ACTA UNIVERSITATIS AGRICULTURAE ET SILVICULTURAE MENDELIANAE BRUNENSIS

Volume 65 8 Number 1, 2017
https://doi.org/10.11118/actaun201765010067

THE ISSUES OF APRICOT (*PRUNUS ARMENIACA* L.) MICROPROPAGATION

Martina Kudělková[1], Radka Pavelková[1], Eva Ondrušiková[1], Miroslav Vachůn[1]

[1]Mendeleum-Institute of Genetics, Faculty of Horticulture, Mendel University in Brno, Zemědělská 1, 613 00 Brno, Czech Republic

Abstract

KUDĚLKOVÁ MARTINA, PAVELKOVÁ RADKA, ONDRUŠIKOVÁ EVA, VACHŮN MIROSLAV. 2017. The Issues of Apricot (*Prunus Armeniaca* L.) Micropropagation. *Acta Universitatis Agriculturae et Silviculturae Mendelianae Brunensis,* 65(1): 0067–0072.

The effect of four modified mediums for apricot multiplication was observed in this study. A total number of 1864 single nodes of 20 Prunus armeniaca L.varieties were established. Explants surface was disinfected with 0.2 % mercuric chloride for 5 minutes. MS (1962) medium with 0.5 mg.l^{-1} BA, 0.01 mg.l^{-1} NAA and 0.5 mg.l^{-1} GA3 was used as a medium for primary culture. 'Velkopavlovická', 'Bergeron', genotype 1128 and genotype LE 2927 Š9 were successfully transferred to aseptic conditions and multiplied. Modified MS medium (1962), DKW/Juglans medium, Quoirin, Lepoivre (1977) medium and Marino *et al.* (1991) medium were used for multiplication. Modified MS medium and modified DKW/Juglans medium were not suitable for apricot multiplication at all and explants did not grow. The best results were observed in the case of Quoirin, Lepoivre (1977) medium with 0.4 mg. l^{-1} BA and 0.01 mg.l^{-1} NAA. Young plants multiplied well, were fresh and vital and no damage was observed. The highest number of new shoots was observed in the case of Marino *et al.* (1991) medium. The average growth of new shoots after the last passaging was 600 %, rate 7.33 (Velkopavlovická); 566 %, rate 7.0 (Bergeron); 475 %, rate 6.25 (1128) and 483 %, rate 6.33 (LE 2927 Š9)). However, new shoots in clusters were too dense and stunted and this medium is not recommended for apricot multiplication.

Keywords: *Prunus armeniaca*, *in vitro* propagation, culture medium, plant grow regulators, single node culture, shoot regeneration, multiplication

INTRODUCTION

Micropropagation is one of the modern possibilities for use of plant vegetative multiplication. It enables us to propagate a high quality plant material quickly and economically (Moudrá *et al.*, 2012). Plant material obtained during a period of vegetative growth is very important for a culture establishment. Culture establishment is less successful in July and August because of a lower growth activity and oncoming dormancy of woody plants. In later terms, microorganisms are a big problem and a percentage of sterile shoots decreases (Křižan *et al.*, 2011). Different chemicals have been used for microorganisms eliminate, e.g. NaOCl, 70% ethanol, mercuric chloride (Koubouris and Vasilikakis, 2006; Křižan *et al.*, 2011; Perez-Tornero *et al.*, 1999; Yildirim *et al.*, 2011). There have been several researches about Prunus armeniaca L. micropropagation which are focused on media compounds (Gago *et al.*, 2011, Jain and Haggman, 2007; Koubouris and Vasilakakis, 2006; Kramarenko, 1999) for instance carbon energy source, effect of hormones (Marino *et al.*, 1991 regeneration from different organs (Escalettes and Dosba, 2003; Peixe *et al.*, 2004; Pérez-Tornero *et al.*, 1999; Pérez-Tornero *et al.*, 2000; Yildirim *et al.*, 2007; Yildirim *et al.*, 2011), hyperhydricty (Pérez-Tornero *et al.*, 2001) and grafting (Errea *et al.*, 2001) as well as many papers focused on virus elimination (Brison *et al.*, 1997; Hauptmanová and Polák, 2011). Plant transfer to aseptic culture and their multiplication for their elimination from viruses were the goals of this research.

