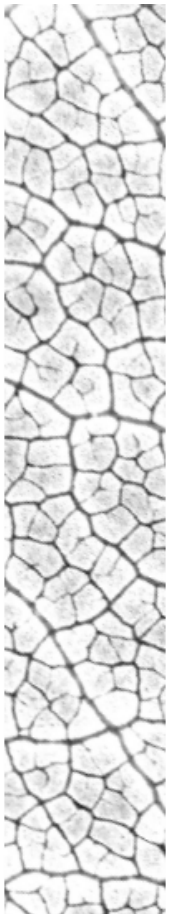

EurAsian Journal of BioSciences
Eurasia J Biosci 13, 1071-1073 (2019)

In vitro clonal micropropagation of Aronia L. varieties from the collection of the botanic garden of the National Research University "BelSU" (Belgorod, Russia)

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Abstract
Studying the characteristics of *in vitro* cultivation of species and varieties of Aronia made it possible to determine the optimal conditions for sterilizing plants, the composition of the medium for propagation and rhizogenesis of explants. Adaptation plant varieties of Aronia obtained using the developed media and cultivation regimes is characterized by a high survival rate (97-99%) under unsterile conditions, which makes them potential objects for mass replication by clonal micropropagation.

Keywords: clonal micropropagation, species, varieties, *Aronia L., in vitro*

Petrova IV, Tokhtar LA, Kulko SV, Borodayeva ZA (2019) *In vitro* clonal micropropagation of Aronia L. varieties from the collection of the botanic garden of the National Research University "BelSU" (Belgorod, Russia). Eurasia J Biosci 13: 1071-1073.

INTRODUCTION

The harmful effect of environmental and anthropogenic factors on the environment, as well as the low guarantee of successful reproduction of plant species and varieties using traditional methods necessitates the creation of technologies of clonal micropropagation of plants *in vitro* to maintain biodiversity, preserve rare and critically endangered plant species, produce food biomass and drugs (Belokurova et al. 2005, Muratova et al. 2017, 2018).

Chokeberry (*Aronia L.*) is a rare, unconventional orchard crop. Plants of this genus are of great value, because their fruits contain a large number of bioactive substances. They are a source of multivitamins, they can boost immune system, regulate the work of the thyroid gland, protect the body from radiation injury. In addition, these varieties are of great decorative value throughout the growing season. The seeds of Aronia plants are not distinguished by good germinating capacity. Therefore, to clone them, cuttings are most often used. The development of the methods for *in vitro* clonal micropropagation of chokeberry makes it possible to carry out works throughout the year and to reduce the areas required for the cultivation of planting set. The success and effectiveness of the micropropagation stage per se depends on the characteristics of the genotype of the parent plant, the mineral composition of the nutrient media, as well as the effect of physical factors during cultivation (light, temperature, humidity) (Kalinin 1992).

The study aims at developing the ways to optimize clonal micropropagation of varieties of *Aronia* genus from the collection of the Botanic Garden of the National Research University "Belgorod State University".

OBJECTS AND METHODS

The study objects were various species and varieties of *Aronia* genus from the collection of the Botanic Garden of the National Research University "Belgorod State University": *A. mitschurini* «Amit», *A. prunifolia* «Nero», *A. prunifolia* «Viking», *A. melanocarpa* «Hugin», *A. arbutifolia* «Brilliant». They were chosen for research because they are successfully cultivated in the conditions of the Belgorod region and are ranged as successful introduced species to be promising for use in the region.

The main research methods were the methods worked out for clonal micropropagation of plants at different stages of their reproduction and adaptation. (Timofeeva and Nevmerzhitskaya 2012, Tokhtar et al. 2016).

For microclonal propagation of varieties of Aronia genus, modifications of the nutrient Murashige and Skoog medium with mesoinositol — 100 mg / l, agar — 7 g / l and the vitamin complex (Murashige and Skoog

Received: February 2019
Accepted: May 2019
Printed: August 2019

Table 1. Effect of Sterilizing Agent on the Explants of the Varieties of *Aronia* Genus

Variety	Effectiveness of Sterilization, %	
Aronia arboriformis "Brilliant"	Viable	71.43
	Infected	28.57
	Necrosis	14.29
Aronia arbutifolia "Hugin"	Viable	92.31
	Infected	23.08
	Necrosis	7.69
Aronia prunifolia "Nero"	Viable	54.55
	Infected	27.27
	Necrosis	36.36
Aronia prunifolia "Viking"	Viable	85.71
	Infected	14.29
	Necrosis	7.14
Aronia mitschurinii "Amit"	Viable	66.67
	Infected	40
	Necrosis	6.67

1962) were used. The source of carbohydrate was sucrose at a concentration of 30 g / l.

