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ÎNMULȚIREA *IN VITRO* A PORTALTOIULUI „ADAPTABIL”
IN VITRO PROPAGATION OF THE ”ADAPTABIL” ROOTSTOCK

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Abstract

`Adaptabil` is interspecific hybrid (*Prunus besseyi* x open pollination), obtained at RIFG Pitesti that is recomanded like rootstock for peach, nectarin and some plum and apricot variety. *In vitro* propagation phases were realised efficient by using culture initiation on basal medium QL suplimented with 0.1 mg/l GA_3 and 0.01 mg/l IBA. The optimal multiplication rate of the buds was 1/8 and was realised on QL culture media with hormonal balance formed by 0.1 mg/l GA_3, 1.5 mg/l BAP and 0.2 mg/l ANA. Use of the DKW, MS, ½ MS and QL culture media with Walkey vitamins in rooting phase recorded to yield by 98% rooting plants on formula basal culture medium: QL, Walkey vitamins and hormonal balance represented by0.01 mg/l GA_3 and 1 mg/l IBA.

Keywords: macroelements, culture media, growth regulators
Cuvinte cheie: macroelemente, mediu de cultura, regulatori de crestere

1. Introduction

The disponibility for peach vegetalively rootstocks, nectarine and apricot for comercialy propagation till present is limited. Due to good compatibility it has with peach cultivars and some apricot and plum genotypes Adaptabil rootstock represents a good solution for nursery industry (Dutu I.et. al.., 2004)

For many fruit species/genotypes which aims is the propagation in great number, were established protocols for *in vitro* propagation, provideng advantages offered by this technique. One particularity is represented by the conditions that must to be optimisated for most species and genotypes (Damiano et.al., 2000).

As well as in other species, in the *Prunus* species the type of citokinine, and its concentration are important factors for multiplication and elongation rate (Leontiev, Orlov et al., 2000a), the genotype answer is different, undesirable effect can occur as a result of hyperacidity phenomenon (Leontiev, Orlov et al. 2000b; Pérez-Tornero and Bugos, 2000, Ambrose-Turk et al., 1991; Bouza et al, 1992). Many authors report that although it is well known that citokinin promotes cell division and shoots multiplication it is of great importance association with appropriate auxins (Ruzic and Vujovic, 2008). Fotopoulos et al., 2005, Durkovic, 2006 under certain good results obtained in vitro for cherry, concluded that the effect of auxins or citokinins on growth quantity and quality is not well known.

In vitro rooting culture stage is also reported in some culture conditions. Response of cuttings to application of exogenous auxins is dependent on many internal and external factors. So, the concentration and type of growth phytohormones applied (Zimmerman and Fordham, 1985, Caboni, et al., 1997) and above all, the answer of the genotype may be limiting factors (Dolezelova et al, 1996). Moreover, concentration and mineral composition of the culture medium affects the behavior of biological material *in vitro* evolution. Some researchers have proposed halving the normal concentration for improve the rooting capacity (Dimassi-Theriou, 1995).

Rooting of woody species, including *Prunus* genus species, can also be improved by applying a dark regimen during the first weeks of the rooting stage (Rugini et al., 1993; Caboni et al., 1997).

Given that, the propagation by tissue culture usually target the high multiplication rate and good rooting; the objective of this study was to evaluate the in vitro culture conditions for establishing an efficient propagation protocol.

2. Material and method

The explant source was represented by apically buds from annual branches of `Adaptabil` variety. The explants obtained were meristems with 0.5-1 mm size.

Desinfections of biological material consist of: washising with water and liquid detergent Tween 80 for 5 min; immersion in 6 % (w/v) $Ca(OCl)_2$ for 20 min; immersion in 90 % ethanol for 10 min; rinsed three times in sterile distilled water.

Culture media were represented by Murashige & Skoog (MS -1962), Quoirin & Lepoivre (QL-1977), vitamins Walkey, 1972, and Driver, J., A. Kuniyuki (DKW-1984). All media contained 40g/l dextrose, 8 g /l agar and 32 mg/l Na Fe EDTA. Growth regulators in various combinations and concentrations were added to each medium Resulted variants are shown in the table 1. After dissection and inoculation the cultures were maintained at 22-24^0 C, 16 light / 8 dark hours photoperiod.

3. Result and discussion

The observations effectuated after 30 days from the establishment of culture for initiation phase showed that explants have a maximum differentiation in terms of 95% on V1 (Figure 1). Differences were due to the basic components of culture media which were characterized by different strengths. So, MS culture media it's a media with a higher contents of ammonium and nitrate ions, while QL and DKW have a lower content. Comparing with the results obtained by other authors it is confirmed that an important factor in the response is the cultivar explants. Mohmood A. et al., 2009, obtained a yield of 60% explants differntiated on MS culture medium in peach variety 'Red June'.

