Scientific Papers. Series B, Horticulture. Vol. LXII, 2018
Print ISSN 2285-5653, CD-ROM ISSN 2285-5661, Online ISSN 2286-1580, ISSN-L 2285-5653

ADVENTITIOUS SHOOT REGENERATION FROM PETIOLE EXPLANTS IN BLACK CHOKEBERRY (*ARONIA MELANOCARPA*)

Ionela RUSEA[1], Aurel POPESCU[2], Valentina ISAC[3], Anca Nicoleta ȘUȚAN[2], Dorel HOZA[1]

[1]University of Agronomic Sciences and Veterinary Medicine of Bucharest,
59 Mărăşti Blvd, Bucharest, Romania
[2]University of Pitești, 1 Târgul din Vale Street, Pitești, Romania
[3]Research Institute for Fruit Growing, 402 Mărului Street, Mărăcineni, Romania

Corresponding author email: aurel.popescu@upit.ro

Abstract

Due to outstanding nutritional and health benefits, and also to its ornamental value, black chokeberry was gaining recently high interest from the small fruit growers in Europe. Together with vegetative propagation, in vitro micropropagation from meristems and adventitious shoots offers suitable methods for the rapid clonal propagation of new or improved cultivars, to provide sufficient quantities of planting material to the growers and to accelerate the establishment of large black chokeberry plantings. In this respect, different concentrations of N6-benzylaminopurine (BAP), dichlorophenoxyacetic acid (2,4-D) and indole butyric acid (IBA) in Murashige and Skoog (MS) and Lee-Fossard (LF) basic culture media, respectively, were assessed for their effects on adventitious shoot regeneration of the black chokeberry cultivar 'Nero'. The ability of callus formation and shoot regeneration from petiole segments was assessed using various combinations of BAP (2.5; 5.0; 10 mg L^{-1}), 2,4-D (0.25; 0.5; 1.0 mg L^{-1}), and IBA (0.25; 0.5; 1.0 mg L^{-1}). Data on callus formation and shoot regeneration were recorded after 60 days of culture. The highest percentage of black chokeberry petiole explants forming callus (100%) was found in treatments containing a combination of 2.5 mg L^{-1} of BAP, 0.25 mg L^{-1} of 2,4-D, and 0.25 mg L^{-1} of IBA in MS medium. The only growth regulators combination which resulted in 100% petiole explants forming callus on both MS and LF media was 5 mg L^{-1} of BAP, 0.5 mg L^{-1} of 2,4-D, and 0.5 mg L^{-1} of IBA. Adventitious shoot regeneration from petiole-derived callus was high in treatments with 10 mg L^{-1} and 1.0 mg L^{-1} IBA, on both MS and LF basic media. Excepting the cytokinin-auxin combination of 2.5 mg L^{-1} of BAP, 0.25 mg L^{-1} of 2,4-D and 0.25 mg L^{-1} of IBA, shoot regeneration from petioles of 'Nero' cv. was better on MS medium. However, the best adventitious regeneration and the highest number of shoots formed per explant ocurred by direct organogenesis. Thus, an average number of 4.3 shoots per petiole explant was achieved through direct organogenesis on MS medium supplemented with BAP at 5 mg L^{-1}, 0.5 mg L^{-1} of 2,4-D, and 0.5 mg L^{-1} of IBA.

Key words*: Aronia, in vitro culture, growth regulators, callus, organogenesis.*

INTRODUCTION

Aronia melanocarpa (Michx.) Elliot (black chokeberry), a native North American shrub which was naturalized and is well adapted in Europe, is an extraordinary medicine plant (McKay, 2001). 'Nero', 'Rubina', 'Viking', 'Galicjancka', 'Fertödi', 'Hugin', 'Aron' and 'Melrom' are among the most popular varieties of *A. melanocarpa* in Europe (Strigl et al., 1995; Kulling and Rawel, 2008; Walther and Schnell, 2009; Borowska and Brzoska, 2016; Șuțan et al., 2017). They differ from each other in the efficiency of juice extraction, content of total polyphenols, anthocyanins and proantho-cyanidins, total antioxidative capacity, as well as the weight and diameter of the fruit (Rop et al., 2010; Ochmian et al., 2012; Rugină et al., 2012).

Aronia melanocarpa berries constitute a very rich source of numerous substances exerting a beneficial impact on health, including mainly polyphenols (proanthocyanidins, anthocyanins, flavonoids, and phenolic acids), possessing antioxidative, anti-inflammatory, antiviral, anticancer, antiatherosclerotic, hypotensive, antiplatelet, and antidiabetic properties (Benvenuti et al., 2004; Slimestad et al., 2005; Naruszewicz et al., 2007; Jakobek et al., 2012; Bădescu et al., 2015; Borowska and Brzóska, 2016; Park et al., 2017). Now it is well known that black chokeberry possesses one of the highest antioxidant activities among fruits (Denev et al., 2012).

A. melanocarpa berries are also known to be rich of cyanidin glycosides (Wiczkowski et al., 2010). Therefore, black chokeberries has been extensively investigated for their phenolic compound content, antioxidant properties and potential positive influence on the health (Tanaka and Tanaka, 2001; Jakobek et al., 2007; Rop et al., 2010; Jakobek et al., 2012; Litwinczuk, 2013).

