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The Effectiveness of the Concentration of Fhytohormones in the Micro Propagation of CAB6P (Prunus Cerasus)

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Abstract

This study was carried out to determine the most appropriate culture and plant growth regulators for micro propagation of CAB6P at AUT in the Department of Horticulture & Landscape Architecture. In the scope to evaluate the most efficient and effective protocol for the micro-propagation of the CAB 6P are analyzed four variants of rooting media with auxin additions naphtha acetic acid ANA, benzyl amine Purina BAP, Indol butyric acid IBA and gibberellins GA_3 and without any change in the amount of vitamins in Murashige and Skoog: (i) Macro elements ½ MS, MS microelements, 0.1 mg l^{-1} ANA; (ii) Micro elements ½ MS, Macro elements MS, 0.1 mg l^{-1} ANA; (iii) Macro elements MS, microelements ½ MS, 2 mg l^{-1} ANA, iv) Macro Elements MS, Micro Element ½ MS, BAP 0.3 mg l^{-1}, IBA 0.1 mg l^{-1}, GA3 6.7 mg l^{-1}Explants of species *prunus cerasus L* showed better results during inoculation and organogenesis in rooting media i, where rooting index was very high (90%), while in rooting media ii (75%).In rooting medium iii, high concentrations of ANA auxin induce the formation of callus at the end of the stomach in the plantlets of CAB 6P. Variance Analysis (ANOVA), using JMP software during the rooting phase of explants in four types of media rooting, confirms some changes in terms of rooting index in each media.

***Key words*:** CAB 6P; Proliferation; Micro-propagation; Plantlets, BAP; ANA and GA_3.

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1. Introduction

Rootstock CAB 6P is widely cultivated to be used as a very good rootstock for persistence [1].

It is somewhat short and can be easily maintained at 8-10 ft high, is of early development and with great output, and is easy to manage.

Reference [4] Trees in CAB 6P should be pruned early in life to keep the size of the fruit. It is much easier to form new branches in CAB 6P than in Gisela 5 and is one of the reasons for the popularity of this rootstock. Gisela 5, besides the fact that it is sensitive to bacterial cancer, produces a small tree on which it is difficult to grow the tree and hold energy, and Gisela 12 tends to produce larger trees with somewhat deeper roots [3].

However, CAB 6P is a rootstock with priority in features to and our study pays attention [8]. The choice of nutrient media in "In vitro" propagation depends on plant species, tissue or organ in culture and the purpose of culture [6]. Success depends on knowing the nutritional needs of the tissues.

Reference [7] As a universal media for the beginning of callus from the tissues of dicotyledonous is considered basal media Murashige and Skoog (MS).

Reference [2] Its characteristic is the relatively high concentrations of nitrates, potassium and ammonium [5]. As we have noted above, there is still a smoke in the nurseries of eastern Europe for the addition of rootstocks of prunus and more on CAB 6P as a rootstock recently released in the market [9].

1.1 Material and Methods

For rootstock CAB 6P there are no references to micro propagation but only for Gisela 6, for which references point out that the IBA increased in MS we have successful rollout

In earlier experiments at the In-Vitro Laboratory of AUT Albania we tried with IBA but the result was not significant.

The rooting response was evaluated after 4-5 weeks of cultivation in each rooting medium.

In all media, the pH is specified at the value of 5.6 and sucrose and agar is added respectively to 30 g l^{-1} and 3%.

When the explants reached lengths of 2 to 4 cm, they were transferred to rooting media.

To evaluate the most efficient and effective protocol for the micro-propagation of the CAB 6P are analyzed four variants of rooting media with auxin additions naphtha acetic acid ANA, benzyl amine Purina BAP, Indol butyric acid IBA and gibberellins GA3 and without any change in the amount of vitamins in Murashige and Skoog:

(i) Macro elements ½ MS, MS microelements, 0.1 mg l^{-1} ANA;

(ii) (ii) Micro elements ½ MS, Macro elements MS, 0.1 mg l^{-1} ANA;

(iii) (iii) Macro elements MS, microelements ½ MS, 2 mg l^{-1} ANA;

(iv) iv) Macro Elements MS, Micro Element ½ MS, BAP 0.3 mg l^{-1}, IBA 0.1 mg l^{-1}, GA3 6.7 mg l^{-1}

Nutritional media for four alternatives:

Table 1: Nutritional media and concentrations of synthetic hormones added in the variant (i)

Macro element 1/2		**25ml**
Microelement		**50ml**
Fe-EDTA		**5,0ml**
Vitamin		**10ml**
ANA	0,1mg.l^{-1}	0,05mg
Sugar	**30g.l^{-1}**	**15gr**
Agar	**6,7g.l^{-1}**	**3,35g**
ph	**5,6**	