The evolution of the multiplication phase was influenced by hormonal balance reported to quantities of citokinine, auxinic and gibereline combinations. Axillary buds appeared in greater number when the amount of BAP was 1.5 mg/l, combined with NAA 0.2 mg/l and GA_3 0.1 mg/l (fig 2). Hormonal balance characterized by 1.5 mg/l BAP combined with 0.5 mg/l NAA and 0.2 mg/l GA3 although gave approximately the same number of buds, shoots which developed were looking less vigorous than if V3.

The lowest multiplication rate was recorded in V1 multiplication in which hormonal balance BAP is 0.5 mg/l. Studies efectuated by Mansseri-Lamrioui A. et al. (2011) on cherry rootstock recorded the highest multiplication rate fluctuations with BAP ranging from 2-4 mg/l. In this case increasing the amount of BAP at 2.5 mg/l did not lead to a significant increase in the number of plants but was signalated a unwanted phenomenon, namely the vitrification emergence of the plants (photo 1-a, b).

A factor less studied in the literature on multiplication phase is the influence of the repeated subcultures. A decrease of the multiplication potential was observed after subculture 4, reported to V5 variant that occurred the best results (Fig. 3). However other authors ex. Debnath SC (2004), observed in the dwarf raspberry bush (*Rubus pubescens* Raf.) that multiplication rate increased by subculture to subculture until third then remained constant. In similarly mode, an increase of the multiplication rate was reported by Grant and Hamm (1999) in cherry and apple rootstocks. In experiments performed to rooting study was found that both types of auxinic used had influence in the evolution of risogenesis process (Table 2).

Rooting was induced in greater proportion by the use of IBA in the amount of 1 mg/l based on QL medium, in which it was obtained an average of 5 roots/plant. Increased IBA at 2 mg/l gives to alteration of the phenomenon of inducing roots. Incresing of the auxinic concentration up to 3 mg/l gives toxicity and manifestation of declining cuttings without rooting.

For NAA the amount of 1 mg/l in basal medium were recorded the maximum results that established an average of 2.3 roots/plant. In this case increasing the amount up to 3 mg/l give unrooted shoots. The influence of increasing IBA/NAA auxinic quantityes was also observed in both types of basic culture medium ½ QL and QL.

On the control media, without auxin was not registered rooting.

Another influencing factor was the concentration of macro and microelements in basic medium. The best results were recorded on culture medium with complete macro and microelements.

The benefical effect of using concentrated solutions can be attributed to the participation of inorganic ions in adjustment processes in regulating of hormonal balance (Amzallag et al., 1992). In this case QL is a medium with a low concentration compared to other basic medium, ex. for MS wich is recommended by some authors halved for different species. Dimassi-Theriou (1995), reported that reducing of the concentration of minerals in half for MS medium, increase the percentage of rooting and stimulate the root elongation for another rootstock, GF 677 peach.

4. Conclussions

Researches shows that the rootstock `Adaptabil` is suitability for propagation by *in vitro* culture with good results in optimized conditions.

By comparison with literature it is confirming that genotypes give contradictory answers by *in vitro* evolution.

For a success in micropropagation it is necessary suplimentary research regarding to establishment of a protocol corresponding in efficient mode to as many genotypes.

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Tables and Figures

Tabele 1. The experimental variants

Variant	***Basal medium***	***Phase of the culture***			
		Regeneration phase			
		Vitamins	***Growth regulators***		
			GA_3 (mg/l)	**IBA (mg/l)**	
V1	QL	Walkey	0.1	**0.01**	
V2	MS	MS	0.1	**0.01**	
V3	DKW	DKW	0.1	**0.01**	
			Multiplication phase		
		Vitamins	**Growth regulators**		
			BAP (mg/l)	**ANA (mg/l)**	**GA_3(mg/l)**
V1	QL	Walkey	0.5	0.2	**0.1**
V2	QL	Walkey	0.5	0.5	**0.2**
V3	QL	Walkey	1	0.2	**0.1**
V4	QL	Walkey	1	0.5	**0.2**
V5	QL	Walkey	1.5	0.2	**0.1**
V6	QL	Walkey	1.5	0.5	**0.2**
V7	QL	Walkey	2.5	0.2	**0.1**
V8	QL	Walkey	2.5	0.5	**0.2**
		Rooting phase			
		Vitamins	***Growth regulators***		
			GA_3 (mg/l)	**IBA (mg/l)**	**ANA (mg/l)**
V1	½ QL	Walkey	0.01	1	-
V2	QL	Walkey	0.01	1	-
V3	½ QL	Walkey	0.01	2	-
V4	QL	Walkey	0.01	2	-
V5	½ QL	Walkey	0.01	3	-
V6	QL	Walkey	0.01	3	-
V7	½ QL	Walkey	0.01	-	-
V8	QL	Walkey	0.01	-	-
V9	½ QL	Walkey	0.01	-	1
V10	QL	Walkey	0.01	-	1
V11	½ QL	Walkey	0.01	-	2
V12	QL	Walkey	0.01	-	2
V13	½ QL	Walkey	0.01	-	3
V14	QL	Walkey	0.01	-	3
V15	½ QL	Walkey	0.01	-	-
V16	QL	Walkey	0.01	-	-