From the researches on the antioxidant (Kahkonen et al., 2001; Wu et al., 2004; Oszmianski and Wojdylo, 2005; Olas et al., 2008; Denev et al., 2012), anti-inflammatory (Zapolska-Downar et al., 2012), hepato-protective (Kowalczyk et al., 2003; Valcheva-Kuzmanova et al., 2004), cardioprotective (Bell and Burt, 2007; Naruszewicz et al., 2007), hypotensive and lipid lowering (Hellstrom et al., 2010; Park and Park, 2011), hypoglycaemic and antidiabetic effects (Simeonov et al., 2002; Rugină et al., 2011; Bădescu et al., 2015; Banjari et al., 2017), to those on antimutagenic (Gasiorowski et al., 1997; Duthie et al., 2007) and antitumoral effects (Malik et al., 2003; Bermudez-Soto et al., 2007; Olas et al., 2010; Sharif et al., 2013), or those on protective action against degenerative diseases, the scientific literature is rich in information highlighting their prophylactic and therapeutic properties, without suggests on any unwanted or side effect of their use (Kokotkiewicz et al., 2010).

Zielińska-Przyjemska et al. (2007) studied the *in vitro* effects of *Aronia melanocarpa* juice on oxidative metabolism and apoptosis of neutrophils from obese and non-obese individuals, and reported that *Aronia* juice exert beneficial effects in cells and may, therefore, be useful in the treatment of obesity disorders.

Bijak et al. (2011) and Sikora et al. (2012) reported the anticoagulant properties of *Aronia* extract, based on the results of their studies, which showed significant inhibition of platelet aggregation after black chokeberry extract administration.

Experimental data indicate that not only the fruit but also the leaves of *A. melanocarpa* and their products may be effective means for prevention and treatment of the effects of toxic action of some xenobiotics in humans (Borowska and Brzoska, 2016).

Recently, Park et al. (2017) reported that *A. melanocarpa* show beneficial effects against hepatic lipid accumulation along with improvements in body weight, liver functions, lipid profiles and antioxidant capacity suggesting the potential therapeutic efficacy of its juice on nonalcoholic fatty liver disease (a hepatic manifestation of metabolic syndrome). *Aronia* phenolics are considered to be also beneficial for cardiovascular health (Wu et al., 2017).

Successful plant micropropagation from *in vitro* cultured meristems has been reported for *Aronia* species, including *A. melanocarpa* (Brand and Cullina, 1990; Brand and Cullina, 1992; Petrovic and Jacimovic-Plavsic, 1992; Velchev and Mladenova, 1992; Staniene et al., 1999; Litwińczuk, 2002; Mahečić, 2009; Litwinczuk, 2013; Kwak et al., 2015; Șuțan et al., 2017) and *A. arbutifolia* (Kane et al., 1991). However, there is no relevant information on adventitious shoot regeneration using somatic tissue explants in *Aronia melanocarpa*.

Adventitious shoot organogenesis and somatic embryogenesis are the basis for implementing new genetic variability and biotechnological approaches in woody species, particularly if somatic tissues from valuable cultivars are used (Silvestri et al., 2016). Regardless of the type (direct organogenesis, indirect organogenesis or somatic embryogenesis), regeneration process is usually influenced by biotic factors including genotype, explant type, and abiotic factors such as culture media and environmental conditions. Although plant cell totipotency theoretically enables any of the cells to retain the ability to regenerate whole new plants through organogenesis or somatic embryogenesis, the regeneration capacity of plant cells usually varies between species, cultivars, and explant types (Ganeshan et al., 2002). Proper regeneration of adventitious shoots rely on composition of nutrient medium, plant grow regulators and types of explant (Popescu and Isac, 2000; Isac and Popescu, 2009).

In the present study, we investigated the ability of adventitios shoot regeneration of 'Nero', one of the most valuable black chokeberry cultivars in both central and south-eastern Europe, using petiole explants, and found that both basic culture medium and growth regulators

combination and concentration affected adventitious shoot regeneration.

MATERIALS AND METHODS

Petiole segments of about 10 mm in length, excised from *in vitro* micropropagated shoots of chokeberry cultivar 'Nero', were cultured on Murashige and Skoog (MS) (1962) and Lee and Fossard (LF) (1977) basic culture media, respectively, supplemented with N6-benzylaminopurine (BAP) in concentration of either 2.5, 5.0 or 10 mg L^{-1}, dichloro-phenoxyacetic acid (2,4-D) in concentration of either 0.25, 0.5 or 1.0 mg L^{-1}, and indole butyric acid (IBA) in concentration of either 0.25, 0.5 or 1.0 mg L^{-1} (Table 1).

For medium preparation, separate stock solutions of macronutrients and micro-nutrients were used. Iron was added to the medium as separate stock solution of ferric sodium salt EDTA (32 mg L^{-1}). BAP and IBA were disolved in 1N HCl and 1N NaOH, respectively. Dextrose was used as carbon source in the culture media (40 g L^{-1}). The pH of the culture medium was adjusted to 5.7 with 0.1N KOH before autoclaving for 20 minutes at 121°C.

The freshly prepared explants were placed aseptically onto regeneration medium in 100 ml glass jars (four petiole segments per jar), each containing 40 ml of sterile medium solidified with 0.9% plant agar (Duchefa Biochemie).

Based on our previous experience with raspberry petiole segments cultured *in vitro* (Popescu and Isac, 2000), the cut ends of chokeberry petiole segments were slightly deeped into the culture medium in order to prevent dehydration and promote the nutrients and growth regulators uptake. The explants from the same donor plantlet were randomly distributed in different jars in order to avoid the possible errors in interpretation of results due to the differences in physiological state of the source of explants. Each treatment consisted of 20 petiole segments, in five replications.

