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TEHNOLOGIA DE ÎNMULȚIRE *IN VITRO* LA SCORUȘUL NEGRU *ARONIA MELANOCARPA* (Michx.) ELLIOT

IN VITRO PROPAGATION TECHNOLOGY FOR THE BLACK CHOKEBERRY *ARONIA MELANOCARPA* (Michx.) ELLIOT

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Abstract

The fruits of *Aronia melanocarpa* (Michx.) Elliot are recognized for their many benefits on the human body due to their medicinal and nutritional qualities. In order to ensure the need for propagating material recently requested by the market, an attempt was made to make the multiplication more efficient. Classical methods of multiplication have given poor results, which has led to the approach of multiplication by in vitro cultures. The varieties of interest were 'Nero' and 'Melrom'. The research carried out so far has established effective culture media for the initiation, multiplication and rooting phases as follows: MS mineral salts and LS vitamins with a hormonal balance consisting of GA_3, BAP and IBA for the initiation and multiplication stages. The phytohormones GA_3 and IBA were used for rooting. The regeneration of the explants in the initiation stage reached an average of 86%. The multiplication rate was on average at 'Nero' of 6 shoots / explant and at 'Melrom' 12.6 shoots / explant. The rooting on the established *in vitro* culture media was 97% for 'Nero' and 80.19% for 'Melrom'. The acclimatization percentages were 92.15% for 'Nero' and 90.41% for 'Melrom'. *In vitro* propagation of *Aronia melanocarpa* (Michx.) Elliot has proven to be the method that gives the best results for the multiplication of this species.

Cuvinte cheie: scorușul negru, *in vitro*, tehnologie, genotip, mediu de cultură, rată de multiplicare.
Key words: black chokeberry, *in vitro*, technology, genotype, culture medium, multiplication rate.

1. Introduction

Aronia melanocarpa, popularly called black chokeberry, wild currant or wild rose, is native to eastern North America, including Canada (Andrzejewska et al., 2015), having been imported into Europe at the beginning of the 20th century due to its potential its beneficial for health (Poyraz and Mert, 2020). Due to the presence and high content of different bioactive compounds, such as vitamins, minerals and polyphenolic compounds, *Aronia melanocarpa* fruits and leaves show a wide range of positive health effects (Jurikova et al., 2017; Szopa et al., 2017; Jurendić and Ščetar, 2021).

The cultivation area of *Aronia melanocarpa* species has expanded substantially in Europe, USA, Canada and Asia. The species was introduced into cultivation in Romania after 1986 (https://www.madr.ro/docs/agricultura/legume-fructe/Ghid-Pomicultura-final.pdf), when scientific research was carried out on the improvement and propagation of the species, and nurseries were established to obtain quality planting material (Bălan et al., 2017). Currently, there are no official statistics regarding black chokeberry culture at the global level, as these are niche cultures in continuous development.

Plant biotechnologies currently have a very important role in the development and modernization of agriculture and horticulture. Among these, micropropagation has become in recent decades a reliable and routine approach for the rapid multiplication of plants, which is based on cultures of plant cells, tissues and organs on well-defined culture media under aseptic conditions (Jain and Häggman, 2007).

Aronia can be propagated by seeds, but the method is not recommended because uneven plants are obtained that come into fruit late, the fruiting shrub species being genetically heterozygous. Internationally, studies on the in vitro micropropagation of this species have been carried out by several researchers since the 1990s (Brand and Cullina, 1992, Ruzic, 1993, Velchev and Mladenova, 1992; Staniene et al., 1999) and established that micropropagation is much more efficient than other propagation methods.

In Romania, concerns for the micropropagation of this species were developed mainly at I.C.D.P. Pitesti in the Tissue Culture Laboratory. Research was developed for the establishment and improvement of micropropagation technologies, as well as development activities for the application of the research results. We report the results published in *Aronia arbutifolia (Photinia pyrifolia)* by Neculae, L. in 1988; Rusea et al., 2018, 2019, 2022; Şuţan et al., 2017; Borsai et al., 2021. After the introduction of the *A. melanocarpa* species into the study as a species of interest, research was developed for the establishment and improvement of the micropropagation technology as well as development activities for the application of the research results to the 'Nero' variety in the 90s and the Romanian variety 'Melrom'

created at ICDP Pitesti in 2016. 'Melrom' has similar morphological characteristics to the 'Nero' variety, but it is differentiated by the larger fruits.