Table 2: Nutritional media and concentrations of synthetic hormones added in the variant (ii)

Macro element		**50ml**
Microelement 1/2		**25ml**
Fe-EDTA		**5,0ml**
Vitamin		**10ml**
ANA	0,1mg.l^{-1}	0,05mg
Sugar	**30g.l^{-1}**	**15gr**
Agar	**6,7g.l^{-1}**	**3,35g**
ph	**5,6**	

Table 3: Nutritional media and concentrations of synthetic hormones added in the variant (iii)

Macro element		**50ml**
Microelement 1/2		**25ml**
Fe-EDTA		**5,0ml**
Vitamin		**10ml**
ANA	2,0mg.l^{-1}	2,0mg
Sugar	**30g.l^{-1}**	**15gr**
Agar	**6,7g.l^{-1}**	**3,35g**
ph	**5,6**	

Table 4: Nutritional media and concentrations of synthetic hormones added in the variant (iv)

Macro element		**50ml**
Microelement 1/2		**25ml**
Fe-EDTA		**5,0ml**
Vitamin		**10ml**
BAP	0,3mg.l^{-1}	0,15ml
IBA	0,1mg.l^{-1}	0,05ml
GA-3	6,7g.l^{-1}	0,15gml
Sugar	**30g/l**	
Agar	**3,35g^{--}**	
Ph	**5,6**	

a b c d

Figure 1: (a, b, c, d). a) Media Preparation b) Weighting of micro macro elements and c) Adjustment of pH d) inoculation of explants

a b

Figure 2: Autoclaved media R1, R2, R3, R4 (a and b)

1.2 Results and Discussions

The rooting stage

Plants of *prunus cerasus L* species showed better results during rooting cultivation i, where the rooting index was very high (90%), while in rooting ii (75%).

In the latter case (rooting medium iii), high concentrations of ANA auxin induce the formation of the shoots at the end of the stem in the CAB 6P plantlets. In this case, the number of roots is high, but they have an abnormal appearance, as they are too short and thick.

The variance analysis (ANOVA), during the rooting stage of the four types of rootstock rootstocks, confirms some changes with respect to rooting index in each field (Table 3).

Based on the Variance Analysis Table ($P < 0.05$), since the value F is much greater than the theoretical value (Prob> F) then there is a statistical difference in the comparative data.

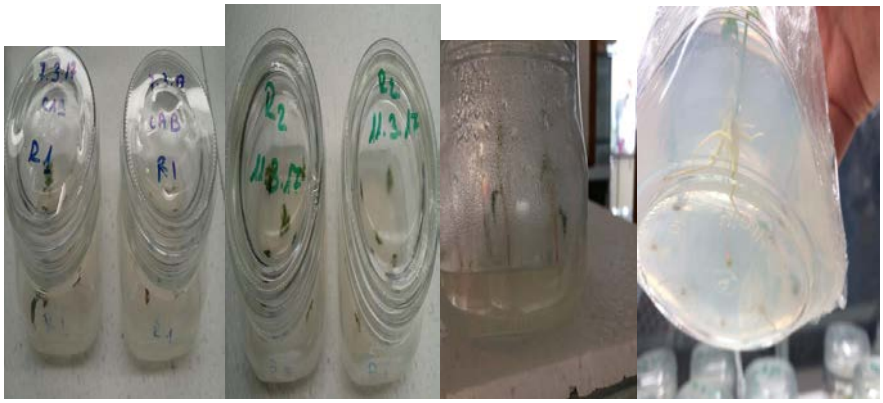

Figure 3: Rooting plantlets on media (i) with Macro elements ½ MS, microelements MS, vitamins MS combined with 0.1 mg l^{-1} ANA; (ii) Macro elements ½ MS, microelement MS, vitamins MS combined with 0.1 mg l^{-1} ANA;

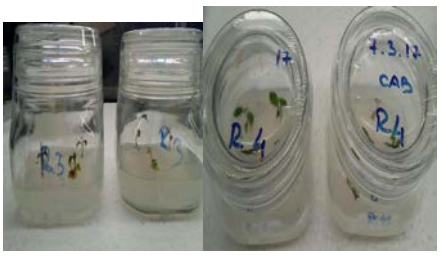

Figure 4: Rooting Planlets on media (iii) with Macro elements MS, microelement ½ MS, vitamins MS combined with 2 mg l^{-1} ANA; (iv) with Macro elements MS, microelement ½ MS, vitamins MS of combined with BAP 0,3mg.l^{-1}, IBA 0,1mg.l^{-}, GA-3 6,7g.l^{-1}