For all treatments, the petiole segments cultured *in vitro* were subjected to an initial dark treatment for one week, at 20-22°C. Subsequently, the cultures were maintained in the growth chamber at 23±1°C, under a 16 hours photoperiod of 30 lE m^{-2} s^{-1} from cool white fluorescent tubes. Transfer of the explants to fresh medium was performed every 4 weeks.

Table 1. Composition of the culture medium used for *in vitro* adventitious regeneration of shoots in 'Nero' cultivar of *A. melanocarpa* (Michx.) Elliot

Experiment	Basal medium	Growth regulators (mg L^{-1})		
		2,4-D	IBA	BAP
E1	MS	0.25	0.25	2.5
E2	MS	0.5	0.5	5.0
E3	MS	1.0	1.0	10.0
E1	LF	0.25	0.25	2.5
E2	LF	0.5	0.5	5.0
E3	LF	1.0	1.0	10.0

The observations on adventitious shoot formation by direct organogenesis or via callus were made weekly, and after two months of culture, when regeneration frequency (percentage of the petiole explants with at least one shoot) and number of shoots per explant were recorded. For all treatments were calculated both the percentage of petiole explants forming shoots and the average number of shoots per explant. Data for shoot regeneration were analyzed for significance by the standard analysis of variance (ANOVA) with mean separation by Duncan's test ($p > 0.05$).

RESULTS AND DISCUSSIONS

The *in vitro* response of black chokeberry petiole explants from cultivar 'Nero' was significantly influenced by both the culture medium and concentration of growth regulators tested. The highest percentage of petiole explants forming callus (100%) was found in

treatments containing a combination of 2.5 mg L^{-1} of BAP, 0.25 mg L^{-1} of 2,4-D, and 0.25 mg L^{-1} of IBA in Murashige-Skoog medium.

The only growth regulators combination which resulted in 100% petiole explants forming calli on both Murashige-Skoog and Lee-Fosard media was 5 mg L^{-1} of BAP, 0.5 mg L^{-1} of 2,4-D, and 0.5 mg L^{-1} of IBA.

While petiole explants cultured on MS medium formed small amounts of callus in all treatments, only a few on those cultured on LF medium were induced to form callus in treatments with IBA and 2,4-D in concentrations of 0.25 mg L^{-1} and 0.5 mg L^{-1}, respectively. Although the presence of two different auxins in the culture medium, which is not usual, would be expected to induce a higher potency of callus formation, a synergistic effect of IBA and 2,4-D was not obvious. This observation is important in the context of recent reports showing the production and accumulation of hydroxy-benzoic acids and other biologically active phenolic acids in shoot and callus cultures of *Aronia melanocarpa* (Michx.) Elliott (Szopa et al., 2013; Kwiecien et al., 2013; Szopa and Ekiert, 2014).

We did not find a strong correlation between the concentrations of the auxins in the culture medium, and type and amount of callus formed by the petiole explants. However, the green calli derived from petiole explants cultured on MS medium containing auxins in smaller concentrations showed a higher ability to form adventitios buds and regenerate shoots by indirect organogenesis (Figure 1C). Highly proliferative petiole-derived calli (Figure 1B) did not show ability to form adventitious buds.

Depending on the treatment, shoot regeneration was observed either through direct organogenesis where the regenerants emerged mostly at the cut edge of explants or wound sites (Figure 1 and Figure 2), or indirect organogenesis (via callus), where regenerants appeared on the entire surface of the petiole-derived calli (Figure 1).

Optimum combination of plant growth regulators for shoot regeneration was medium-dependent (Table 2).

Table 2. Frequency of adventitious shoot regeneration *in vitro* by either direct organogenesis or indirect organogenesis (via callus) from petiole explants of black chokeberry cultivar 'Nero'

Experimental variants	Average number of adventitious shoots regenerated per petiole explant	Average length of adventitious shoots (cm)	Average number of calli formed per petiole explant	Average number of shoots regenerated per petiole-derived callus
E1-MS	0.9±0.41 c	2.23±0.15 ab	1.1±0.20 a	0.6±0.15 b
E2-MS	4.3±0.62 a	2.80±0.48 a	0.9±0.06 ab	1.0±0.00 a
E3-MS	3.4±0.42 ab	2.03±0.60 abc	1.0±0.00 ab	0.9±0.10 a
E1-LF	1.4±0.36 c	0.97±0.19 cd	0.1±0.06 d	0.9±0.06 a
E2-LF	2.1±0.80 bc	0.92±0.12 d	0.4±0.20 cd	1.0±0.05 a
E3-LF	2.6±0.48 bc	1.56±0.21 bcd	0.6±0.17 bc	0.9±0.06 a

*Values presented are mean ± SE. Means followed by the same letter are not significantly different (Duncan test, $p>0.05$)

The best development of adventitious buds and formation of shoots was achieved on MS medium supplemented with 0.1 mg L^{-1} IBA, 0.1 mg L^{-1} 2,4-D, and 0.5 mg L^{-1} BAP. Excepting the cytokinin-auxin combination of 2.5 mg L^{-1} of BAP, 0.25 mg L^{-1} of 2,4-D and 0.25 mg L^{-1} of IBA, shoot regeneration from petioles of 'Nero' cv. was better on MS medium. However, the statistical analysis showed that there is no difference between the percentages of adventitious shoots regenerated *in vitro* by direct organogenesis from petiole explants of black chokeberry cultivar 'Nero' cultured onto LF medium, and those of adventitious shoots regenerated on MS medium (Table 2). The best adventitious regeneration and the highest number of shoots formed per explant ocurred by direct organogenesis (Figure 1 and Figure 2). Thus, an average number of 4.3 shoots per petiole explant was achieved through direct organogenesis on MS medium supplemented with BAP at 5 mgL^{-1}, 0.5 mgL^{-1} of 2,4-D, and 0.5 mg L^{-1} of IBA (Table 2).