The purpose of this scientific article is to present an efficient micropropagation technology for 'Nero' and 'Melrom' cultivars of *Aronia melanocarpa* which was developed through research carried out in the laboratory of in vitro cultures at ICDP Pitesti, Maracineni, Arges. We optimized all the stages of in vitro rapid propagation, in order to provide a modern and highly improved technology for the production of planting material.

2. Material and methods

The *in vitro* multiplication technology was carried out in a laboratory specially set up for in vitro culture activities, with specialized staff.

Explants of axillary buds from mature, authentic plants are used to initiate cultures. A very good time for harvesting the biological material and inoculation is between February and April.

The presented micropropagation technology goes through 5 stages: 3 in vitro and 2 in vivo: in vitro cultures initiation, in vitro multiplication, in vitro rooting, acclimatization, fortification in the greenhouse or in the field.

The annual shoots are sectioned into segments that contain at least one vegetative bud. Black chokeberry nodal explants were washed in tap water with 2-3 drops of Domestos, dipped in ethyl alcohol 96 volume % for 5 minutes, rinsed with sterile distilled water, and sterilizes for 10 minutes in calcium hypochlorite 6% for 10 minutes. After three rinses with sterile distilled water the nodal explants were transferred on culture media. After four weeks of in vitro culture, new black chokeberry shoots will be transferred on multiplication media. For in vitro rooting the biological material consisted of shoots from micropropagation stage. All operations were carried out in the horizontal laminar air flow hood. The vessels used for in vitro culture initiation were test tubes containing 5 ml of medium. For the in vitro multiplication and rooting experiments 720-mL jars containing 100 ml of culture media vessel were used.

The composition of culture media for chokeberry micropropagation is shown in Table 1. The cultures were incubated in the growth chamber at 23±3°C and 2,400 Lux light intensity and a photoperiod of 16 hours of light and 8 hours of darkness.

In order to determine the behavior in acclimatization stage to in vivo conditions the rooted seedlings were removed from the culture medium, rinsed with sterile distilled water, dried on sterile paper towels, and transferred to perlite under greenhouse conditions with artificial mist.

The data recorded for establishing the most adequate conditions for all stages of micropropagation were: (i) the regeneration percentage; (ii) the multiplication rate – RM (number of plants / explant); (iii) the rooting percentage; (iiii) the survival percentage in acclimatization stage.

3. Results and discussions

In vitro cultures initiation

The process of growth and differentiation manifests itself after 4 weeks of culture. The optimal balance between the growth regulators (mg/l) that ensured the success of the initiation of black chokeberry culture in vitro is: 1.0 BAP and 0.1 GA_3 on a basic medium consisting of macroelements and microelements Murashige - Skoog (1962) and vitamins Linsmaier - Skoog (1965). The composition of this medium allows the regeneration of explants into viable shoots in an average percentage of over 86% (Table 2) for the two varieties. The newly formed seedlings are 10-20 mm long and have 4-5 leaves (Fig. 1).

In vitro multiplication

To stimulate the differentiation of axillary buds, the basic medium with mineral salts Murashige - Skoog (1962) and vitamins Linsmaier - Skoog (1965), in the presence of the growth regulators (mg/l) combination consisting of: 0.1 mg/l GA_3, 1, 0 mg/l BAP and 0.1 mg/l IBA offer the best conditions (Fig. 1).

A good MR of 12.6 shoots per bush is obtained in 'Nero' and 6 shoots per bush in 'Melrom' (media), after a total of 5 subcultures (Table 2). For black chokeberry, the data regarding the MR show that the proliferation starts right from the 2nd subculture. Despite the significantly higher number of shoots formed/explant in the second subculture, there were no significant differences in the vigor of chokeberry shoots from the second subculture compared to the first subculture. A higher MR is obtained in the 'Nero' variety compared to the 'Melrom' variety. The difference in shoot multiplication in the two black chokeberry varieties can be attributed to the influence of the genotype. Similar results were obtained by Kwak et.al, 2015, who reported a higher rate of multiplication in cultivar 'Nero' compared to other cultivars ('Viking', 'Purple', 'Mackenzie').