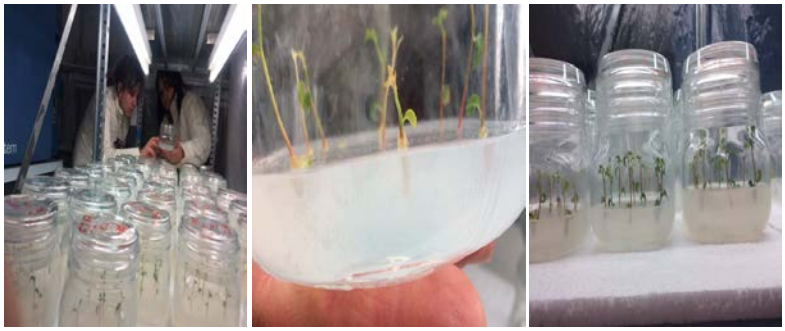

Figure 5: Plantlets proliferated and with callus on (iii) dhe (iv) without roots

Table 5: Statistical changes min, max, Dv st in 2017 for (i) (SPSS)

Trajtimi	Min	Max	Mean ± Dv St
Nr i bimeve	**5**	**5**	**5 ± 0**
Gjatesi rrenjeve	**2**	**3**	**2.8 ± 0.2**
Nr I gjetheve	**5**	**7**	**6 ± 0.81**

Table 6: Statistical changes min, max, Dv st in 2016 for (i) (SPSS)

Trajtimi	Min	Max	Mean ± Dv St
Nr i bimeve	**3**	**5**	**4.2 ± 0.9**
Gjatesi rrenjeve	**2**	**4**	**2.8 ± 0.8**
Nr I gjetheve	**3**	**5**	**4 ± 0.8**

Table 7: Statistical changes min, max, Dv st in 2017 for (ii) (SPSS)

Trajtimi	Min	Max	Mean ± Dv St
Nr i bimeve	**4**	**5**	**4.5 ± 0.6**
Gjatesi rrenjeve	**2**	**5**	**2.9 ± 1.4**
Nr I gjetheve	**3**	**5**	**4 ± 0.8**

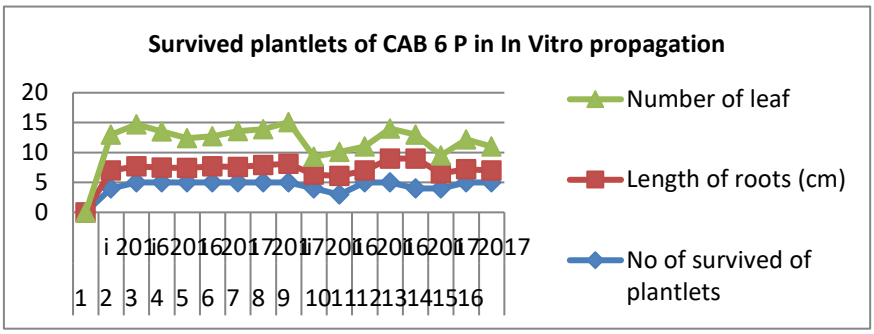

Figure 6: Index of rooting in four different media of sp. *Prunus avium cerasus L*, rootstock CAB 6 P

Scatterplot Matrix

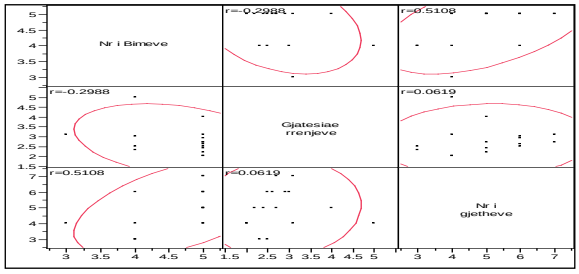

Figure 7: Correlation of Search Indicators for rooting planlets in In vitro culture in two different media of sp. Prunus cerasium L rotstock CAB 6

2. Conclusions

1. MS1 with BAP 0.5 mg/L^{-1} and ANA 0.1 mg/L^{-1} mg, MS2 with BAP 0.5, IBA 0.1, GA_3 0.1, suitable for micro-propagation.
2. The best medium for proliferation was MS 1 with: BAP 0.5, NAA 0.1, GA_3 0.1, shown to be good.
3. Rootstock CAB 6P, in MS1 media, which containing: 0.5 mg l^{-1} BAP and NAA 0.1 mg/l^{-1}, had the best proliferation and rooting too.
4. For rooting the best combination of culture medium was achieved with treatment in MS 1/2 with 1 mgl^{-1} IBA, followed by transfer to a hormone medium BAP 1 mg l^{-1}, NAA 0.1 mg l^{-1} and GA_3 0.1 mg l^{-1} after 45 days, that resulting in 88% success.

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