STUDIES REGARDING POTATO MICROPROPAGATION BY SINGLE-NODE CULTURE

STUDII PRIVIND MULTIPLICAREA CARTOFULUI PRIN MICROBUTĂŞIRE

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Summary: *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.*

Rezumat: *Studierea răspunsului la cultura in vitro a mai multor soiuri de cartof pe medii conţinând diverse balanţe hormonale a avut ca scop accelerarea micropropagării soiurilor de cartof recalcitrante. În timp ce adiţia de 0,5 mg/l GA_3+0.1 mg/l AIA în mediul de cultură a determinat creşterea taliei plantelor la majoritatea soiurilor studiate, varianta hormonală conţinând 0,1 mg/l GA_3+0,1 mg/l IAA+ 1,5 mg/l BAP a fost cea mai eficientă în creşterea numărului de lăstari. Prin folosirea mediului PM, un mediu de cultură pus la punct special pentru multiplicarea cartofului, s-a reuşit accelerarea ritmului de multiplicare şi la soiurile recalcitrante, cum e soiul Amelia, comparativ cu rezultatele obţinute pe mediul bazal Murashige&Skoog. Soiurile studiate au evidenţiat o mare variabilitate a răspunsurilor la balanţele hormonale folosite, cele mai bune rezultate fiind obţinute la soiul Nicoleta.*

Key words: *potato micropropagation, single node cuttings, hormonal variants, PM and MS culture media*
Cuvinte cheie: *micropropagarea cartofului, butaşi uninodali, variante hormonale, medii de cultură PM şi MS*

INTRODUCTION

A number of different approaches have been successfully used in the regeneration of potato plants from *in vitro* cultures. Roest and Bokelman (1976) obtained plantlet regeneration from potato stem segments when explants were cultured on a MS medium supplemented with 10 mg/l GA_3, 1.0 mg/l BA, and 1.0 mg/l IAA. Espinoza *et al*. (1992) have reported on the micropropagation of potato by either nodal section or shake cultures. They found that when nodal sections were inoculated onto a MS culture medium supplemented with 0.25 mg/l GA_3 and 2.0 mg/l calcium pantothenic acid, the number of nodes increased six fold within 3-4 weeks. When nodal sections were cultured on a liquid MS medium supplemented with 0.4 mg/l GA_3, 0.5 mg/l BA, 0.01 mg/l NAA, 2.0 mg/l calcium pantothenic acid, and 2% sucrose, there was a 10- to 20-fold increase in the number of shoos in 2-3 weeks.

Ranalli (1997) reported a typical method for potato micropropagation namely single node propagation or micro grafting. The micrografts are constituted of single node cutting 5-10 mm long that inoculated on solid culture medium generate shoots and finally one plant.

Several Romanian potato cultivars were studied for regenerative capacity by Chiru *et al*. (1993) and recalcitrance for *in vitro* culture was found for some of them.

The aim of these studies was to establish the protocols for micropropagation of four important Romanian potato cultivars, Amelia, Christian, Nicoleta and Roclas created at Potato and Sugar Beet Research - Development Institute from Braşov, Romania.

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.

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 shoots used as initial material for micropropagation were obtained from meristem culture. The single node cuttings were shaped in accordance with the protocol described by Ranalli (1997). 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 single-node micropropagation system: L1 – 0,5mg/l gibberellic acid + 0,1mg/l alpha indolil acetic acid; L2 -0,1mg/l gibberellic acid + 0,1mg/l alpha indolil acetic acid + 1,5 mg/l kinetin; L3 -0,1mg/l gibberellic acid + 0,1mg/l alpha indolil acetic acid + 1,5 mg/l benzyl amino purine; L4 -0,1mg/l gibberellic acid + 0,1mg/l alpha indolil acetic acid + 1,5 mg/l zeatin.

RESULTS AND DISCUSSION

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.

The highest regenerated shoots number, of about 7,46 shoots/inoculum, was obtained for the cultivar Nicoleta, using single-node 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

Differences significance between potato micropropagation results obtained on MS and PM basal media

Medium / Cultivar	MS	PM	X	%	Difference	Signification
Amelia	0,89	1,54	1,22	100	-	Control
Christian	1,25	1,68	1,47	120,49	0,25	-
Nicoleta	1,26	1,79	1,53	125,41	0,31	-
Roclas	1,12	1,76	1,44	118,03	0,22	-
X	1,13	1,69				
%	100	149,55				
Difference	-	0,56				
Signification	Control	-				

Medium $DL_{5\%} = 2,08$ $DL_{1\%} = 3,21$ $DL_{0,1\%} = 4,24$
Cultivar x medium $DL_{5\%} = 1,84$ $DL_{1\%} = 2,25$ $DL_{0,1\%} = 3,67$
Cultivar $DL_{5\%} = 1,26$ $DL_{1\%} = 2,32$ $DL_{0,1\%} = 4,05$

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 (Loebenstein and Alper, 1985).