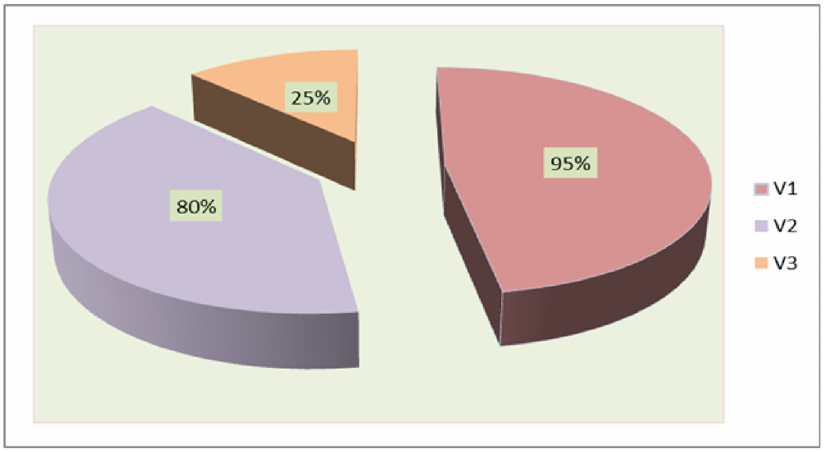

Fig. 1. Evolution of `Adaptabil` rootstock by basal culture media

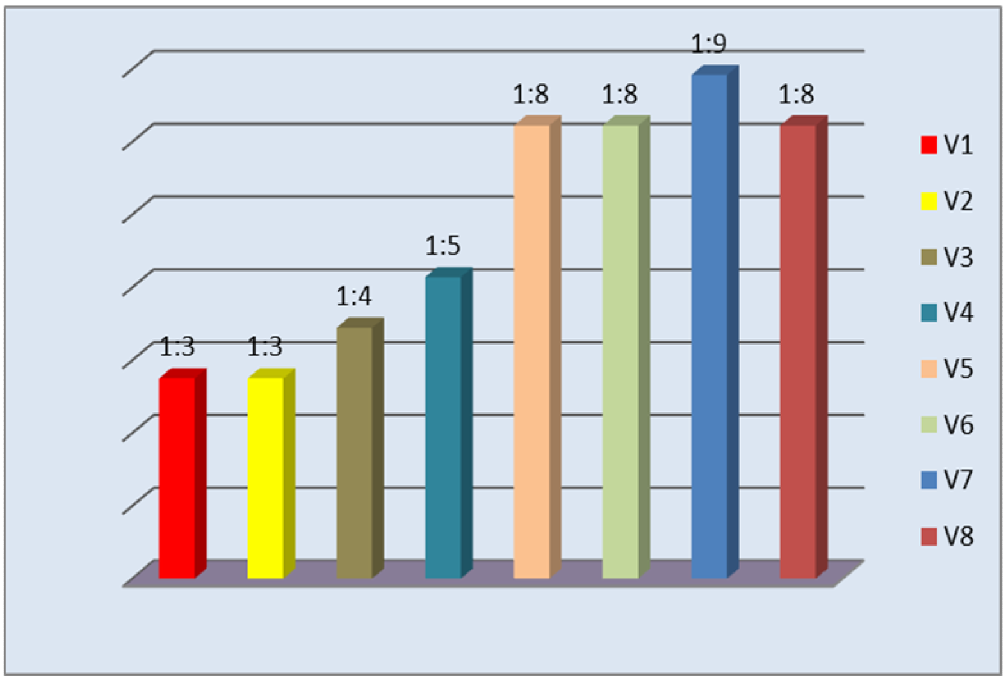

Fig. 2. The influence of hormonal balance on multiplication of the `Adaptabil` rootstock

