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Optimization of the ground for CAB 6P (prunus cerasus)

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Abstract. Rooting is a process that is induced by many difficulties, especially in fruit trees. For this reason, four nutrient terrains that contain different concentrations of auxin ANA (α -naphthaleneacetic acid), BAP, GA3 and macro and micronutrient MSs are compared. Rizogenesis is observed after three to four weeks of cultivation on the ground of rupture. Explants react differently in each field used (Figures 4,5,6,7). The concentration of inorganic and auxinous ANA salts on the nutrient terrains affects the rooting index and the characteristics of the roots. Plants of the *prunuscerasium* L species showed better results during rooting cultivation I, where the rooting index was very high (90%), while in rooting field II (75%) (Mirrors, Graphs a, b). In the latter case (the rooting ground III), the high concentrations of ANA auxin induce the formation of the colon at the end of the stem in the CAB 6P herb. In this case, the number of roots is high, but they have an abnormal appearance, as they are too short and thick (Fig. B). The variance analysis (ANOVA), during the rooting stage of the four types of rootstocks, confirms some changes with respect to rooting index in each field (Table 3, Chart 1a, b). 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.

Introduction

The biotype of *PrunusCerasus* (CV Maraska di vigo) developed by the University of Bologna is used as a rootstock for cherry as well as for sour cherry. It is middle-class productivity the rootstock, the best results are achieved with a T-bar or a sleeping chip. Cherry compatibility with this rootstock is very good, while the variety of Sour Cherry has excellent compatibility. It has a moderately developed root system, adapts to heavy soils with low perspiration while resistance to limestone reaches up to 9% of active lime. It is sensible by *Armellariamellea* and solid from *phytophoracactorus* and *Verticillium*.

The varieties of cherries, grafted in this rootstock have a time of blooming and maturing for 2-3 days earlier than those grafted in the seedbed. Fruits are of good quality and of optimal size. It is a suitable rootstock for specialized cherry orchards where the number of herbs must reach 500-800 plants per hectare.

Nutrition terrain selection

Choosing a particular field depends on plants specifics, tissue or organ in culture and the purpose of culture. Success depends on knowing the nutritional needs of the tissues. As a universal ground for the start of the callus from the dicotyledonous tissues is considered the terrain BasalMurashige and Skoog (MS). Its characteristic is the relatively high concentrations of nitrates, potassium and ammonium (George, 1993).

The mineral composition of the terrain has been studied as an important factor affecting in vitro addition of the 'Gisela 5' from Ružić et al. (2000) that received a better growth and development in MS and MS × 2 than ½ MS and ¼ MS.

Šiško (2011) reported that the WPM terrain shows the highest degree of addition (4.2 explants) in the 'Gisela 5', while MS with lower values (3.0 explants).

Bošnjak Et al. (2012) successfully added 'Gisela 5' to the Quorin and Lepoivre - QL terrain (1977). Terrain DKW medium contains 0.5 mg • l-1 BAP was used for slow augmentation of Gisele (Lambardi et al., 2006).

Dorić et al. (2014) used this field In vitro addition of various cherry subgroups, including 'Gisela 6'. Although MS is still more widely used, it is often replaced with low salt concentration, especially those with lower nitrogen content, including ammonium nitrate content, which is 1650 mg l-1 in MS and 1416; 400 mg l-1 in DKW and WPM.

In Vitro Addition remains a priority since the phytosanitary situation is of paramount importance in the production of new uninfected cultivars. Regarding the spread of viruses in fruit trees, appearing in many recent publications, prunus tree tests in Albania reveal that 42% of the tested trees are infected with at least one virus. Cherries and plums are the most infected species, with 56% and 47% respectively

Treatment of seeding materials of fruit trees with the use of in vitro meristemic methods for the elimination of viruses for important species of Prunus, Malus and Pyrus grown in Albania and Kosovo remains to be a future challenge. That's why I have the ambition to bring successful results, in researching the objectives and develop a successful method for Kosovo which does not have a micro propagation laboratory.

Nutritional grounds

The success of "in vitro" culture depends heavily on the chemical composition of the field of culture. For the optimal plant growth, it is necessary the presence of relatively large quantities of macro elements, small quantities of microelements, iron supply, carbohydrates, vitamins and especially phytohormones.

Microelements

Plant tissue cultures require continuous supply of some inorganic salts. Essential elements are N₂, K, Ca, Mg and S (Table 3) (Taiz&Zeiger, 2006), Nitrogen, Phosphorus, Magnesium, Sulfur Potassium, Calcium, etc. Many authors present 5-9 microelements in the form of sulphates or chlorides as indispensable in very small quantities (usually several mg l-1). The most prominent and most present in the nourishing culture are Mn, Zn, B, Cu, Co, J and Mo (Mirror 3). Agar is also a source of many microelements, it has traces of vitamins, and possibly toxic substances (Pierik, 1988).