MATERIALS AND METHODS

Plant material

The basic materials for this experiment were obtained from apricot trees *Prunus armeniaca* L. from different places in the area of the Faculty of Horticulture in Lednice. The first group of samples came from the trees cultivated in the quarantine room of the technical isolate, the second one was from a greenhouse and the third one was from an orchard (Tab. I–IV). One budded herbaceous shoots from trees (single node culture) were taken from 2012 to 2015 (Tab. I).

Explant disinfection

Explants were cut in 1 single nodes and pre-sterilized in warm water with commercial detergent. Explants' surface was disinfected with 0.2 % mercuric chloride for 5 minutes and cleaned with sterilized distilled water three times. After the disinfection, the nodes were transferred into test-tubes on the Murashige and Skoog, MS (1962) medium with 6 $g.l^{-1}$ agar, 30 $g.l^{-1}$ sucrose, plant regulators (0.5 $mg.l^{-1}$ BA, 0.01 $mg.l^{-1}$ NAA and 0.5 $mg.l^{-1}$ GA_3), 100 $mg.l^{-1}$ myo-inositol; pH 5,7. Medium also contained 1 $ml.l^{-1}$ of the antibiotics ProClin 200 (Sigma-Aldrich, St. Louis, USA).

Culture conditions

Cultures were maintained at 21 ± 1 °C with a photoperiod of 16/8 h. Fluorescent tubes with light intensity 22 $\mu mol.m^{-2}.s^{-1}$ were used for lighting.

Shoot multiplication

Unfolded buds and shoots were promoted by various medium types (a–d) after the successful culture establishment (Tab. I–IV). Basal MS medium and DKW medium were obtained from Duchefa Biochemie (Haarlem, Netherlands). Shoots were cultivated on the fresh medium every three weeks.

a) MS (1962) medium + 13 $g.l^{-1}$ sucrose + 11 $g.l^{-1}$ sorbitol + 4 $g.l^{-1}$ K_2SO_4 + 500 $mg.l^{-1}$ myo-inositol + Jacquiot (1950) vitamins + 0.75 $mg.l^{-1}$ BA ribozid; pH 5.7

b) DKW/Juglans medium + 30 $g.l^{-1}$ sucrose + 500 $mg.l^{-1}$ myo-inositol + 0.6 $mg.l^{-1}$ BA + 0.01 $mg.l^{-1}$ IBA; pH 5.7

c) Quoirin, Lepoivre (1977) medium with 6 $g.l^{-1}$ agar + 30 $g.l^{-1}$ sucrose + 100 $mg.l^{-1}$ myoinositol + MS (1962) microelements + 0.4 $mg.l^{-1}$ BA + 0.01 $mg.l^{-1}$ NAA; pH 6.3. This medium was used as a control medium. It was used successfully in the past in our lab for apricot and peach cultivation.

d) Marino *et al.* (1991) medium with 5.5 $g.l^{-1}$ agar + 40 $g.l^{-1}$ sorbitol + 100 $mg.l^{-1}$ myoinositol + 1 $mg.l^{-1}$ BA; pH 5.7

I: *The number of single nodes of apricot varieties established in 2012*

term	variety	source	number	a)	c)
4. 7.	Velkopavlovická	TI	75	10	9
	Leskora	TI	75	15	14
	Aurora	TI	75	6	5
	LE 3276	TI	75	12	12

II: *The number of single nodes of apricot varieties established in 2013*

term	variety	source	number	a)	c)
20. 3.	Aurora	TI	75	10	9
	Velkopavlovická	TI	30	4	4
	LE 3276	TI	75	8	8