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 2). The lowest umber 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 indolil 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 zeatin respectively.

Table 2

Differences significance between cultivar x hormonal variant combinations regarding *in vitro* micropropagation

Cultivar	Hormonal variants	Nr. shoots/ inoculum	Difference towards the hormonal variant			
			L1	L2	L3	L4
Amelia	L1	1,28	-	$-1,39^{000}$	$-2,34^{000}$	$-0,83^{000}$
	L2	2,67		-	$-0,95^{000}$	$0,56^{***}$
	L3	3,62			-	$1,51^{***}$
	L4	2,11				-
Christian	L1	1,45	-	$-5,22^{000}$	$-6,08^{000}$	$-4,35^{000}$
	L2	6,67		-	$-0,86^{000}$	$0,87^{***}$
	L3	7,53			-	$1,73^{***}$
	L4	5,8				-
Nicoleta	L1	1,52	-	$-7,72^{000}$	$-9,72^{000}$	$-6,36^{000}$
	L2	9,24		-	$-2,00^{000}$	$1,36^{***}$
	L3	11,24			-	$3,36^{***}$
	L4	7,88				-
Roclas	L1	1,46	-	$-6,97^{000}$	$-7,84^{000}$	$-6,37^{000}$
	L2	8,43		-	$-0,87^{000}$	$0,6^{***}$
	L3	9,30			-	$1,47^{***}$
	L4	7,83				-

$DL_{5\%}$ = 0,26 (nr. shoots/inoculum) $DL_{1\%}$ = 0,34 (nr. shoots/inoculum)
$DL_{0,1\%}$ = 0,45 (nr. shoots/inoculum)

The type of cytokinin or generally the phyto-hormone type influence plants *in vitro* regeneration response, thus for the same hormone different genotypes give variable results (Bhojwani and Razdan, 1996).

This might be the explanation why the same cultivar, on the same basic medium regenerates a higher number of shoots on a hormonal variant containing one type of cytokinin comparing with the other hormonal variants containing other types of cytokines. Another explanation might be given by the fact that some genotypes are capable of metabolizing one type of cytokinin comparing with other genotypes that can metabolize different cytokinin (Neamțu and Irimie, 1991).

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 lateral shoots formation (Neamțu and Irimie, 1991).Very good results were obtained as well on the hormonal variant L2 that contains kinetin instead of BAP, in the same concentration of 1.5 mg/l, being the best hormonal variant for the cultivar Roclas.

The lowest results for all the hormonal variants used and also for both basic media were registered by the cultivar Amelia. The cultivar Christian was rather constant in reaction to the different hormonal balances, its results being satisfactory. It was observed that the red color cultivars are more recalcitrant to *in vitro* culture comparing with white or yellow cultivars, the genetic correlation between *in vitro* recalcitrance and genotype color was not found yet (Del Avila *et al.*, 1996).

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. The cultivar Amelia raised the lowest *in vitro* culture regeneration and multiplication capacity.

REFERENCES

1. BHOJWANI., S.S., RAZDAN., M.K. (1996). Plant Tissue Culture: Theory and Practice. Elsevier, The Netherlands
2. CHIRU, N., POP L., CACHIȚĂ-COSMA, D. (1993). Regenerarea de plante prin cultura de țesuturi la cartof. Anale ICPC Braşov. 20, 1-8
3. DANCI, M. AND DANCI, O. (2007). Ghid practic de culturi *in vitro*, Ed. Eurobit, Timisoara
4. DEL AVILA, A., PEREYRA, S. M. AND ARGÜELLO, J. A. (1996). Potato micropropagation: Growth of cultivars in solid and liquid media, Potato Research 39: 253-258
5. ESPINOZA, N., LIZARRAGA, R., SIGUENAS, C., BUITRON, F. (1992). Tissue culture: Micropropagation, Conservation and Export of Potato Germplasm. CIP Research Guide 1, International Potato Center, Lima, Peru
6. LOEBENSTEIN, G., ALPER, M. (1985). Phytopathology, 53: 349
7. NEAMȚU, G., IRIMIE, F. (1991). Fitoregulatori de creştere, Editura Ceres, Bucureşti
8. RANALLI, P. (1997). Innovative propagation methods in seed tuber multiplication programmes. Potato Research 40: 439-453
9. ROEST, S. AND BOKELMAN, G.S. (1976). Vegetative propagation of *Solanum tuberosum* L. in vitro, Potato Res 19, pp. 173–178