Phytohormones and growth regulators

From culture tissue often is required a combination of other auxins and phytohormones.. The term "phytohormone" is reserved for natural plant hormones. Growth regulators are called synthetic as 2,4-D (dichlorophenoxyacetic acid) or kinetin (Salisbury & Ross, 1992). Phytohormones and growth regulators are used in very small quantities, ranging from 0.01 to 10 mg l-1, and greatly determine the cruciality of the growth during embryogenesis or morphogenesis

(Tech & Seiler, 2004). But their use in the nourishing ground depends mainly on the type of the plant and plant species and by endogenous phytohormones. Mainly three phytohormone categories are used:

Auxines

It plays a role in cell growth and division, differentiation of encephalopathy, different bones and roots tropisms, fruit growth etc. In tissue cultures, auxins are used to stimulate the growth of boulders, start initiation of roots and extension, induction of somatic embryogenesis and the onset of the callus formation. Auxin causes tissue swelling and stiffens the formation of roots in high concentrations. (Tech & Seiler, 2004).

Among the natural auxins, indolacetic acid (AIA) is used in culture. 3-indol butyric acid (AIB) are highly effective. AIB is the root-rooting agent. Naphthylacetic acid (ANA) and dichlorophenoxyacetic acid (2,4-D) are synthetic auxin. 2,4-D is the most effective auxin for callus proliferation but is used in very small quantities, 10^{-7} - 10^{-5} M, as it is highly toxic (Satyavathi et al., 2004; Taiz&Zeiger, 2006).

Cytokine

They are used to stimulate plant growth and development, because they favour cell division, cytokinesis and callus organization, especially when combined with auxins. Cytokinins are involved in cell division, morphogenesis, the onset of the formation of shoots, affecting apical dominance, and so on. (Tech & Seiler, 2004). Of the cytokines, most useful in the soil is kinetin (6-furfurylaminopurine), BAPP or BAP (6-benzylaminopurine or 6-benzyladenine) and zeatin. The first two are synthetic, while zeatin is natural (Taiz&Zeiger, 2006).

Giberelins

Not widely used in "in vitro" culture, but GA3 (gibberellic acid) seems to be more usable. After autoclaving, its activity loses 90%. Generally, gibberellins induce protuberance and increase the meristems or buds. They also interrupt the deep sleep of embryos or isolated seeds (Tech & Seiler, 2004; Taiz&Zeiger, 2006).

It should be noted that the auxin / cytokine ratio is an instrument in regulating cell division, prolongation, cell differentiation, and organ formation. In general, low concentrations of auxin and high levels of cytokines stimulate cellular growth.

Some cultures are capable of rapidly forming in an autotrophic way after several recurrent subcultures. The advantage of using these tissues is the high intensity of their growth and the low cost of the field without phytohormone.

Also, in some cases, no phytohormone is used on the ground eg. in the "in vitro" genetic banks, when vegetative material is kept in a state of minimum growth for several months to a year (Kongjika et al., 1998) or during subcultures when cultures have been developed in-vitro, have sucked sufficient amount of phytohormones during the first stages of development (Kongjika et al., 1995). The PH is normally adjusted between 5.5 and 6.0 before autoclaving, but varies during autoclaving and during the culture period.

Material and Method

When the implants reached lengths of 2 to 4 cm, they were transferred to the rooting ground. Three variants of terrains were analyzed with auxin supplements ANA, BAP, IBA and GA3 during root formation :

- ½ MS macroelement, MS microelements, MS vitamins combined with 0.1 mg l⁻¹ ANA;
- ½ MS macroelement, MS microelements, MS vitamins combined with 0.1 mg l⁻¹ ANA;
- MS macroelement, micro-element ½ MS, MS vitamins combined with 2 mg l⁻¹ ANA;
- MS macro-elements, micro-nutrients ½ MS, MS vitamins combined with BAP 0.3mg.l⁻¹, IBA 0.1mg⁻¹, GA-3 6.7µl

For CAB 6P rootstock there is no reference in the microscope but only for gyrus 6, for which the references emphasize that with an increased percentage of IBA in MS we have a successful root formation.

In Experiment II we tested with IBA but the result was not significant

The rooting response was evaluated after 4-5 weeks of cultivation on each rooting ground. In all the fields, the pH is specified at the value of 5.6 and sucrose and agar is added respectively to 30 g l⁻¹ and 3%.