III: *The number of single nodes of apricot varieties established in 2014*

term	variety	source	number	a)	b)	c)	d)
21. 5.	Bergeron	TI	27	0	0	0	0
	Lednická	TI	169	27	25	25	25
	Lednická 37	TI	48	5	5	4	4
	Veselka	TI	110	13	12	12	11
	Velkopavlovická	TI	30	3	2	2	2

IV: *The number of single nodes of apricot varieties established in 2015*

year	term	variety	source	number	a)	b)	c)	d)
2015	13.3.	Bergeron	GH	35	2	1	1	1
		Leskora	GH	25	0	0	0	0
		Lednická 37	GH	20	0	0	0	0
		Velkopavlovická	GH	30	1	1	1	0
		Lednická	GH	20	3	3	3	3
		Veselka	GH	15	3	3	2	2
	8.6.	4-395	ORCH	30	3	3	2	2
		Velkopavlovická	ORCH	30	1	1	1	1
		Paviot	ORCH	30	1	1	0	0
		rootstock A	ORCH	25	0	0	0	0
		MO1	ORCH	25	0	0	0	0
		LE 2927 Š9	TI	30	4	3	3	3
	19.VIII	1002 Gold	TI	40	0	0	0	0
		1125	TI	40	1	1	0	0
		1128	TI	40	4	4	4	4
	25.8.	Bergeron	GH	55	0	0	0	0
		Leskora	GH	25	0	0	0	0
		Lednická 37	GH	25	0	0	0	0
		Velkopavlovická	GH	30	0	0	0	0
		Lednická	GH	20	0	0	0	0
		Veselka	GH	25	0	0	0	0
	4.9.	Bohutická	TI	35	0	0	0	0
		LE 3276 Š4	TI	30	0	0	0	0
		LE 2926 (TI)5	TI	40	0	0	0	0
		LE 2927 Š5	TI	40	0	0	0	0
		LE 3276 Š3	TI	40	0	0	0	0
		LE 3241 Š1	TI	25	0	0	0	0
		LE 3241 Š6	TI	35	0	0	0	0
		LE 2926 (TI)2	TI	40	0	0	0	0
	14.9.	Zemliansky	ORCH	100	0	0	0	0

GH – material from green house
ORCH – material from orchard
TI – material from technical isolation
number – the number of single nodes transferred to in vitro culture
a) – d) – the number of the young explants transferred on different types of multiplication medium

RESULTS

Culture establishment

A total number of 1864 single nodes of *Prunus armeniaca* L. were established in the plant tissue culture laboratory at the Faculty of Horticulture in Lednice between 2012 and 2015. Together in all the years, primary cultures were established from 20 different varieties, overall 42 times. The group of 'Velkopavlovická', 'Bergeron', genotype 1128 and genotype LE 2927 Š9 were the only surviving groups and they could be maintained on multiplication medium. The number of these four genotypes that established shoots (2012–2015) is expressed in Tab. V. 'Leskora', 'Aurora', genotype LE 3276, 'Lednická', 'Lednická 37', 'Veselka', genotype 4-395, 'Paviot' and genotype 1125 were transferred on multiplication medium as well, however, they did not survive the first month of the cultivation and were not evaluated. The samples from the rest of varieties were attacked with pathogenic microorganisms, however, blacking of the primary cultures or no unfolding of single nodes were the most common problems (70 % on average).

The most suitable terms for primary explants obtaining were 4. 7. 2012 and 21. 5. 2014. The lowest success rate for plant material obtaining was observed in 2015. The last three terms of the plant material obtained from mother trees (25.8., 4.9. and 14.9. 2015) were not suitable for in vitro culture establishment at all.

