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USE OF BIOTECHNOLOGIES METHODS IN MULTIPLICATION OF NATIVE *PRUNUS* SP. GERMPLASM

SUMMARY

The present study was carried out to investigate the use of "in vitro" methods as an alternative form of propagation for two different *Prunus* germplasm native resources. Shoot tips explants collected in the Tirana region were cultured in an MS medium supplemented with different types of plant growth regulators (ANA, BAP and GA_3) during the proliferation and multiplication phases. Results were elaborated with factorial experiment with two factors in completely random design layout. During proliferation, apricot explants were recorded as having higher values for the shoot length parameter (±10.6mm) in comparison with that of plum explants (± 6.3mm). Different results were observed for leaf parameters, where plum shoot tips presented a higher number of leaves (±8.6) than apricots (±5.2). During subculture, medium composition positively affects the new plantlets biometry, giving plum plantlets the highest number of leaves (±16) and new apricot plantlets the highest value of shoot length (±2.2cm). The multiplication coefficient recorded for the two *Prunus* plantlets was more than 4 buds/explants. Our results suggested that the method used in this study was appropriate for the multiplication of the studied *Prunus* germpalsm.

Keywords: propagation, *Prunus*, germplasm, growth regulators, medium

INTRODUCTION

The application of tissue culture methods plays an important role in the evaluation of different genetic resources. Advantageous in comparison with other multiplication methods, tissue culture methods assuring clone material to evaluate through rapid propagation over a short period (Kongjika et al. 2002). *In vitro* micro-propagation is useful for the clone multiplication of endangerous species; *in vitro* tissue cultures for stone-fruits represent an alternative propagation method separate from traditional techniques used for woody plants.

Micro-propagation has been used for commercial production of stone-fruit species since late 1970. Initially it was used for a small fruit species, such as strawberries and raspberries, and the rootstocks of some stone-fruits, particularly the peach (Zimmerman, 1991). Reports on the tissue culture of *Prunus* species,

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like apricots and plums, are encountered very little in the literature and most of them are recent (Murai et al. 1997; Balla and Vértesy, 2001; Mahmood et al. 2009). Most commercial production is focused on the production of rootstocks.

In Albania, given the difficult transitory conditions which influence genetic erosion, the use of tissue culture methods may be important not only for the preservation of biodiversity, but also for the use of autochthonous plant genetic resources (Kongjika et al. 2010).

Published studies on *in vitro* propagation of the native germplasm species of the genus *Prunus* in our country are scarce (Spahiu 2008; Bode et al. 2010; Kongjika et al. 2011). The study in question will contribute to the assessment of the native species of the genus *Prunus*, through application of the *in vitro* technique.

MATERIAL AND METHODS

Plant material: Active shoot tips and lateral bud explants were collected during the spring of 2010 from apricot and plum populations in the Tirana region. A tissue culture experiment was conducted in the Tissue Culture Laboratory of Biotechnology Department, Natural Science Faculty, Tirana.

Explants disinfection: Before undergoing the inoculation process under laminar flux, explants of two populations were treated with a 70º ethanol solution (1 minute) and rinsed three times in distilled water, followed with 0.01% $HgCl_2$ solution for 5 minutes (again rinsed three times in distilled water).

Proliferation phase: The composition of two different nutrient media:

1.Basal nutrient media, MS (Murashige and Skoog, 1962), was supplemented with 0.7 mg L^{-1} BAP, ANA 0.01 mg L^{-1} and GA_3 0.1 mg L^{-1}. Other elements in this media were saharoze 3%, agar 0.6% and pH 5.6.

2.A Modified nutrient media for woody plants (WPM) (Lloyd and McCown, 1981), supplemented by $MgSO_4$ of MS media and only in conjunction with the 1 mg l^{-1} BAP. Other elements in this media were saharoze 3%, agar 0.6% and pH 5.9.

Multiplication phase: Based on the behavior of the two populations of *Prunus* during in the proliferation phase, the media used in the second phase was WPM for the apricot explants and MS for the plum explants (supplemented by 0.3 mg L^{-1} BAP). The data recorded during the proliferation and multiplication phases dealt with biometric parameters that were statistically elaborate using a factorial experiment with two factors in a completely random design layout.

RESULTS AND DISCUSSION

Disinfections results:

The plum and apricot explants reacted positively to the disinfectant method used, with a high survival rate of 58.97% and 66.6%, respectively. The contamination level of the plum explants was 17.3%; for apricots, it was 13.5%. Our results are lower than those reported by Rodrigues et al. (1999), in which the contamination level of the *Prunus* rootstocks ranged from 50-95.8%. These

authors found that the high percentage of contamination is a significant effect related to the genotype during *in vitro* establishment process. Our results agree with the survival rate of the *Prunus persica* explants, as reported by other authors (Silva et al. 2003; Hammerschlag, 1982).