***In vitro* rooting**

To stimulate rhizogenesis, the shoots are excised from the bush of multiplied plants and grown individually on a nutrient medium without BAP. *Aronia melanocarpa* responds quickly to the rooting stage and the results are effective. The induction of rhizogenesis is carried out with IBA in a concentration of 0.7 mg/l on the same basic medium used for the multiplication of micro cuttings. The in vitro rooting percentage of chokeberry seedlings is over 80.0%, with a number of 2.8 roots/plant. Rooting on the established medium formula was 97% at 'Nero' and 80.19% at 'Melrom' (Table 2, Fig. 2).

Acclimatization

Acclimatization consists in the transition of the plants from the heterotrophic state to the autotrophic one. The duration of the acclimatization is about 45 days, of which the first 3-4 days can be constituted as a pre-acclimatization stage that consists in discovering the pots with plants. For acclimatization, the plants are removed from the culture vessels and placed in trays and either planted immediately, or kept in cold rooms or refrigerators. Planting is done in perlite and atmospheric humidity and shading are ensured. Since the plants are small, the planting operation is done carefully avoiding the strong tightening of the substrate at the level of the roots. To prevent dehydration of the leaves, as they are planted, sprinkle them with water and cover them with polyethylene film. The acclimatization process is considered complete after the appearance of new leaves and root growth. The percentage of acclimatized plants is between 90-93% in high humidity conditions (70 – 80 %), in June (Table 2, Fig. 2).

Fortification can be done in pots or alveolar plates with soil mixture. Plant sizes upon delivery, minimum 20 cm. The duration of acclimatization is approx. 45 days.

4. Conclusions

We optimized all the stages of in vitro rapid propagation of *Aronia melanocarpa*, in order to provide a modern and highly improved technology for the production of planting material.

This simple and easy *in vitro* technology prove it that micropropagation is an effective technique for the production of *Aronia melanocarpa* planting material.

In vitro culture initiation can be done, successfully, in spring, using single-node shoot fragments.

The stages of *in vitro* culture initiation, multiplication and rooting can be achieved using the basic medium consisting of macroelements and the microelement MS with LS vitamins with agar, dextrose and iron chelate.

Analyzed in general, the results show that the rates between auxins, cytokinin and giberelic acidis used ensured good shoot growth and good rooting in vitro.

It has been proven once again that the influence of the genotype is important in establishing the in vitro micropropagation technology for chokeberry. The 'Nero' variety had a greater capacity for regeneration and proliferation *in vitro* than the 'Melrom' variety

The established in vitro propagation technology also allowed the development activity through which a number of 203 758 plants were produced and delivered to beneficiaries in the period 2014-2022. From this total, 105 642 plants were sold in the certified category according to the legislation in force.

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Tables and Figures

Table 1. Composition of culture medium used for *in vitro* propagation of *Aronia melanocarpa* (Michx.) Elliot cultivars 'Nero' and 'Melrom'

Compound	In vitro stages (mg/l)		
	Cultures initiation	Multiplication	Rooting
Macroelements			
NH_4NO_3	1650	1650	1650
KNO_3	1900	1900	1900
Ca Cl_2 x 2 H_2O	440	440	440
Mg SO_4 x 7H_2O	370	370	370
KH_2NH_4	170	170	170
Microelements			
Mn SO_4	22.3	22.3	22.3
Zn SO_4	8.6	8.6	8.6
H_3BO_3	6.2	6.2	6.2
Cu SO_4	0.025	0.025	0.025
Na_2MoO_4	0.250	0.250	0.250
Co Cl_2	0.250	0.250	0.250
KI	0.830	0.830	0.830
Vitamins			
Thiamine	0.4	0.4	0.4
Inositol	100	100	100
Growth regulators			
6- Benzylaminopurine	1.0	1.0	0
Gibberellic acid	0.1	0.1	0
Indole-3-butyric acid	0	0.1	0.7
NaFeEDTA	32	32	32
Agar Duchefa (g/l)	9	9	9
Dextrose	20	20	20

Table 2. The behavior of of *Aronia melanocarpa* (Michx.) Elliot cultivars 'Nero' and 'Melrom' in micropropagation technology application

Cultivar	Parameters			
	The regeneration percentage	*In vitro* multiplication - RM	The rooting percentage	Acclimatization percentage
Nero	88	12,6	97	93
Melrom	86	6	80,19	91

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Fig. 1. *In vitro* explant regeneration and multiplication of *Aronia melanocarpa* 'Nero' and 'Melrom' cultivars

Fig. 2. *In vitro* rooting and acclimatization of *Aronia melanocarpa* 'Nero' and 'Melrom' cultivars