Collezioni *in vitro* di alcune specie autoctone del genere *Prunus* in Albania

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RIASSUNTO

La conservazione della biodiversità delle piante autoctone del genere *Prunus* (*Prunus avium, P. webbii, P. mahaleb*) è molto importante per soddisfare le esigenze nutrizionali umane. Per valorizzare meglio questa risorsa genetica è necessaria un'attenta valutazione al fine di conservare quei genotipi più produttivi e dotati di un elevato valore nutraceutico e salutistico. La crescente disponibilità di prodotti alimentari derivati da piante selvatiche e genotipi autoctoniè di grande importanza per le aziende agrituristiche e per i ristoranti rurali e locali, i quali rappresentano parte del patrimonio tradizionale locale. La conservazione *ex situ* della diversità genetica vegetale, attraverso la coltura *in vitro* consente una rapida moltiplicazione delle accessioni con l'impiego di un quantitativo trascurabile di materiale vegetale necessario per avviare la coltura *in vitro* che non determina alcun impatto sulle popolazioni selvatiche. La ricerca in questo campo è iniziata in collaborazione con il Centro Sperimentale di Frutticoltura, CREA, di Roma con la finalità di produrre collezioni di germoplasma con tecniche *in vitro* di crescita lenta, basate sulla riduzione del metabolismo cellulare durante la fase di proliferazione dei germogli di accessioni appartenenti ad alcune specie del genere *Prunus*. Per la conservazione di breve e medio termine sono state saggiate tre tecniche differenti: 1) - riduzione della concentrazione di saccarosio e dei sali del substrato MS; 2) - riduzione della temperatura e della intensità luminosa; 3) - sottrazione di fitormoni o regolatori di crescita nei terreni di coltura. La percentuale di sopravvivenza e di crescita sono stati valutati in diversi momenti. Le diverse tecniche hanno influito significativamente sulla percentuale di sopravvivenza degli espianti. La combinazione bassa temperatura (4°C) e riduzione della intensità di luce è risultata la tecnica migliore di conservazione a medio termine, permettendo una sopravvivenza dei germogli fino a 14 mesi. La riduzione delle concentrazioni di saccarosio e di sali nel substrato di coltura (1/2 MS) è risultata, invece, efficace per un periodo non superiore a 5 mesi.

Parole chiave: *Prunus* spp. , micropropagazione, conservazione *in vitro*, tecniche *in vitro* di crescita lenta

INTRODUCTION

Maintenance and preservation of diversity in autochthons *Prunus* sp. (*Prunus avium, P. webbii, P. mahaleb,*) plants is very important to human being for fulfilling their nutritional developments. Ethnobiological studies conducted in the Western Balkans in recent years have reported rich biocultural diversity and a remarkable vitality of traditional knowledge concerning the local flora in this region (Pieroni *et al*, 2013). Even nowadays are conserved over than 70 plant species and subspecies, with more than 1200 primitive populations of fruit trees and bushes. Although the traditional use of wild edibles is largely decreasing due to socioeconomic and ecological changes, wild plants are becoming a part of the new thinking about food: they are very important as health food, and in food security and slow food movements (Luczaj *et al*., 2012). Currently, the edible spontaneous species are even more in the focus of researchers due to: (i) re-interest for local traditional foods and forgotten nutrient food sources; (ii) the "terroir" concepts related even with cultural heredity; (iii) the potential of these foods with alimentary and medicinal values. By the economical point of view, this resource of germplasm needs the discovery, the evaluation, the conservation and the utilization of those forms with higher levels of production and quality and with a high value of dietary and therapeutic components. These species induct a great interest for the development of biological and eco-touristic horticulture in the mountain and hilly areas of our country, so necessary in the conditions of eco-tourism progress. Nowadays, different plant conservation methods are developed (Engelmann & Engels, 2002), especially as *in situ* and *ex situ* ones. Recent decades, progress in biotechnology allows successful involvement of tissue culture techniques in plant germplasm conservation. The aim of medium term storage is to increase the interval period between subcultures by reducing growth. This might be achieved by the use of modified environmental conditions, modified culture medium, growth retardants, osmotic regulators and/or reduction of oxygen concentration (Kameswara 2004).

MATERIALS and METHODS

Plant Materials: Cultures of different populations of *Prunus* avium and *Prunus mahaleb* were established from apical and lateral buds removed from adult field-grown trees. The preferred explants are shoot tips due to their genetic stability. In general, the buds were collected between January and March, when buds were starting to swell from shoots in dormancy. For *Prunus webbii* were used zygotic embryos as primary explants.