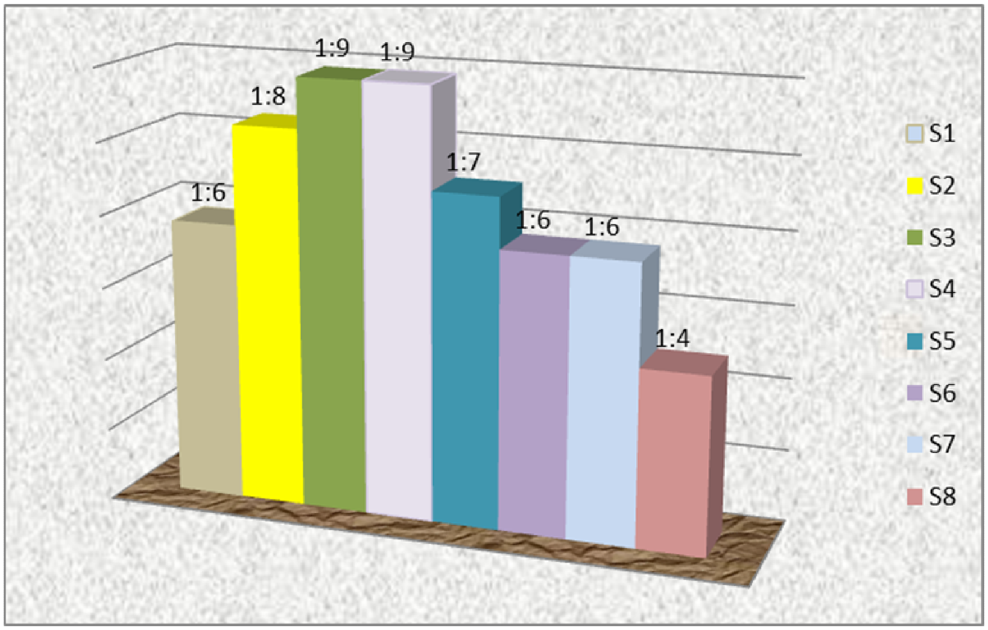

Fig. 3. Effect of the succesiv subcultures to `Adaptabil` rootstock on QL culture media suplimented with 0,1 mg/l GA_3, 1.5 mg/l BAP and 0,2 mg/l ANA

Table 2. Effect of hormonal balance and salts concentration on rooting phase for `Adaptabil` rootstock

Experimental variant	Rooting coefficient (%)	Average of roots number	Average of roots length (mm)
V1	98	5	25
V2	80	4.2	20
V3	65	3	14.9
V4	50	3	14
V5	-	-	-
V6	-	-	-
V7	-	-	-
V8	-	-	-
V9	70	2.3	23.5
V10	50	2	12.8
V11	30	2	12.6
V12	25	1.7	8.5
V13	-	-	-
V14	-	-	-
V15	-	-	-
V16	-	-	-

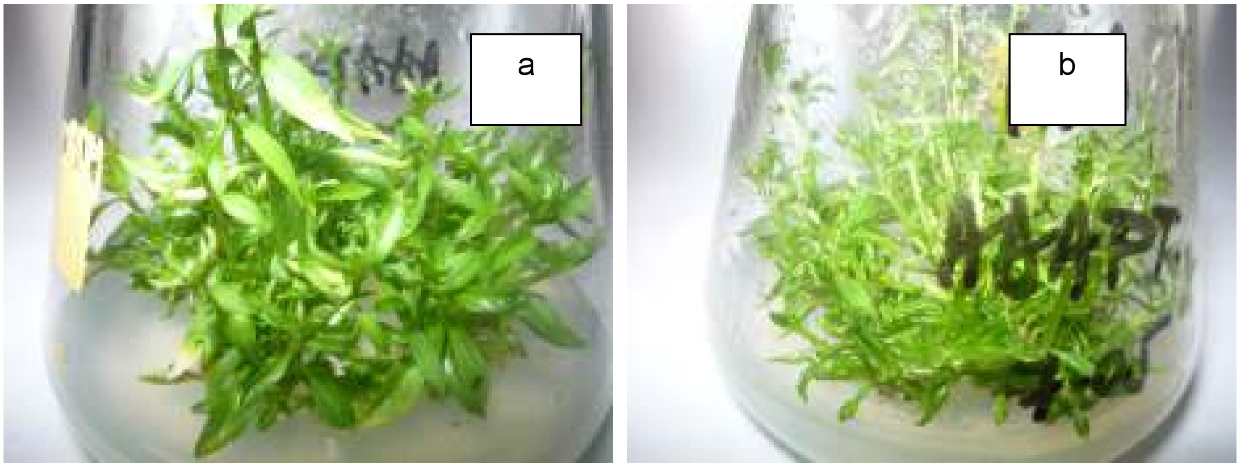

Foto 1 – Multiplication phase to `Adaptabil` rootstock: a) culture with normal aspect; b) cultute with vitrification aspect