Cytokinin was used to induce morphogenesis: 6-benzylaminopurine (6-BAP); auxins: indolyl-3-butyric acid (IBA), indolyl-3-acetic acid (IAA); gibberellins: gibberellic acid (GA) (Reshetova and Kashin 2013, Litwinczuk 2002).

Micro cuttings of 1.5–2 cm grown in the propagation medium were used for rooting. At the stage of establishment of shoot cuttings, the concentration of carbohydrate and macrosalts was halved. Hormone-free nutrient media were used or β-indolylbutyric acid (IBA) and β-indolylacetic acid (IAA) were added to the rooting medium at a concentration of 0.5-1.0 41 mg / l (Brand and Cullina 1992, Chalupa 1983).

In the experiments on clonal micropropagation of plants, the number of germinating, failed and infected explants were taken into account, as well as the number of newly formed shoots and buds, the terminal shoots of 1.5 – 2 cm long, the number and length of rooted shoots, the number and length of roots of rooted shoots, the number of plants after adaptation in unsterile conditions.

To determine the reproduction factor, the ratio of the total number of newly formed shoots per passage to the number of explants that formed at least one additional shoot was determined, We have selected 25–30 explants for each variant of the sample All experiments on the reproduction and rooting of shoots have been repeated at least three times.

MAIN PART

Resting buds of Aronia as the primary explants for bringing under cultivation were used. Aronia cuttings were cut and placed in water for germination.

The sprouted buds were removed from the upper cover scales, washed in a weak solution of potassium permanganate (KMnO4) and placed in a laminar box. The explants were placed in a sterile glass and being processed with "Belizna" for 4 minutes, then washed with sterile distilled water three times for 5 minutes. The sterile explants were placed in a test tube on a nutrient medium containing salts and vitamins according to Murashige and Skoog. The tubes covered with foil and film were placed in the light room for further explants' germinating.

The analysis of the data suggests that "Belizna" can be successfully used to sterilize chokeberry explants. This sterilizer had a severe effect on the meristem of Aronia prunifolia of "Nero" variety, which killed 36.36% of the explants. However, other varieties showed a rather high viability (**Table 1**).

To make the plants reproduction factor definite, the number of new shoots formed and their length was determined. The greatest number of newly formed shoots was found in Aronia prunifolia "Viking" - 5.08 pcs with an average shoot length of 0.86 cm.

As with many cultures, the morphogenesis of chokeberry in tissue culture remains a subject matter requiring further development. This is due to the species and varietal specificity of plants. The genotypes included in our studies require an individual salt selection and hormonal composition of the nutrient medium.

It is established in the course of analyzing literature data that for clonal micropropagation of plants of different varieties of different species of the *Aronia* genus, several variants of media with different phytohormone contents may be suitable (Kuklina et al. 2003, Milekhin and Rubtsov 2015, Shipunova 2001).

The most important factor in the formation of shoots is the correct choice of growth regulator and its concentration. We have selected 6-benzylaminopurine of the 52 phytohormones of the cytokinin series as one of the most active and successfully used cytokinin in the cultivation of plants *in vitro*. As the result of study, the composition of the nutrient medium for the most active shoot formation have been chosen. **Table 2** presents the results obtained.

CONCLUSION

In the course of research of different species and varieties of chokeberry, it was established that sterilization of plants at the stage of their *in vitro* cultivation, carried out with the help of the household sterilizer "Belizna", made it possible to achieve a fairly high viability of explants, which was 55-92%. The major number of newly formed shoots has been revealed in Aronia prunifolia "Viking" – 5.08 ± 2.57 pcs with an average shoot length of 0.86 ± 0.55 cm.

To propagate the explants of all Aronia varieties that have been studied, Murashige and Skoog medium with different level of concentration of cytokinin 6-BAP is recommended: for the "Brilliant", "Nero" and "Viking" varieties – 1.5 mg / l; "Amit" varieties – 1 mg / l; "Hugin" – 0.5 mg / l for maximum shoot formation or 1.5 mg / l for maximum explant extraction.

It has been established that to optimize the process of rhizogenesis of different varieties of chokeberry, it is necessary to use different types and concentrations of

Table 2. The Influence of Cytokinin on the Number (pcs) of the Formed Shoots of Varieties of Aronia Species