Explant disinfection: The stem sections were washed carefully with water and were shaken for 5 min. in ethanol 70 % followed by 20 min treatment with $HgCl_2$ 0.01% and two drops of Tween 20. The sections were rinsed 3 times in sterile water. The buds were dissected up to 3 mm. Thereafter the explants were rinsed out three times with sterilized distilled water. All the procedures are carried out in sterile conditions in the laminar flow.

Micropropagation: The isolated shoot tips were cultured onto glass tubes containing MS salts and vitamins (Murashige & Skoog 1962) or in the nutrient medium QL (Quoirin *et al.,*1977), or LP medium (Quoirin and Lepoivre, 1977) and WPM medium (Lloyd & McCown, 1980), combined with different ratios of plant growth regulators (auxins, cytokinins and gibberellins) according the fruit tree species. The proliferation nutrient media were supplemented with 3% sucrose and solidified with 0.55% agar. The pH of the media was adjusted 5.7 – 5.8 before autoclaving. The incubation conditions were at 25°C in a 16 h light/24 h regime with cool, white fluorescent light of 1500 lux. Plant-derived shoots were subcultured monthly into fresh medium in order to obtain a great number of plantlets. The subculture medium was similar with the proliferation medium.

In vitro storage: In order to evaluate the effect of low temperature and light regime, the proliferated shoots were incubated at 4°C in dark conditions. The medium under these conditions was the same with the multiplication medium. The cultures were stored in these conditions for different periods (3, 6, 10, 14 months). The effect of reduced sucrose concentrations and MS salts strength was also evaluated. The cultures were transferred onto ½ MS medium without sucrose and supplemented with the same rate of plant regulators and agar as in the multiplication medium. The incubation conditions were the same as in the multiplication stage. For long-term storage was used encapsulation - dehydration as a pretreatment method before freezing in liquid nitrogen. For encapsulation the explants, initially, were inoculated in Na-alginate containing solution and after that in 100mM $CaCl_2$ solution for 30 min. For dehydration, the encapsulated explants were treated with 0,25 - 1,25mM sucrose solution and after that were dried in laminar flow.

Statistical analysis: Data collections in experiment were evaluated by computer using the statistical evaluation program JMP 7.0.

RESULTS and DISCUSSION

Wild cherry (*Prunus avium* L., *Cerasus avium* L. var. *silvestris* Ser., *P. cerasus*), vulnerable VU (A_1b)

Distribution in Albania: Përmet, Tirana, Llogora, Çika, Skrapar, Kruja, Mat, Mirdita

Use: Food: the fleshy part of the fruit consumed raw; syrups (Pieroni *et al.*, 2013). Cherries contain a number of very effective antioxidants including chlorogenic acid, quercetin, and kaempferol. Cherries also help to reduce arthritis and gout pain. Cherries are an excellent source of fiber, potassium, and many B-vitamins. Eating cultivars are usually grafted onto wild cherry rootstocks.

Figure 1. Micropropagation cycle of wild cherry, *Prunus avium* L.

Table 1. *In vitro* culture of some autochthonous *Prunus sp.* germplasm

Species and status	Explant, nutrient medium	Phytohormones, mgl^{-1}			Authors
		Proliferation	Organ formation, subculture	Rooting	
Prunus avium L. Vulnerable VU (A_1b)	Apical buds MS LP leaf pieces MS	BAP 0.3, IBA 0.1, GA_3 0.3 BAP 0.25, IBA 0.6, GA_3 0.3 NAA 10^{-4} M, BAP 10^{-5}M	BAP 0.7, NAA 0.01 GA_3 0.1 BAP 0.25, IBA 0.6, GA_3 0.3 NAA 10^{-5} M, BAP 10^{-4} M	**1**. Macro MS/2, micro MS, NAA 0.1 **2**. Macro MS/2, micro MS/2, NAA 0.1 **3.** Macro MS, micro MS/2, NAA 2 -	Kongjika *et al*., 2009; Bode *et al.*, 2010
Prunus webbii (Spach) Vierh. Endangered E	Mature Embryos MS Cotyledons MS Buds MS	**1.** Without phytohormones **2.** BAP 1, IBA 0.1 **1.** BAP 1, IBA 1 **2.** BAP 0.5, IBA 1 BAP 0.3, IBA 0.1, GA_3 0.3	BAP 0.7, NAA 0.01, GA_3 0.1 BAP 0.7, NAA 0.01, GA_3 0.1 BAP 0.7, NAA 0.01, GA_3 0.1	Macro MS/2, micro MS, NAA 0.1	Sota & Kongjika, 2014
Prunus mahaleb L.	Apical buds MS, LP Lateral Buds, MS, LP	(MS) BAP 0.3, IBA 0.1, GA_3 0.3 (LP) BAP 0.25, IBA 0.6, GA_3 0.3	(MS) BAP 0.7, NAA 0.01 GA_3 0.1 (LP) BAP 0.25, IBA 0.6, GA_3 0.3	**1**. Macro MS/2, micro MS, NAA 0.1 **2**. Macro MS/2, micro MS/2, NAA 0.1 **3.** Macro MS, micro MS/2, NAA 2	Sota (Mata) & Kongjika., 2011 Sota & Kongjika., 2014