Results of the proliferation phase:

Plant explants were inoculated in two different media. The plum explants reacted positively, with 40-70% of proliferation. They were only exposed to the MS media, emphasising the idea that composition with micro and macro elements combined with the presence of different types of hormones, can affect the proliferation process of plant material. On the contrary, in the WP media, the plum explants reacted negatively (0 %) (Fig. 1). Shoot tips and lateral apricot buds proliferated on different levels in the two nutrient media used. WP media was accelerated: the apricot explants proliferated at a rate of 62%, whereas for MS media it was 20% (Fig. 1).

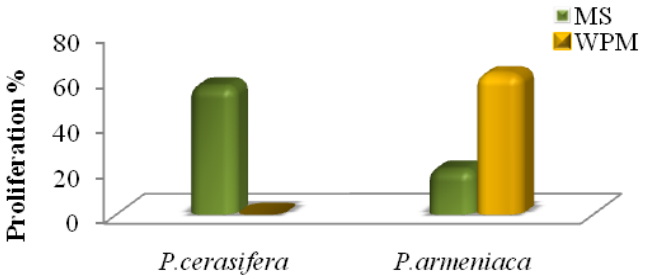


Figure 1. Proliferation of two Prunus species in different nutrient media
(10 explants with 4 replicates each)

According to Murai et al. (1997) the reason full strength MS was not successful at culturing apricot explants might be attributed to a high concentration of nitrogen and/or high total salts. Nitrogen concentration in WPM is less than that of MS medium. Reports from other studies suggest the use of MS media in comparison with different nutrient media for the establishment and *in vitro* multiplication of *Prunus* species. According to Mante et al. (1989) the proliferation level of plum explants on basal MS media was 20-80%. Mahmood et al. (2009) reported that *Prunus persica* explants react positively on MS full media (60% proliferation) when compared with half the MS media. Biometric parameter results (shoot length, and number of leaves), obtained during the proliferation phase, are illustrated in Figures 2 and 3.

The explant and genotype can affect the development dynamic of our two *Prunus* species. Plantlets originated from apricot shoot tips, presenting a mean shoot length higher in value than plum plantlets. The results illustrated in Figure 2 (Table 1) express different reactions not only between species but within the same species, whereas lateral buds originated in plantlets with higher values of length. The opposite was true for apricot shoots.

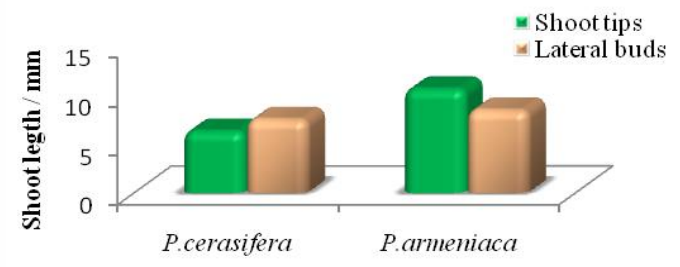


Figure 2. Effect of explants type on shoot length parameter
(10 explants with 4 replicates each)

Table 1. Effect of the explant type on plantlet shoot length originated from active shoot tips during the proliferation phase.

Source of variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	185	1	185	15.3343518**	0.0009284	4.3807497
Columns	280	19	14.7	1.221737	0.3334417	2.1682516
Error	229	19	12.1			
Total	694	39				

LSD 005-2.3, *LSD 001-3.14, Cv %- 49.9.*

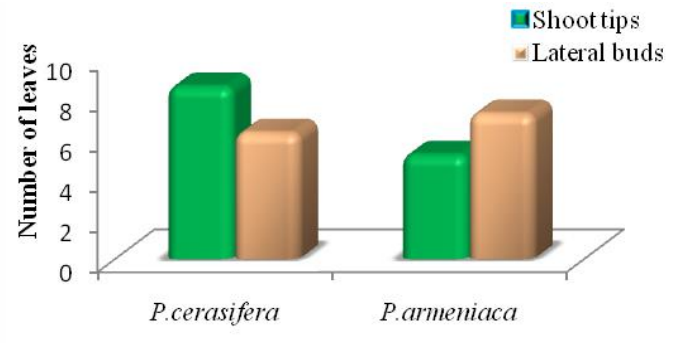


Figure 3. Effect of explant type on number of leaves/plantlets
(10 explants with 4 replicates each)

Table 2. Effect of explant type on the number of leaves parameter, learned from plantlets that originated from active shoot tips during the proliferation phase.