Variety	Mineral Composition of Medium and Concentration of Cytokinin, mql							
	½ MS 0.5	½ MS 1	½ MS 1.5	MS 0.5	MS 1	MS 1.5	MS 2	MS 0 (Control)
Aronia arboriformis "Brilliant"	1.12±0.33	1.18±0.39	1.24±0.44	1.06±0.24	1.17±0.39	1.53±0.71	2.06±1.6	1.06±0.24
Aronia arbutifolia "Hugin"	1.24±0.44	1.41±0.51	1.06±0.24	1.71±0.77	1.41±0.71	1.35±0.7	1.18±0.53	1.06±0.25
Aronia prunifolia "Nero"	1.05±0.25	1.24±0.56	1.18±0.39	1.18±0.53	1.41±0.62	1.47±0.62	1.24±0.43	1.05±0.26
Aronia prunifolia "Viking"	1.21±0.54	1.1±0.31	1.26±0.45	1.11±0.31	1.53±0.7	1.74±0.93	2±0.88	1.16±0.38
Aronia mitschurinii "Amit"	1.26±0.45	1.79±0.92	1.16±0.37	1.16±0.37	2.05±1.03	1.37±0.6	1.53±0.77	1.74±0.99

auxins: for "Nero" – 0.5 mg / l of IAA; "Brilliant" and "Viking" – 0.5 mg / l of IBA or 0.5 mg / l of IAA; "Hugin" – 1 mg / l of IBA or 1 mg / l of IAA; "Amit" –0.5 mg / l or 1 mg / l of IBA.

Adaptation of plants of Aronia varieties obtained using the developed media and cultivation regimes is characterized by a high survival value (97-99%) to unsterile conditions, which makes them promising objects for mass replication by clonal micropropagation.

ACKNOWLEDGEMENT

The investigation was carried out with the financial support from the State Program No. 6.4854.2017/Basic Part, the Ministry of Science and Higher Education of the Russian Federation.

REFERENCES

Belokurova VB, Listvan EV, Maistrov PD, Sikura YY, Gleba YuYu, Kuchuk NV (2005) Using the Methods of Plant Biotechnology for the Preservation and Study of Biodiversity of the World Flora. Cytology and Genetics, (1): 41-51.

Brand MH, Cullina WG (1992) Micropropagation of red and black chokeberry (Aronia spp.). HortScience, 27: 81–89. https://doi.org/10.21273/HORTSCI.27.1.81

Chalupa V (1983) In vitro propagation of willows (Salix spp.), European mountain ash (Sorbus aucuparia L.) and black locust (Robinia pseudoacacia L.). Biol. Plant, 29: 305–307. https://doi.org/10.1007/BF02902879

Kalinin FL, Kushnir GP, Sarnatskaya VV (1992) Technology of Microclonal Propagation of Plants. Kiev: "Naukova Dumka", 232.

Kuklina AG, Semerikova EA, Molkanova OI (2003) Experience of Clonal Micro-Propagation of Blue Honeysuckle. Bulletin of the Central Botanical Garden, (185): 160-167.

Litwinczuk W (2002) Propagation of black chokeberry (Aronia melanocarpa Elliot) through in vitro culture. EJPAU, 5: 2–7.

Milekhin AV, Rubtsov SL (2015) Technology of In Vitro Microclonal Reproduction of Chrysanthemum. Young Scientist, (24): 335–338.

Murashige T, Skoog F (1962) A revised medium for rapid growth and bioassays with tobacco tissue cultures. Physiol Plant, 15(13): 473–497. https://doi.org/10.1111/j.1399-3054.1962.tb08052.x

Muratova SA, Budagovskiy AV, Tokhtar LA, Tokhtar VK, Deineka LA (2017) The research of clonal micropropagation efficiency of Schisandra chinensis under the influence of low-intensity coherent radiation. International Journal of Green Pharmacy, 11(3): 634. Retrieved from http://www.greenpharmacy.info/index.php/ijgp/article/view/1187/722

Muratova SA, Subbotina NS, Tokhtar LA, Tokhtar VK, Yatsenko VM, Petrunova TV (2018) The influence of the spectral composition on the root development of ornamental plants in vitro. Indo American Journal of farmaceutical sciences (AJPS), 05(07): 6979-6984. Retrieved from https://zenodo.org/record/1324380#.W-f3OtWPmUk

Reshetova AS, Kashin AS (2013) Peculiarities of Clonal Micropropagation of Mountain Ash of "Granatum" Varieties. Proceedings of the Saratov University, 13: 58–65.

Shipunova AA (2001) Selection of the Mineral Basis of Nutrient Media for In Vitro Clonal Honeysuckle Micro Reproduction. Collection of Scientific Papers of the VSTISP "Fruit and Berry Growing of Russia": 158–163.

Timofeyeva OA, Nevmerzhitskaya YuYu (2012) Clonal Plant Micropropagation: Teaching Aid. Kazan: The Kazan University, 59 p.

Tokhtar VK, Zhang D, Tokhtar LA, Safronova GI (2016) Momordica cochinchinensis (Lour.) Spreng. (Cucurbitaceae) in culture in vitro. Research Journal of Pharmaceutical, Biological and Chemical Sciences, 7(6)(6): 3243. Retrieved from http://dspace.bsu.edu.ru/bitstream/123456789/19581/1/Tokhtar_Momordica.pdf