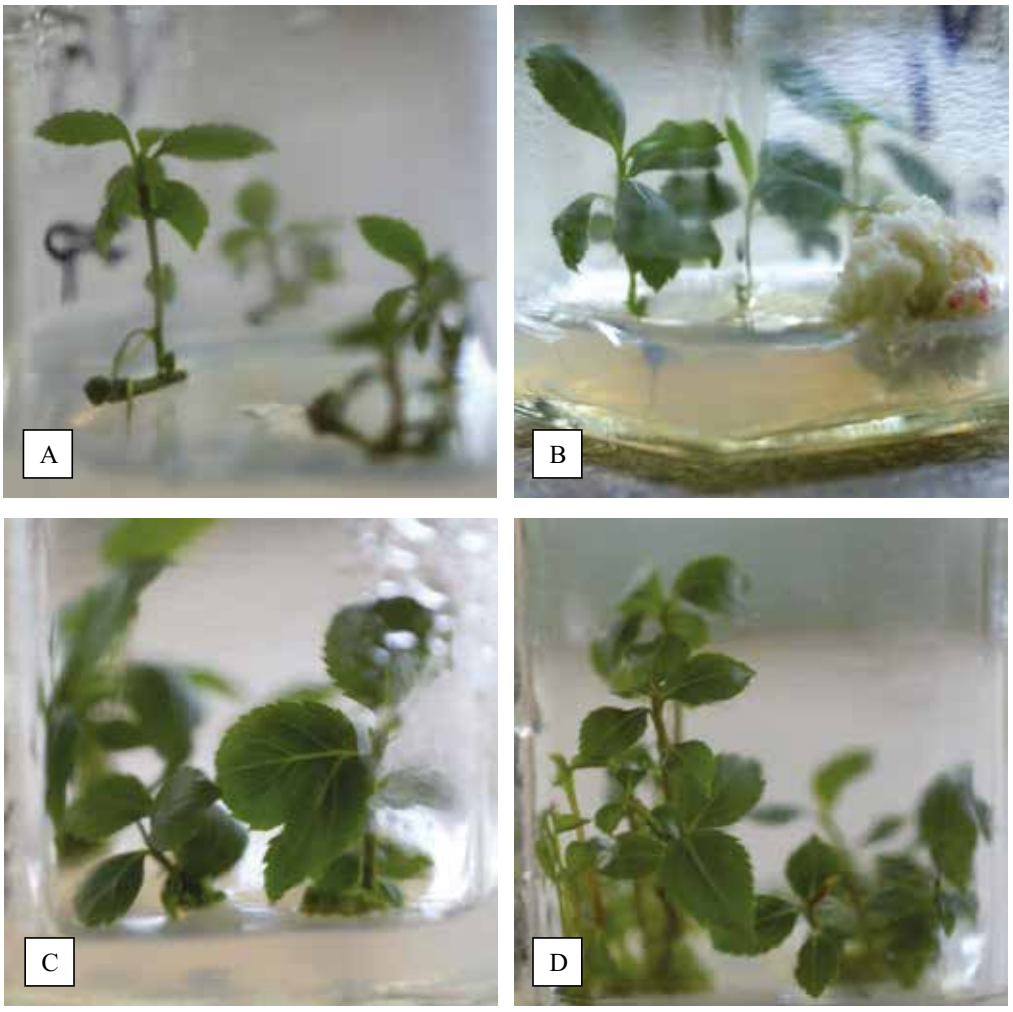

Figure 1. A-D: Adventitious shoot regeneration of black chokeberry cultivar 'Nero' through direct organogenesis from petiole segments

The petiole-derived shoots formed from adventitious buds through direct organogenesis were not uniform in appearance (Figure 2), probably due to interaction between the endogenous hormones in the plantlets used as source of explants and plant growth regulators added in the shoot induction (regeneration) medium.

Regardless of the culture medium, when petiole explants from chokeberry cultivar 'Nero' formed multiple adventitious buds (Figure 1D), only a few shoots grew and suppressed the development of the rest. Although a high frequency of shoot formation is most often desired even from the initial tissue explants, individual shoots rather than clusters are generally advantageous, because they have a higher vigor (and consequently a better ability to multiplicate), and also because the thin and crowded clusters of shoots could not be separated easily for the stage of multiplication.

