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Potato (*Solanum tuberosum* L.) regeneration using the technique of meristem tip culture

Danci Oana Marcela[1], Baciu Anca[2], Danci M.[1]

[1]Banat's University of Agricultural Sciences and Veterinary Medicine Timişoara, Faculty of Horticulture and Forestry; [2]Potato Research and Development Station – Targu Secuiesc, Romania

*Corresponding author. Email: oana_research@yahoo.com

Abstract Technique of meristem tip culture is one of the most used for in vitro culture initiation. It is one of the most used techniques to obtain virus free plantlets. Four Romanian potato cultivars and ten artificial nutritive medium variants were used in this study, in order to establish the best protocol for potato shoots regeneration starting from meristems. The best results have been obtained on PM medium added with 1mg/l indolyl acetic acid, 1mg/l indolyl butyric acid, and 0.3 mg/l giberelic acid for the genotype Nicoleta when the meristem was constituted of meristematic dome and four leaf primordia.

Key words

meristem tip culture technique, in vitro regeneration, potato, culture media

Potato is one of the most important culture plants from our country, being very important for alimentary and chemical industry and in animal feat, due to its richness in vitamins (especially vitamin C), hydrocabonates, and starch. Potato is a very sensitive plant to viral infection thus, it was imperative to establish a proper protocol for its regeneration from meristems. It is known that meristem culture is the only way to obtain virus free biological material, chemicals having no effect in this topic (Loebstein *et al.*, 2001). Meristem tip culture was first used to produce virus-free plants on assumption that viruses were unable to invade the meristematic tissues of the bud (Jha and Ghosh, 2005). Apical meristems are domes of actively dividing cells, located at the apices of shoots and roots (Badea and Săndulescu, 2001), that remain in an active state of division throughout the vegetative phase of the plant, forming new tissues and organs; they have therefore the capability of producing complete plants *in vivo* and *in vitro*.

The optimum size of meristem is ranging from 0.2 to 1 mm (meristematic dome without or with few leaf primordia). The larger the size of the meristem cultured, the greater is the number of regenerated plant produced, thus the survival rate for potato meristems without leaf primordia is very low (Chiru *et al,*1993).

Biological Material and Method

Four important alimentary and agronomical Romanian potato cultivars were used in order to establish the best regeneration protocol from meristem tip explants. All four cultivars have been created between 1999 and 2001 in Potato Breading Institute from Braşov and provided by. The cultivars used in this study are Amelia, Cristian, Nicoleta, and Roclas.

The meristems were isolated from tuber sprouts sterilized by immersion for several seconds in 70% ethanol followed by immersion in mercuric chloride 0.1% for four minutes. The explants rinsing has been done using sterile distilled water for three to five times 2 minutes each. The best artificial nutritive culture medium was selected from ten culture media variants constituted from different basal media and different hormonal concentrations (table 1). The two basal media used in this study were MS (Murashige and Skoog, 1962) and PM (Loebstein, 1985) that differ in vitamin and macroelements concentration and composition.

Table 1

Growth regulators variants and concentrations used for meristem culture regeneration

Basal medium	Growth regulators variants	Growth regulators concentration (mg/l)				
		IAA	IBA	GA_3	KIN	BA
MS	CM 1	0,1	-	0,3	-	-
	CM 2		0,1	0,3	-	-
	CM 3	0,05	0,05	-	-	-
	CM 4	-	-	0,3	-	1
	CM 5	-	-	0,3	1	-
PM	CM 6	0,1	-	0,3	-	-
	CM 7		0,1	0,3	-	-
	CM 8	0,05	0,05	-	-	-
	CM 9	-	-	0,3	-	1
	CM10	-	-	0,3	1	-

Explants suitable for *in vitro* culture initiation have been variously designated: meristematic dome without leaf primordia, meristematic dome plus single leaf primordia, meristematic dome plus two leaf primordia and meristematic dome plus four leaf primordia (figure 2). The meristem tip explants were excised under a stereomicroscope (x 8-40) with suitable instruments (forceps, needles and pieces of razor blades fixed to handles), in a sterile laminar-flow cabinet (figure 1). Excision was done as fast as possible to avoid desiccation of the explants by heat from the microscope light.