Source of variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	115.6	1	115.6	8.6677**	0.00833	4.38075
Columns	228.6	19	12.03	0.902131	0.58765	2.168252
Error	253.4	19	13.34			
Total	597.6	39				

LSD 005-2.41, *LSD 001-3.3, Cv %- 56.73.*

Our results are in agreement with those of Rogalski et al. (2003). It was also reported that the use of cytokinin (BAP) was necessary for the shoot development of *Prunus sp*. Plum plantlets that originated from shoot tips presented a higher number of leaves in comparison with apricot plantlets, while apricot lateral buds create new plantlets with a higher number of leaves (Table 2. Figure 3).

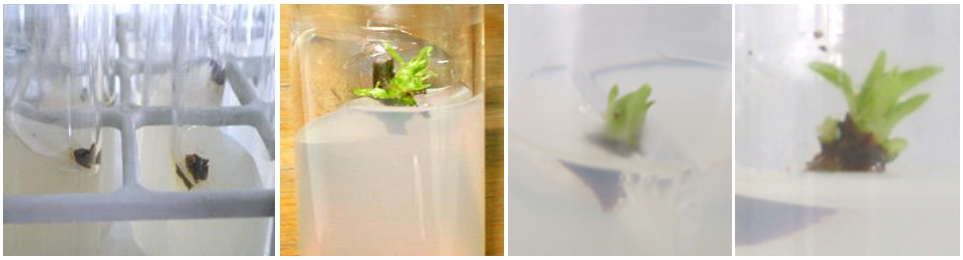
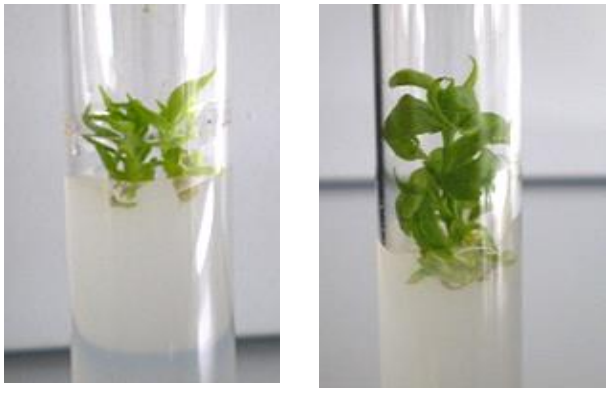


Figure 4. Lateral buds and shoot tips of *Prunus* sp. during proliferation

Multiplication phase:

Plum and apricot plantlets during the multiplication phase presented a high coefficient micro-propagation number. Plum plantlets originating from lateral buds had an average of 4.14 buds/plant, while those originating from shoot tips had 3.17 buds/explant. Our result for this parameter, recorded from *Prunus cerasifera* plants, inoculated with the MS media, and supplemented with cytokinin BAP 0.3 mg L^{-1}, is higher than that reported from Rogalski et al. (2003) on the *in vitro* micro-propagation of plum cultivars (2.8-3.6 buds/explant).

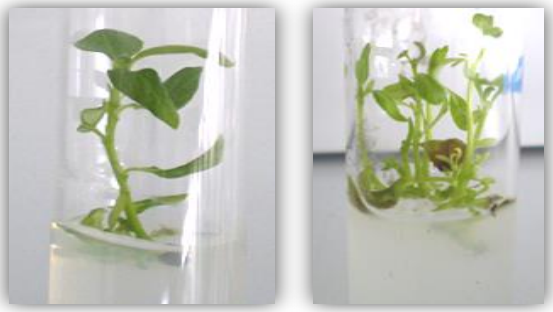


a b

Figure 5. Plum plantlets
(a) during (b) at the end of subculture.

The micro-propagation coefficient of apricot plantlets originating from shoot tips was 4.25, quadruplicating the initial number of plants (Figure 6) inoculated with the WPM media and supplemented by 1 mg L^{-1} BAP, while the

coefficient value of the plantlets that originated from lateral buds was 3.6. Arena and Caso (1992), Leontiev-Orlov et al. (2000b), and Pérez-Tornero e Burgos (2000) suggested that the differences found in the *in vitro* micro-propagation coefficient of various *Prunus* species depends on their genotype.



a b

Figure 6. Apricot plantlets (a) during (b) at the end of the subculture.

During the second phase, plantlets of two stone fruits presented not only higher values of shoot length when compared to the first phase, but an increased number of leaves (Figure 7). Among the two species in our study, plum *in vitro* plantlets show the highest number of leaves/explants (16.1) (Table 3).

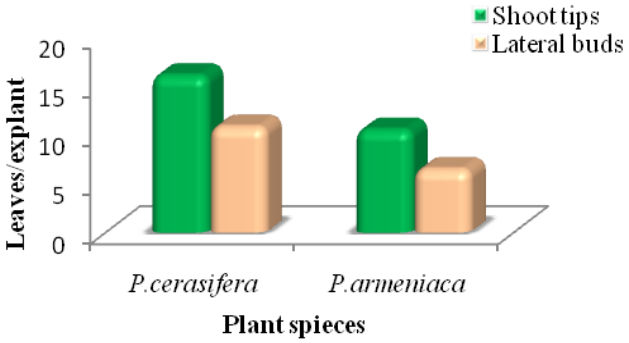


Figure 7. Effect of explants type on number of leaves/plantlets during multiplication phase.