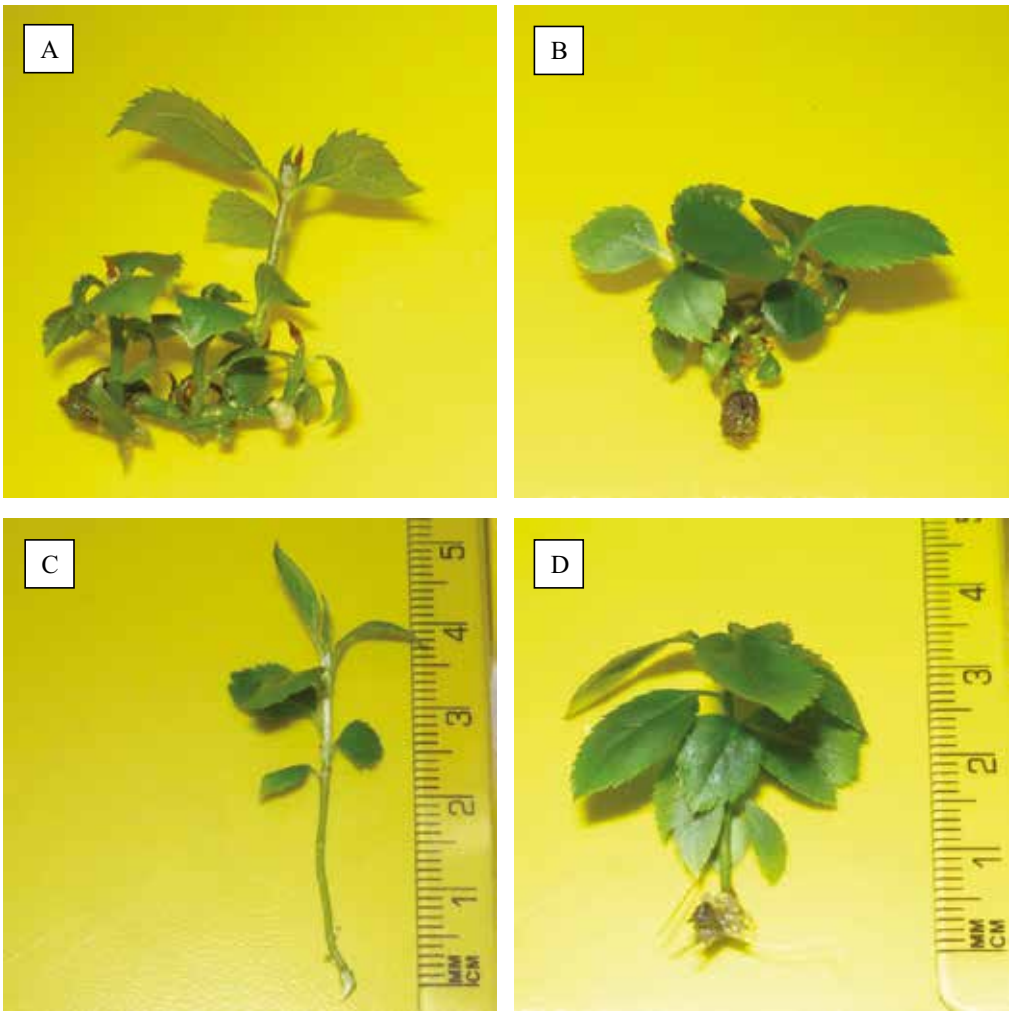

Figure 2. A-D: Length and vigour of the adventitious shoots regenerated *in vitro* by direct organogenesis from petiole explants of black chokeberry cultivar 'Nero'

As with micropropagation of *Aronia*, which is considered far more efficient than other conventional cloning methods like layering or softwood cuttings (Litwinczuk, 2013), adventitious shoot regeneration from somatic tissue explants (e.g. petiole segments) proved to be effective by direct organogenesis and even from tissue-derived calli (at a lower frequency) on Murashige and Skoog medium, and much more less effectively on Lee and Fossard medium. The effectiveness of MS medium for the *in vitro* culture of chokeberry was emphasized in many published reports (Brand and Cullina, 1990; Kane et al., 1991; Brand and Cullina, 1992; Petrovic and Jacimovic-Plavsic, 1992; Velchev and Mladenova, 1992; Staniene et al., 1999; Litwińczuk, 2002; Mahečić, 2009; Litwinczuk, 2013; Şuțan et al., 2017). Even the use of Woody Plant Medium (WPM) (Lloyd and McCown, 1980) became frequent with woody plants, including *Aronia* (Kwak et al., 2015; Chen, 2017), MS medium is a choice with reliable results, supported by many authors, such as Brand and Cullina (1990; 1992), who reported that both MS medium and WPM medium supported vigorous shoot proliferation in *Aronia arbutifolia* and *A. melanocarpa*.

Currently, experiments are underway in our laboratory to improve the conditions of *in vitro*

culture of *A. melanocarpa*, in order to achieve higher regeneration percentages per petiole explant.

CONCLUSIONS

We have undertaken the first studies to investigate the ability of adventitious shoot regeneration by either direct or indirect organogenesis from somatic tissues of *Aronia melanocarpa*.

In the current study, shoots were induced by both direct and indirect organogenesis from petiole segments of *in vitro* cultured plants of *A. melanocarpa*, cultivar 'Nero'.

Our results showed the possibility of establishing an effective *in vitro* adventitious shoot regeneration system for *Aronia melanocarpa*, which holds great promise for either micropropagation and/or genetic transformation studies in black chokeberry.

Also, the results of this investigation may be useful in optimizing shoot regeneration systems for other cultivars of *Aronia melanocarpa*, and also as an alternative allowing rapid mass propagation of elite genotypes independent from seasonal influences.

ACKNOWLEDGEMENTS

This research was supported by a Doctoral Research Support grant from the Ministry of National Education. We greatly appreciate the support received through the collaborative work undertaken with the Laboratory of Tissue Culture at the Research Institute for Fruit Growing, and University of Pitești.

REFERENCES

Banjari I., Misir A., Šavikin K., Jokić S., Molnar M., De Zoysa H.K.S., Waisundara V.Y., 2017. Antidiabetic effects of *Aronia melanocarpa* and its other therapeutic properties. Frontiers in Nutrition, 4:53.