V: *Established shoots of four genotypes used for multiplication on different types of medium*

2012–2015	a)	b)	c)	d)	total
Velkopavlovická	19	4	17	3	43
Bergeron	2	1	1	1	5
1128	4	4	4	4	16
LE 2927 Š9	4	3	3	3	13

a)–d) – the number of the young explants transferred on different types of multiplication medium
total – the number of established shoots used for multiplication in total (2012–2015)

Shoot multiplication

The results were evaluated after 10 sub-cultivations. Sub-cultivation on a fresh medium was implemented every 3 weeks.

Modified MS medium (a) and modified DKW/Juglans medium (b) were not suitable for apricot multiplication. Explants faded and did not multiply. The period of cultivation was shortened to one week. Some of the cultivated shoots began to grow and to be vigorous, however, they did not multiply well. Multiplication on modified Quoirin, Lepoivre (1977) medium (c) and Marino *et al.* (1991) medium (d) was more successful. The use of Marino *et al.* (1991) medium was statistically better than the other mediums in all varieties. However, new shoots in clusters were too dense and stunted. The average growth of new shoots after the last passaging was 600 %, rate 7.33 (Velkopavlovická); 566 %, rate 7.0 (Bergeron); 475 %, rate 6.25 (1128) and 483 %, rate 6.33 (LE 2927 Š9). The average number of new shoots on Quoirin, Lepoivre (1977) medium was significantly lower than Marino *et al.* (1991) medium, however, new shoots were strong and vital and no damage or dwarfism were observed. The average growth of new shoots after the last passaging was 138 %, rate 2.53 (Velkopavlovická); 100 %, rate 2.0 (Bergeron); 125 %, rate 2.25 (1128) and 133 %, rate 2.33 (LE 2927 Š9). The average number of new shoots on Quoirin, Lepoivre (1977) medium was significantly higher than DKW/Juglans medium (b) in all varieties. Statistically significant differences are displayed in Tab. VI.

DISCUSSION

Stem nodal segments method is the most commonly used method to establish in vitro culture. However, microorganisms' contamination at the beginning and during the cultivation poses a significant problem (Perez-Tornero *et al.* 1999). We also observed problems with microorganisms in a primary one nodal segment culture in most of the twenty apricot varieties. Only four varieties were established successfully. The higher concentration or time of disinfection with mercuric chloride damaged apricot nodal segments as well

VI: *The effect of different medium on shoot multiplication after the last passaging. Means in each column followed by different letters are different according to Duncan's multiple range test (P < 0.05)*

Variety	Medium	Mean (%)	S.D.
Velkopavlovická	A	$21{,}05^{ijk}$	
	B	50^{ghijk}	
	C	$138{,}24^{f}$	14.7
	D	$566{,}67^{a}$	66.7
Bergeron	A	50^{fghi}	
	B	0^{i}	
	C	10^{h}	
	D	600^{a}	
1128	A	75^{fghij}	
	B	0^{l}	
	C	125^{ef}	
	D	475^{a}	50.0
LE 2927 Š9	A	25^{ij}	
	B	$33{,}33^{hij}$	
	C	$133{,}33^{ef}$	
	D	$483{,}33^{a}$	50.0

as peach segments, grape segments or chestnut segments (unpublished results). Yildirim *et al.* (2011) tried to use NaOCl solution in different concentration for 30 minutes. The highest survival rate (94.12%) was observed by 40-s immersion in 70% ethanol, and then 10-min immersion in 5% NaOCl. Similar concentrations in apricot were also applied successfully Perez-Tornero *et al.* (1999) and Koubouris and Vasilikakis (2006).