Table 1. Nutritional sites and concentrations of synthetic hormones added to them (R1, R2, R3 and R4)

(R 1)

Microelements ½		25ml
Microelement		50ml
Fe-EDTA		5,0ml
Vitamin		10ml
ANA	0,1mg.l⁻¹	0,05mg
Sucrose	30g.l ⁻¹	15gr
Agar	6,7g.l ⁻¹	3,35g
Ph.	5,6	

(R 2)

Microelements		50ml
Microelement ½		25ml
Fe-EDTA		5,0ml
Vitamin		10ml
ANA	0,1mg.l⁻¹	0,05mg
Sucrose	30g.l ⁻¹	15gr
Agar	6,7g.l ⁻¹	3,35g
Ph.	5,6	

(R 3)

Microelements		50ml
Microelement ½		25ml
Fe-EDTA		5,0ml
Vitamin		10ml
ANA	2,0mg.l⁻¹	2,0mg
Sucrose	30g.l ⁻¹	15gr
Agar	6,7g.l ⁻¹	3,35g

Ph.	5,6	
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(R 4)

Microelements		50ml
Microelement ½		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
Sucrose	30g/l	
Agar	3,35g ⁻	
Ph.	5,6	



Figure 1 (a, b, c).a) Field preparation b) Micro- macro elements and weighing c) PH adjustment.



Figure 2. Laminar flushes before the initiation of inoculation for root-formation, stinging sterilizers.



Figure 3. Laminar flushes before the initiation of inoculation for root-formation, stinging sterilizers.



Figure 4. Plants at the stage of root formation in the laboratory, vegetation rooms of *P. avium* L.

Results and Discussion

Rooting is a process that is induced by many difficulties, especially in fruit trees. For this reason, four nutrient terrains that contain different concentrations of auxin ANA (α -naphthaleneacetic acid), BAP, GA3 and macro and micronutrient MSs are compared.

Rizogenesis is observed after three to four weeks of cultivation on the ground of rupture. Explants react differently in each field used (Figures 4,5,6,7). The concentration of inorganic and auxinous ANA salts on the nutrient terrains affects the rooting index and the characteristics of the roots. Plants of the *prunuscerasium* L species showed better results during rooting cultivation I, where the rooting index was very high (90%), while in rooting field II (75%) (Mirrors, Graphs a, b).

In the latter case (the rooting ground III), the high concentrations of ANA auxin induce the formation of the colon at the end of the stem in the CAB 6P herb. In this case, the number of roots is high, but they have an abnormal appearance, as they are too short and thick (Fig. B). The variance analysis (ANOVA), during the rooting stage of the four types of rootstocks, confirms some changes with respect to rooting index in each field (Table 3, Chart 1a, b). Based on the Variance Analysis Table ($P < 0.05$), since the value F is much greater than the theoretical value ($\text{Prob} > F$) then there is a statistical difference in the comparative data.

	CAB 6 P						
Root formatio n terrain I	90 ± 2,3367 St dev 5,2249 4	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob> F
		Species	1	8294,4000	8294,40	256,0000	<,0001
		Error	8	259,2000	32,40		
		C.Total	9	8553,6000			
Root formatio n terrain II	10 ± 1,9235 St dev 4,3011 6	Source	DF	Sum of Squares	Mean Square	F Ratio	Prob> F
		Species	1	10562,500	10562,5	211,2500	<,0001
		Error	8	400,000	50,0		
		C.Total	9	10962,500			
Root formatio n terrain III							
Root formatio n terrain IV							

The positive effect on the induction of rhizogenesis of one of the auxin, α -naphthaleneacetic acid (ANA), has been reported in several authors' studies in the in vitro fertilization of apple (Nemeth, 1981, Monter, 1992). Comparison of different terrains showed that the use of high concentrations of auxin (2-3 mg l⁻¹) favours the formation of the callus and limits the growth of the roots. Also in other studies with P. avium species (Shatnavi et al., 2007), the same phenomenon was observed using ruminant hormones (AIB, ANA and AIA). As a result, it is recommended to use in lower than 0.5 mg l⁻¹ doses.

Conclusions

Based on this research we have reached to the following conclusions:

1. The "in vitro" (microshum) culture of the Prunusavium species results as an efficient method for the propagation of these plant species;
2. P. exteries exhibit better results during the rooting cultivation I containing ½ MSmacroelements, MS microelements, MS vitamins combined with 0.1 mg l⁻¹ ANA;
3. P. aviumexteries exhibit better results during the Rooting II cultivation that contains ½ MS macroelement, MS ½ microelements, MS vitamins combined with 0.1 mg l⁻¹ ANA;
4. The in-vitro (microshumium) culture of Prunuscerasium species results as an efficient method for propagating this species plant.
5. During the direct organogenesis of the organized systems results in a high micro-shrinkage coefficient (6-7 bushes) and thus it is possible to obtain a large number of herds after some subcultures.
6. High rooting index (75%) is observed using the first tested variant of the feeding environment. This variant also represents the highest percentage of survival (73%).
7. Problematic is a very low index of acclimatization for which the next work will be done.

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