Bădescu M., Bădulescu O., Bădescu L., Ciocoiu M., 2015. Effects of *Sambucus nigra* and *Aronia melanocarpa* extracts on immune system disorders within diabetes mellitus. Pharmaceutical Biology, 53(4):533-539.

Bell D.R., Burt T.D., 2007. Phenolic acids contained in anthocyanin enriched extracts from elderberry, bilberry and chokeberry possess endothelium dependent and independent vasorelaxation properties in porcine coronary arteries. FASEB Journal, 21:366.

Benvenuti S., Pellati F., Melegari M., Bertelli D., 2004. Polyphenols, anthocyanins, ascorbic acid, and radical scavenging activity of *Rubus, Ribes* and *Aronia*. Journal of Food Science, 69: FCT 164.

Bermúdez-Soto M.J., Tomás-Barberán F.-A., García-Conesa M.-T., 2007. Stability of polyphenols in chokeberry (*Aronia melanocarpa*) subjected to *in vitro* gastric and pancreatic digestion. Food Chemistry, 102 (3): 865-874.

Bijak M., Bobrowski M., Borowiecka M., Podsedek A., Golanski J., Nowak P., 2011. Anticoagulant effect of polyphenols-rich extracts from black chokeberry and grape seeds. Fitoterapia, 82: 811-817.

Borowska S., Brzóska M.M., 2016. Chokeberries (*Aronia melanocarpa*) and their products as a possible means for the prevention and treatment of noncommunicable diseases and unfavorable health effects due to exposure to xenobiotics. Comprehensive Reviews in Food Science and food safety, 15 (6): 982-1017.

Brand M.H., Cullina W.G., 1990. Micropropagation of *Aronia arbutifolia* and *A. melanocarpa*. Hort Science, 25 (9):1096.

Brand M.H., Cullina W.G., 1992. Micropropagation of red and black chokeberry (*Aronia* spp.). Hort Science, 27 (1): 81.

Cheng Y., 2017. Tissue culture of *Aronia melanocarpa*. Protection Forest Science and Technology.

Denev P.N., Kratchanov C.G., Ciz M., Lojek A., Kratchanova M.G., 2012. Bioavailability and antioxidant activity of black chokeberry (*Aronia melanocarpa*) polyphenols: in vitro and *in vivo* evidences and possible mechanisms of action. Comprehensive Reviews in Food Science and Food Safety, 11: 471-489.

Duthie S.J., 2007. Berry phytochemicals, genomic stability and cancer: evidence for chemoprotection at several stages in the carcinogenic process. Molecular Nutrition & Food Research, 51:665-674.

Ganeshan S., Caswell K.L., Kartha K.K., Chibbar R.N., 2002. Shoot regeneration and proliferation. In: Khachatourians G.G., McHughen A., Scorza R., Nip W.K., Hui Y.H. (Eds), Transgenic Plants and Crops. Marcel Dekker, Inc., New York, 69-84.

Gasiorowski K., Szyba K., Brokos B., Kołaczynska B., Jankowiak-Włodarczyk M., Oszmianski J., 1997. Antimutagenic activity of anthocyanins isolated from *Aronia melanocarpa* fruits. Cancer Letters, 119 (1): 37-46.

Hellström J., Shikov A.N., Makarova M.N., Mattila P., 2010. Blood pressure-lowering properties of chokeberry (*Aronia mithurinii*, var. Viking). Journal of Functional Foods, 2 (2): 163-169.

Isac V., Popescu A., 2009. Protocols for *In Vitro* Micropropagation of Raspberry, and Plant Regeneration by Organogenesis. In: Mezzetti B., Ružić Đ., and Gajdosova A. (Eds), A Guide to Some *In Vitro* Techniques – Small Fruits. Grafika Jureš, Čačak, Serbia.

Jakobek L., Seruga M., Medvedovic-Kosanovic M., Novak I., 2007. Antioxidant activity and polyphenols of *Aronia* in comparison to other berry species.

Agriculturae Conspectus Scientificus, 72 (4): 301-316.

Jakobek L., Drenjancevic M., Jukic V., Seruga M., 2012. Phenolic acids, flavonols, anthocyanins and antiradical activity of 'Nero', 'Viking', 'Galicianka' and wild chokeberries. Scientia Horticulturae, 147 (12): 56-63.

Kahkonen M.P., Hopia A.I., Heinonen M., 2001. Berry phenolics and their antioxidant activity. Journal of Agriculture and Food Chemistry, 49: 4076-4082.

Kane M.E., Dehgan B., Sheehan T.J., 1991. In vitro propagation of Florida native plants: *Aronia arbutifolia*. Proceedings of Florida State Horticultural Society, 104: 207-290.

Kokotkiewicz A., Jaremicz Z., Luczkiewicz M., 2010. *Aronia* plants: A review of traditional use, biological activities, and perspectives for modern medicine. Journal of Medicinal Food, 13 (2): 255-269.

Kowalczyk E., Kopff A., Fijałkowski P., Kopff M., Niedworok J., Błaszczyk J., Kedziora J., Tyślerowicz P., 2003. Effect of anthocyanins on selected biochemical parameters in rats exposed to cadmium. Acta Biochimica Polonica, 50 (2): 543-548.

Kulling S.E., Rawel H.M., 2008. Chokeberry (*Aronia melanocarpa*) – A review on the characteristic components and potential health. Planta Medica, 74: 1625-1634.

Kwak M.C., Choi C.H., Choi Y.E., Moon H.K., 2015. Micropropagation of *Aronia* (*Aronia melanocarpa* Elliot, black chokeberry) and its 5 varieties. Journal of Plant Biotechnology, 42: 380-387.