Modified Quoirin, Lepoivre (1977) medium with sucrose and 0.4 $mg.l^{-1}$ BA + 0.01 $mg.l^{-1}$ NAA produced healthier shoots and it is possible to recommend that for apricot multiplication. The highest growth of new shoots was observed on modified Marino *et al.* (1991) medium with 1 $mg.l^{-1}$ BA and with sorbitol instead of sucrose in our experiment. However, it is not possible to recommend this medium for apricot multiplication because of new shoots of all varieties were stunted and too dense. Kramarenko (1999) obtained a high number of new shoot using 1 $mg.l^{-1}$ of BA, however, plantlets were shortened and often in a rosette form. Using 0.1 $mg.l^{-1}$ BA decreased a number of new shoots, internodes were normal, leaves were large and of deep green colour. Koubouris and Vasilikakis (2006) also confirmed extensive rosetting and hyperhydricity of apricot shoots treated with a high concentration of BA. On the other hand, the lack of BA caused slow growth, necrosis and death after 4 subcultures. According to Murai *et al.* (1997), BA is the most used cytokinin for micropropagation of apricot shoots. BA as the most effective cytokinin for multiplication of apricot at amount 0.5 $mg.l^{-1}$ observed Yildirim *et al.* (2011). Marino *et al.* (1993) published that a number of new apricot shoots in apricot was increased up to 2 mg. l^{-1} of BA. Koubouris and Vasilikakis (2006) observed optimum results with 2.2 µM.

Improved apricot proliferation and better development of lateral shoots with the use of sorbitol were observed by Marino *et al.* (1993). Yildrim *et al.* (2011) obtained better results for apricot shoot multiplication using sucrose rather than glucose, fructose, and lactose.

As Koubouris and Vasilikakis (2006) reported, multiplication can be increased with short-term chilling of explants at 4 °C before culture. Explants produced deep green, large leaves and appeared more vigorous compared to the control. A success of multiplication is determined on genotypes as well as Perez-Tornero *et al.* (1999) reported.

We only evaluated general composition of mediums, not individual compounds. We might have observed better results with the use of different basal mediums, concentration or combination of plant growth regulators or carbon sources.

CONCLUSION

The effect of four modified mediums for apricot multiplication was observed in this study. A total number of 1864 single nodes of 20 Prunus armeniaca L.varieties were established. 'Velkopavlovická', 'Bergeron', genotype 1128 and genotype LE 2927 Š9 were successfully transferred to aseptic conditions and multiplied. Modified MS medium (1962), DKW/Juglans medium, Quoirin, Lepoivre (1977) medium and Marino *et al.* (1991) medium were used for multiplication. Modified MS medium and modified DKW/Juglans medium were not suitable for apricot multiplication at all and explants did not grow. The best results were observed in the case of Quoirin, Lepoivre (1977) medium. Young plants multiplied well, were fresh and vital and no damage was observed. The highest number of new shoots was observed in the case of Marino *et al.* (1991) medium, however, new shoots in clusters were too dense and stunted.

Acknowledgements

This paper was supported by the NAZV project QJ1210175: Research and development of standard methodic procedures of recovering of fruit trees and grapevines by chemotherapy of in vitro cultures for certification system of healthy status of planting material is gratefully acknowledged.

REFERENCES

BRISON, M., DE BOUCAUD, M. T., PIERRONNET, A. and DOSBA, F. 1997. Effect of cryopreservation on the sanitary state of a cv Prunus rootstock experimentally contaminated with Plum Pox Potyvirus. *Plant Science*, 123(1): 189–196.

ERREA, P., GARAY, L. and MARÍN, J. A. 2001. Early detection of graft incompatibility in apricot (Prunus armeniaca) using in vitro techniques. *Physiologia Plantarum*, 112(1): 135–141.

ESCALETTES, V. and DOSBA, F. 1993. In vitro adventitious shoot regeneration from leaves of Prunus spp. *Plant Science*, 90(2): 201–209.

GAGO, J., PÉREZ-TORNERO, O., LANDÍN, M., BURGOS, L. and GALLEGO, P. P. 2011. Improving knowledge of plant tissue culture and media formulation by neurofuzzy logic: a practical case of data mining using apricot databases. *Journal of plant physiology*, 168(15): 1858–1865.

