**
Amelia	2.42	-	-2.94^{000}	-5.04^{000}	-4.33^{000}
Christian	5.36		-	-2.11^{000}	-1.39^{000}
Nicoleta	7.46			-	0.71^{***}
Roclas	6.75				-

$DL_{5\%}$ = 0,108 (no shoots/inoculum) $DL_{1\%}$ = 0,164 ((no shoots/inoculum)
$DL_{0,1\%}$ = 0,263 ((no shoots/inoculum)

At the opposite pole is the cultivar Amelia with lowest results and negative significant differences comparing with the other cultivars. Amelia proved to be a recalcitrant cultivar to *in vitro* culture presenting thin shoots, small leaves and a

small number of roots. The cultivar Amelia showed a low viability in the same culture and needed to be transferred in fresh culture medium every 3-4 weeks.

On suppose that the capacity of being cultured *in vitro* conditions of a cultivar is correlated with each genotype capacity of adaptation to different surrounding conditions, but more over with their capacity of metabolizing the nutritive substances existent in the culture media.

Conclusions

1. The best micropropagation results were obtained for the cultivar Nicoleta, closely followed by the cultivar Roclas.
2. PM medium proved to be the best basic medium for potato micropropagation due to the fact that contains phosphorus and glycine supplementary, necessary for potato plantlets regeneration and growth.
3. BAP proved to be the optimum cytokinin for axillary buds micropropagation for all four potato cultivars tested.
4. The cultivar Amelia raised the lowest *in vitro* culture regeneration and multiplication capacity.

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