Kwiecien I., Szopa A., Madej K., Ekiert H., 2013. Arbutin production via biotransformation of hydroquinone in in vitro cultures of *Aronia melanocarpa* (Michx.) Elliott. Acta Biochimica Polonica, 60 (4): 865-870.

Lee E.C.M., de Fossard R.A., 1977. Some factors affecting multiple bud formation of strawberry Fragaria x ananassa *in vitro*. Acta Horticulturae, 78: 187-195.

Litwińczuk W., 2002. Propagation of black chokeberry (*Aronia melanocarpa* Elliot) through *in vitro* culture. Electronic Journal of Polish Agricultural Universities, 5 (2): #06.

Litwińczuk W., 2013. Micropropagation of chokeberry by *in vitro* axillary shoot proliferation. Methods in Molecular Biology, 11013: 179-186.

Lloyd G., McCown B.H., 1980. Commercially feasible micropropagation of mountain laurel (*Kalmia latifolia*) by use of shoot tip culture. International Plant Propagation Society, Combined Proceedings, 30: 421-427.

Mahečić I., 2009. *In vitro* propagation of black chokeberry (*Aronia melanocarpa* Michx.). Diploma thesis, Faculty of Science, University of Zagreb, Croatia.

Malik M., Zhao C., Schoene N., Guisti M., Moyer M.P., Magnuson B.A., 2003. Anthocyanin-rich extract from *Aronia melanocarpa* E. induces a cell cycle block in colon cancer but not normal colonic cells. Nutrition and Cancer, 46 (2): 186-196.

McKay S.A., 2001. Demand increasing for aronia and elderberry in North America. New York Fruit Quarterly, 9 (3): 2-3.

Murashige T., Skoog F., 1962. A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiologia Plantarum, 15 (3): 473-497.

Naruszewicz M., Laniewska I., Millo B., Dluzniewski M., 2007. Combination therapy of statin with flavonoids-rich extract from chokeberry fruits enhanced reduction in cardiovascular risk markers in patients after myocardial infarction. Atherosclerosis, 194: e179-e84.

Ochmian I., Grajkowski J., Smolik M., 2012. Comparison of some morphological features, quality and chemical content of four cultivars of chokeberry fruits (*Aronia melanocarpa*). Notulae Botanicae Horti Agrobotanici Cluj-Napoca, 40: 253-260.

Olas B., Kedzierska M., Wachowicz B., Stochmal A., Oleszek W., Jeziorski A., Piekarski J., Glowacki R., 2010. Effect of *Aronia* on thiol levels in plasma of breast cancer patients. Central European Journal of Biology, 5: 38-46.

Olas B., Wachowicz B., Nowak P., Kedzierska M., Tomczak A., Stochmal A., Oleszek W., Jeziorski A., Piekarski J., 2008. Studies on antioxidant properties of polyphenol-rich extract from berries of *Aronia melanocarpa* in blood platelets. Journal of Physiology and Pharmacology, 59 (4): 823-835.

Oszmiański J., Wojdyło A., 2005. *Aronia melanocarpa* phenolics and their antioxidant activity. European Food Research & Technology, 221: 809-813.

Park C-H., Kim J-H., Lee E.B., Hur W., Kwon O-J., Park H-J., Yoon S.K., 2017. *Aronia melanocarpa* extract ameliorates hepatic lipid metabolism through PPARγ2 downregulation. PLoS ONE, 12 (1): e0169685.

Park J.M., Park J.B., 2011. The preventive and therapeutic effects of Aronox extract on metabolic abnormality and hypertension. Journal of the Korean Society for Hypertension, 17: 95-102.

Pawlowicz P., Stachowiak G., Bielak A., Wilczynski J., 2001. Application of natural anthocyanins extracted from chokeberry (*Aronia melanocarpa*) for treatment of oligospermia in men having increased levels of antibodies anti-O-LDL (oLAB). Influence on the fructose level in sperm. Ginekologia Polska 72 (12): 983-988.

Petrovic D.M., Jacimovic-Plavsic M.M., 1992. *Aronia melanocarpa* and propagation *in vitro*. *In vitro* culture. XXIII[rd] International Horticultural Congress, Florence, Italy, 30 August 1990. Acta Horticulturae, 300: 133-135.

Popescu A., Isac V., 2000. High frequency shoot regeneration from leaf-derived callus in raspberry (*Rubus idaeus* L.). Acta Horticulturae, 538: 667-670.

Rop O., Mlček J., Juríková T., Valšíková M., Sochor J., Rězníček V., Kramářová D., 2010. Phenolic content, antioxidant capacity, radical oxygen species scavenging and lipid peroxidation inhibiting activities of extracts of five black chokeberry (*Aronia melanocarpa* Michx., Elliot) cultivars. Journal of Medicinal Plant Research, 4(22): 2431-2437.

Rugină D., Sconta Z., Pintea A., Bunea A., Socaciu C., 2011. Protective effect of chokeberry anthocyanin-rich fraction at nanomolar concentrations against oxidative stress induced by high doses of glucose in pancreatic β-cells. Bulletin of UASVM, Veterinary Medicine, 68 (1): 313-319.

Sharif T., Stambouli M., Burrus B., 2013. The polyphenolic-rich *Aronia melanocarpa* juice kills teratocarcinomal cancer stem-like cells, but not their differentiated counterparts. Journal of Functional Foods, 5 (3): 1244-1252.