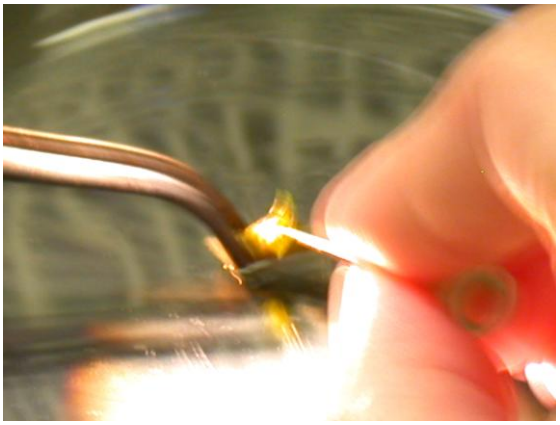


Fig.1 Meristem excision

Fig.2 Meristems with single, two and four leaf primordia

Fig.3 Regeneration of plantlets from meristems

Fig.4 Potato plantlets regenerated from meristems

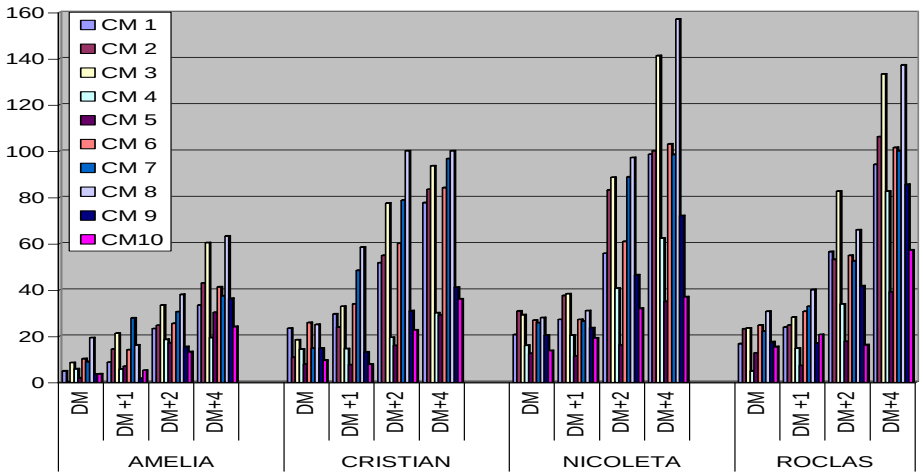
The meristems designated as described before were immediately placed on the nutrient medium and incubated in the growth room under controlled condition of photoperiod (8 hours darkness and 16 hours lightness), temperature (19-21^{o}C), and humidity (70-80%). Plantlets regenerated from meristems were subcultured in higher culture vessels for growth and rooting (figures 3 and 4).

Results and Discussions

The aim of this study was to test the meristems regenerative capacity; meristems viability depends in a great manner on the meristem size but also on the chemical composition of the culture media used. More than three repetitions have leaded us to the following results. Thus, the following graphics show the results obtained for all four genotypes on the culture media studied. The best results have been obtained for the genotype Nicoleta and the smallest number of shoots was regenerated for the genotype Amelia. The best basal culture medium for all the hormonal variants was PM that contains, comparing with MS, a supplementary source of organic nitrogen (glutamine) and phosphor (NaH_2PO4) as important phosphorous source, needed for potato growth (Loebenstein *et al*, 2001). Comparing the results obtained on the hormonal variants used for shoot regeneration from meristem tips it was shown that the best results for all the genotypes studied were obtained when the growth hormones were auxins and gibberellins and the lowest results, in our case were obtained when the media were added with cytokinins. Even if cytokinins are plant hormones that are derivatives of the purine adenine, and without cytokinins in the medium, plant cells will not divide by

mitosis (Badoni and Cauhan, 2009), it was considered that the concentration used in this case were too high and inhibited the apical dominance.

Comparing the results obtained from our four-meristem types can be easily seen that the best results have been obtained when the meristem was constituted of meristematic dome plus four leaf primordia and the smallest number of shoots has been regenerated from the meristems lacking leaf primordia. The formation of new leaves in the apical meristem is initiated by the accumulation of auxin (Badoni and Cauhan, 2009). Already-developing leaves deplete the surrounding cells of auxin so that the new leaves do not form too close to them. In this way, the characteristic pattern of leaves in the plant is established. Since the second pair of leaf primordia probably synthesizes auxins, the meristematic dome of shoot tips is not autonomous for auxins (Jha and Ghosh, 2005). The meristems having more than two leaf primordia regenerated a higher number of shoots even on media added only with cytokinins comparing with the small size meristems that formed more callus than shoots. Even if the number of regenerated shoots was higher, on media added with two auxins (IAA and IBA) the shoots length was low and the internodes were short comparing with the shoots regenerated on the media supplemented with giberelic acid. GA has a number of effects on plant growth, but the most dramatic is its effect on stem growth. When applied in low concentrations to a bush or "dwarf" plant, the stem begins to grow rapidly. The length of the internodes becomes greater. GA seems to overcome the genetic limitations in many dwarf varieties (Mineo, 1990).



The role of GA_3 seems to be great in stems elongation of shoots, its presence in culture media determining a higher growth in length of plantlets (Badea and Săndulescu, 2001). The graphics above show also that the most recalcitrant cultivar for *in vitro* culture, more over for meristem tip culture technique, was Amelia, that surprisingly *in vivo* had the best yield results. It is known that there are species or even genotypes recalcitrant to *in vitro* culture (Chiru *et al*,1993) thus from tissue culture researchers point of view the plants can be divided in two classes: cultivable and non cultivable *in vitro* (Jha and Ghosh, 2005). This "non cultivability" shown by Amelia makes this genotype hard to be multiplied by *in vitro* techniques and hardens the obtaining of free of viruses biological material in order to produce healthy seeds of it.

Conclusions

1. The best regenerative rate using the technique of meristem tip culture was obtained for the potato cultivar Nicoleta whereas the smallest number of shoots was regenerated by the genotype Amelia on all the culture media variants.
2. The highest number of shoots regenerated from meristems was obtained on PM medium supplemented with 1mg/l IAA, 1mg/l IBA and 0.3 mg/l GA_3 for all the genotypes studied.
3. Meristems constituted of meristematic dome and four leaf primordia regenerated the highest number of shoots on both basal media for all the hormonal variants studied.

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