HAUPTMANOVÁ, A. and POLÁK, J. 2011. The elimination of Plum pox virus in plum cv. Bluefree and apricot cv. Hanita by chemotherapy of in vitro cultures. *Hort. Sci.(Prague)*, 38: 49–53.
JAIN, S. M. and HÄGGMAN, H. (eds.). 2007. *Protocols for micropropagation of woody trees and fruits*. Springer Science & Business Media.
KOUBOURIS, G. and VASILAKAKIS, M. 2006. Improvement of in vitro propagation of apricot cultivar 'Bebecou'. *Plant cell, tissue and organ culture*, 85(2): 173–180.
KRAMARENKO, L. A. 1997. Micropropagation of Apricot and Field Performance of In Vitro Propagated Plants. In: *XI International Symposium on Apricot Culture*, 488: 417–420.
KŘIŽAN, B., ONDRUŠIKOVÁ, E., MOUDRÁ, J., EICHMEIER, A. and HOLLEINOVÁ, V. 2011. Elimination of apple chlorotic leafspot virus on peaches and apricots by applying chemotherapy. *Zahradnictví*, 10(12): 18–19.
MARINO, G., BERTAZZA, G., MAGNANINI, E., and ALTAN, E. D. 1993. Comparative effects of sorbitol and sucrose as main carbon energy sources in micropropagation of apricot. *Plant Cell Tissue and Organ Culture*, 34: 235–244.
MARINO, G., MAGNANINI, E., BATTISTINI, S. and RIGHETTI, B. 1991. Effect of Hormones and Main Carbon Energy Sources on In Vitro Propagation of Apricot (Prunus armeniaca L.) cvs. 'San Castrese' and 'Portici'. *Acta Hort.*, 79(2): 335–362.
MOUDRÁ J., ONDRUŠIKOVÁ E. and KŘIŽAN B. 2012. Mikropropagation of apricot cultivars. *Úroda,* 60(9): 53–56.
MURASHIGE, T. and SKOOG, F. 1962. A revised medium for rapid growth and bio-assays with tobacco tissue cultures. *Physiol Plant*, 15(3): 473–497.
PEIXE, A., BARROSO, J., POTES, A. and PAIS, M. S. 2004. Induction of haploid morphogenic calluses from in vitro cultured anthers of Prunus armeniaca cv. 'Harcot'. *Plant cell, tissue and organ culture*, 77(1): 35–41.
PÉREZ-TORNERO, O., BURGOS, L. and EGEA, J. 1999. Introduction and establishment of apricot in vitro through regeneration of shoots from meristem tips. *In Vitro Cellular & Developmental Biology-Plant*, 35(3): 249–253.
PÉREZ-TORNERO, O., EGEA, J., OLMOS, E. and BURGOS, L. 2001. Control of hyperhydricity in micropropagated apricot cultivars. *In Vitro Cellular & Developmental Biology-Plant*, 37(2): 250–254.
PÉREZ-TORNERO, O., EGEA, J., VANOOSTENDE, A. and BURGOS, L. 2000. Assessment of factors affecting adventitious shoot regeneration from in vitro cultured leaves of apricot. *Plant Science*, 158(1): 61–70.
QUOIRIN M. and LEPOIVRE P. 1977. Improved media for in vitro culture of Prunus sp. *Acta Hort.*, 7: 437–442.
YILDIRIM, H., AHMET, O. N. A. Y., TILKAT, E. and AKTÜRK, Z. 2011. Micropropagation of the apricot (Prunus armeniaca L.) cv. Hacıhaliloğlu by means of single node culture. *Turkish Journal of Agriculture and Forestry*, 35(1): 55–64.
YILDIRIM, H., TILKAT, E., ONAY, A. and OZEN, H. Ç. 2007. In Vitro Embryo Culture of Apricot, Prunus armeniaca L. cv. Hacıhaliloğlu. *International Journal of Science & Technology*, 2(2): 99–104.

Contact information
Martina Kudělková: martina.kudelkova@mendelu.cz.