Table 2. *In vitro* conservation of some autochthonous *Prunus sp.* germplasm

Species	Explant	Type of morphogenesis	Conservation method Period of conservation	Authors
Prunus avium L.	Apical buds	Direct organogenesis	**1.** Low temperature,4ºC, darkness (10 months) **2.** Modified medium (Reduction of MS salts, sucrose elimination) (4 months) **3.** Modified medium (phytohormones elimination) (4 months) **4.** Encapsulation – Dehydration (preparatory phase)	Sota (Mata) & Kongjika, 2011
Prunus webbii (Spach) Vierh.	Mature Embryos Cotyledon Buds	Direct, indirect organogenesis Somatic embryogenesis Direct organogenesis	Low temperature, 4ºC, darkness (6 months)	Sota & Kongjika, 2014
Prunus mahaleb L.	Apical buds	Direct organogenesis	**1.** Low temperature,4ºC, darkness (10 months) **2.** Modified medium (Reduction of MS salts, sucrose elimination), (5 months) **3.** Modified medium (phytohormones elimination), (4 months) **4.** Encapsulation – Dehydration (preparatory phase)	Sota (Mata) & Kongjika, 2011

Mahaleb or Rock cherry (*Prunus mahaleb* L., *Cerasus mahaleb*)

Distribution in Albania: Tepelena, Mallakastra, Lunxhëri, Përmet, Kruja, Skrapar etc.

Use: A spice fragrant with the taste of bitter almonds. It is used in small quantities to sharpen sweet foods (El-Dakhakhny, 2006). The bark, wood and seeds have anti-inflammatory, sedative and vasodilatation effects (Philips *et al.*, 1990).

Wild almond (*P. webbii* Spach Vierh.) Endangered E.

Distribution in Albania: Përmet, Mallakastra, Butrint, Konispol, Delvina, Himara, Vlora, Tepelena, Përmet, Leskovik, Skrapar.

Use: rootstock for almond, nectarine and peach, for cultivated almond; antitumor and anti-bacteriological properties (Mobli and Baninasab, 2008). Wild almond trees are cyanogenic, in small quantities stimulate respiration and improve digestion.

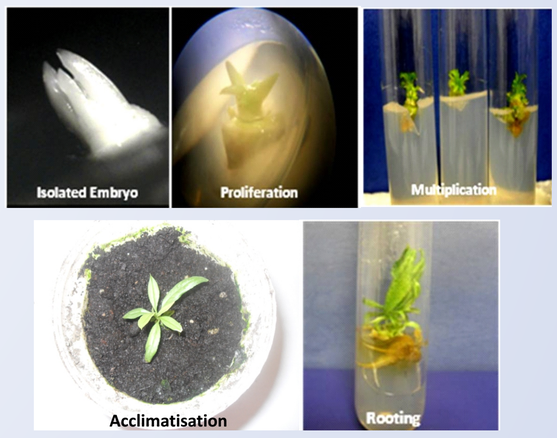

Figure 6. Different stages of embryo culture of wild almond *Prunus webbii*

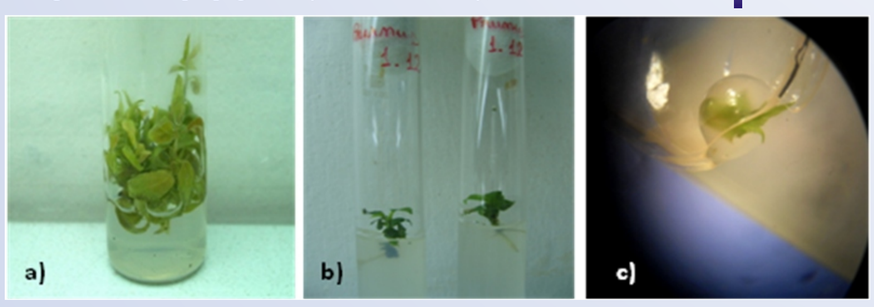

Figure 2. Conservation methods of *in vitro* plantlets of *Prunus avium*:
a- Low temperature and darkness; **b** - Modified nutrient medium and
c – Encapsulation – Dehydration method (preparatory phase of cryoconservation)