Sikora J., Broncel M., Markowicz M., Chałubiński M., Wojdan K., Mikiciuk-Olasik E., 2012. Short-term supplementation with *Aronia melanocarpa* extract improves platelet aggregation, clotting, and fibrinolysis in patients with metabolic syndrome. European Journal of Nutrition, 51 (5): 549-556.

Silvestri C., Cristofori V., Ceccarelli M., Caceres M.E., Escriba-Lacuesta J., Rugin E., 2016. Adventitious shoot organogenesis from leaf and petiole explants of European hazelnut. Plant Cell, Tissue and Organ Culture, 126 (1): 59-65.

Simeonov S.B., Botushanov N.P., Karahanian E.B., Pavlova M.B., Husianitis H.K., Troev D.M., 2002. Effects of *Aronia melanocarpa* juice as part of the dietary regimen in patients with diabetes mellitus. Folia Medica (Plovdiv), 44: 20-23.

Skoczynska A., Jedrychowska I., Poreba R., Affelska-Jercha A., Turczyn B., Wojakowska A., Andrzejak R., 2007. Influence of chokeberry juice on arterial blood pressure and lipid parameters in men with mild hypercholesterolemia. Pharmacology Reports, 59: 177-182.

Slimestad R., Torskangerpoll K., Nateland H.S., Johannessen T., Giske N.H., 2005. Flavonoids from black chokeberries, *Aronia melanocarpa*. Journal of Food Compostion and Analysis, 18: 61-68.

Staniene G., Stanys V., Bobinas C., Duchowski P., Merkys A., 1999. *In vitro* propagation of non-traditional horticultural plants (*Actinidia, Chaenomeles, Aronia*). Zeszyty Problemowe Postepow Nauk Rolniczych, 468: 441-443.

Strigl A.W., Leitner E., Pfannhauser W., 1995. Die schwarze Apfelbeere (*Aronia melanocarpa*) als natürliche Farbstoffquelle. Deutsche Lebensmittel Rundschau, 91: 177-180.

Sueiro L., Yousef G.G., Seigler D., de Mejia E.G., Grace M.H., Lila M.A., 2006. Chemopreventive potential of flavonoid extracts from plantation-bred and wild *Aronia melanocarpa* (black chokeberry) fruits. Journal of Food Science, 71: 480-488.

Şuțan A.N., Isac V., Duminică C., Popescu A., 2017. Studies on the *in vitro* micropropagation ability of *Aronia melanocarpa* (Michx.) Elliot. Current Trends in Natural Sciences, 6 (11): 85-92.

Szopa A., Ekiert H., Muszynska B., 2013. Accumulation of hydroxybenzoic acids and other biologically active phenolic acids in shoot and callus cultures of *Aronia melanocarpa* (Michx.) Elliott (black chokeberry). Plant Cell Tissue and Organ Culture, 113: 323-329.

Szopa A., Ekiert H., 2014. Production of biologically active phenolic acids in *Aronia melanocarpa* (Michx.) Elliott *in vitro* cultures cultivated on different variants of the Murashige and Skoog medium. Plant Growth Regulators, 72: 51-58.

Tanaka T., Tanaka A., 2001. Chemical components and characteristics of black chokeberry. Journal of Japan Society of Food Science and Technology, 48:606-610.

Tolić M.T., Jurčević I.L., Krbavčić I.P., Marković K., Vahčić N., 2015. Phenolic content, antioxidant capacity and quality of chokeberry (*Aronia melanocarpa*) products. Food Technology & Biotechnology, 53 (2): 171-179.

Valcheva-Kuzmanova S., Gadjeva V., Ivanova D., Belcheva A., 2007. Antioxidant activity of *Aronia melanocarpa* fruit juice *in vitro*. Acta Alimentaria, 36: 425-428.

Velchev V., Mladenova O., 1992. Root formation and adaptation of micropropagated Aronia shoots. Rastenievdni Nauki, 29 (5-6): 79-83.

Walther E., Schnell S., 2009. Die Schwarze Apfelbeere (*Aronia melanocarpa*) - eine besondere Obstart [Black chokeberry (*Aronia melanocarpa*) - a special fruit crop]. Zeitschrift für Arznei- & Gewürz-pflanzen, 14 (4): 179-182

Wiczkowski W., Romaszko E., Piskula M.K. 2010. Bioavailability of cyanidin glycosides from natural chokeberry (*Aronia melanocarpa*) juice with dietary-relevant dose of anthocyanins in humans. Journal of Agricultural and Food Chemistry, 58 (23): 12130-12136.

Wu X.L., Gu L.W., Prior R.L., McKay S., 2004. Characterization of anthocyanins and proantho-cyanidins in some cultivars of *Ribes*, *Aronia*, and *Sambucus* and their antioxidant capacity. Journal of Agriculture and Food Chemistry, 52: 7846-7856.

Zapolska-Downar D., Hajdukiewicz K., Bryk D., Sitkiewicz D., Malecki M., 2012. *Aronia melanocarpa* fruit extract exhibits anti-inflammatory activity in human aortic endothelial cells. European Journal of Nutrition, 51 (5): 563-572.

Zielińska-Przyjemska M., Olejnik A., Dobrowolska-Zachwieja A., Grajek W., 2007. Effects of *Aronia melanocarpa* polyphenols on oxidative metabolism and apoptosis of neutrophils from obese and non-obese individuals. Acta Scientiarum Polonorum. Technologia Alimentaria, 6 (3): 75-87.


EX·TERRA·AVRVM