Table 3. The effect of the explant type on the number of leaves found on plantlets that originated from lateral buds during the multiplication phase.

Source of Variation	*SS*	*Df*	*MS*	*F*	*P-value*	*F crit*
Rows	189.2	1	189.2	20.62989**	2E-04	4.381
Columns	229.3	19	12.07	1.31559317	0.278	2.168
Error	174.3	19	9.172			
Total	592.8	39				

LSD 005-2.05, *LSD 001-2.74, Cv %- 44.94*

Among the two species, apricot plantlets originated from shoot tips, were cultured on WP media with BAP-1 mg L^{-1}, represent the highest value of length (±21.6 mm). The explant type, and the quantity of BAP used can affect this parameter, as is shown in Figure 8 (Table 4). There are no differences between the plantlets of two *Prunus* species that originated from lateral buds.

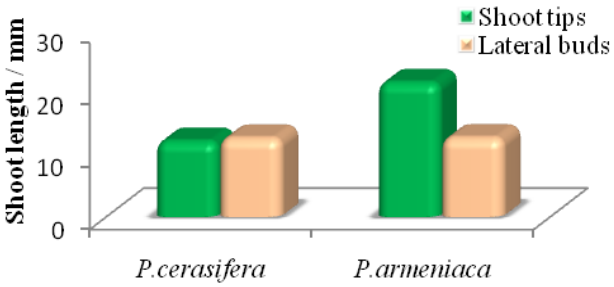


Figure 8. Effect of explant type on shoot length during the multiplication phase.

Table 4. Effect of explant type on shoot length of plantlets originated from active shoot tips during the multiplication phase.

Source of Variation	***SS***	***Df***	***MS***	***F***	***P-value***	***F crit***
Rows	893	1	893	14.0404**	0.001	4.381
Columns	859.3	19	45.23	0.7110408	0.768	2.168
Error	1208	19	63.6			
Total	2961	39				

LSD 005-5.2, *LSD 001*-7.2, *Cv %*- 51.48.

In vitro shoot elongation is a critical process within the micro-propagation system; it considerably depends on the composition of the nutrient media (Chen et al. 2003). The use of saharoze as source of carbohydrates (30 g) in the WP media resulted in plantlets with a height of more than 2 cm. This result is in accordance with the results published by other authors (Yeasenn et al. 2009), for the same type and amount of carbohydrate used.

CONCLUSIONS

The results obtained in the present study suggest that the type of method used for explant disinfections was the right one, indicating a high survival rate. The type of explant and nutrient medium used for the *in vitro* establishment and proliferation of *Prunus* germplasm, affected the rate of proliferation, giving satisfactory results for our two *Prunus* resources. The multiplication rate of the two *Prunus* populations was highly effective, producing a large number of explants through the MS and WPM medium. The technique used in our study might possibly be useful for cloning other woody plants.

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UPOTREBA BIOTEHOLOŠKIH METODA U MULTIPLICIRANJU AUTOHTONIH *PRUNUS* SP. GERMPLASM

SAŽETAK

Ovo proučavanje je izvršeno da bi se istražila upotreba "in vitro" metoda kao alternativnog načina razmnožavanja dva različita prirodna resursa *Prunus* germplasm. Izdanci vrhova rasada sakupljeni u regionu Tirane su bili kultivisani u MS čvrstoj sredini dopunjenoj različitim vrstama regulatora za razvoj biljke (ANA, BAP i GA_3) za vrijeme faza proliferacije i multipliciranja. Rezultati su elaborirani sa dvije faktorske analize varijacija. Za vrijeme proliferacije, rasadi breskve su zabilježili veće vrijednosti parametra dužine izdanka (± 10.6mm) u poređenju sa izdancima šljive (± 6.3mm). posmatrali smo različite rezultate za parametre lisova, gdje izdanak vrha šljjive ima veći broj listova (±8.6) od breskvi (±5.2). Za vrijeme subkulture, sastav sredine popositivno utiče na novu biometriju regeneranata, dajući šljivi regenerante sa najvećim brojem listova (±16) a breskvi nove regenerante sa najvećom vrijednošću dužine izdanka (±2.2cm). Zabilježeni koeficijent multipliciranja za dva *Prunus* regeneranta je bio veći od 4 pupoljka po rasadu. Zabilježeni podaci predlažu da je metoda korišćena u ovom proučavanju bila adekvatna za multipliciranje naših *Prunus* germpalsm.

Ključne riječi: razmnožavanje, Prunus, germplasm, regulatori rasta, sredina