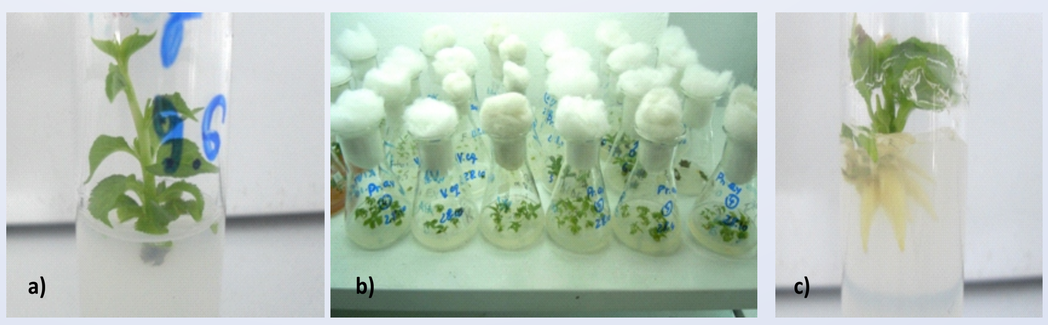

Figure 3. Development of plantlets of Mahaleb cherry in **a)** Proliferation stage **b)** Subculture stage **c)** Rooting stage

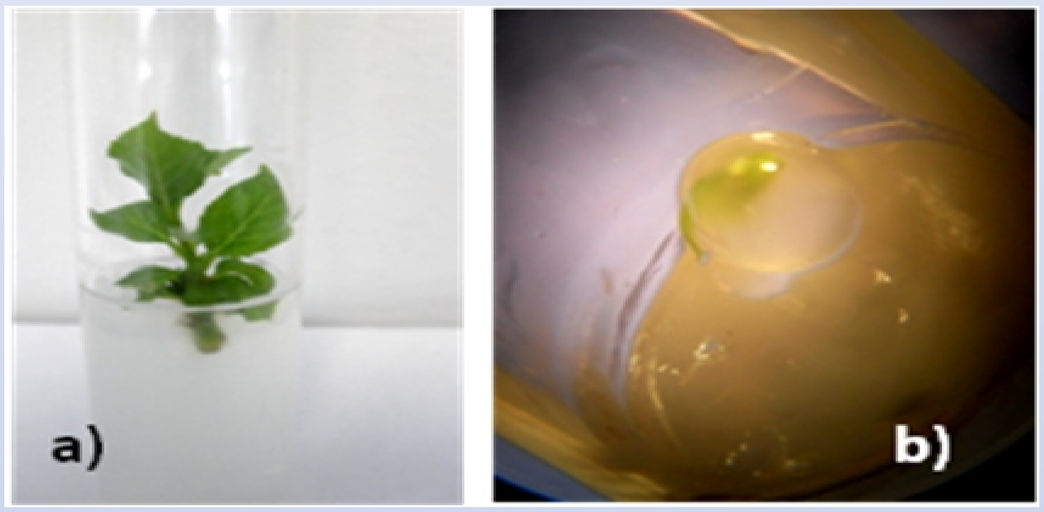

Figure 4. (**a**) - Regenerated plantlets of *Prunus mahaleb* after transferring onto fresh medium after 10 months conservation in low temperature,4ºC and darkness; (**b**) - Encapsulation – Dehydration method

CONCLUSIONS

Micropropagation of autochtonous species of *Prunus* germplasm is an efficient method for their multiplication. The most optimal nutrient media results MS medium combined with PGRs such BAP, NAA and GA_3. During subcultures is observed a very high micropropagation coefficient in plantlets cultivated in MS medium supplemented with BAP 0.7 mg l^{-1}, NAA 0.01 mg l^{-1} and GA_3 0.1 mg l^{-1}. For *Prunus webbii* using zygotic embryo as primary explants, results an optimal method during proliferation in MS medium without PGRs or phytohormones. Induction of somatic embryogenesis by cotiledons results effective in MS medium with BAP 0.7 mg l^{-1}. Better results in rooting percentage are observed during cultivation in ½ MS macronutrients, MS micronutrients, MS vitamins and NAA 0.1 mg l^{-1}. Conservation in 4°C in darkness is an effective method of minimal growth for medium term conservation periods. Conserving of *Prunus* germplasm for 10 months in such conditions gives high percentage of survival and regeneration parameters.

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