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In vitro rooting and acclimatization *ex vitro* of *Aronia melanocarpa* cv. 'Nero'

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Abstract Originally from North America, naturalized and very well adapted in Europe, *Aronia melanocarpa* (Michx.) Elliot (black chokeberry) is an important edible and medicinal species due to its high content of antioxidants. Although Aronia can be easily propagated by seeds, this method is not indicated because it delays fruiting, and therefore micropropagation is recommended for rapid multiplication. The present study was carried out to investigate the rooting response of shoots from different *in vitro* subcultures, as well as the influence of IBA concentration on *in vitro* rooting of chokeberry cultivar 'Nero'. For micropropagation of chokeberry cv. 'Nero', shoots obtained from meristems were used as explants, which were multiplied during three subcultures. *In vitro* rooting of shoots was performed after each subculture on the Murashige & Skoog (MS) basal medium containing MS macroelements and LF microelements, supplemented with IBA (0.7 - 0.2 ml/L^{-1}) and GA_3 (0.1 ml/L^{-1}). The highest rooting percentage (94.99%) was achieved with shoots from the third multiplication subculture, on medium containing 0.7 mg/L^{-1} IBA, which also promoted a higher number of roots (an average of 6.06). However, microshoots cultivated on the MS medium with 0.2 mg/L^{-1} IBA produced the longest roots (3.19 cm on average). In order to acclimatize the vitroplants to *ex vitro* conditions, four variants of substrate were tested, under shading conditions. The overall result of acclimatization of vitroplants showed that the perlite substrate allowed the highest percentage (90%) of rooted plants acclimatized *ex vitro*.

Key words

Aronia melanocarpa, meristems, rooting, subculture, acclimatization

Aronia, a species of fruit shrub relatively recently introduced in culture in our country, with minimum cultivation requirements, has a long history of human use as a food ingredient and also in homeopathic medicine [17; 4; 2]. From an agricultural point of view, chokeberry is one of the world's most promising small fruit species due to the nutraceutical value of its berries, with many health and medicinal benefits, coupled with its ornamental value [3; 24; 8; 22].

Among the multitude of small fruits, chokeberry is one of the most important species, its cultivation becoming more and more popular due to the high content of useful bioactive compounds in its berries, especially antioxidants [25; 1].

Aronia species can be propagated easy by several conventional methods, such as rooting of soft wood and hardwood cuttings, or by layering, but propagation of new selections could be achieved more rapidly using *in vitro* methods of propagation [9; 5; 23; 6]. Micropropagation and *in vitro* culture offer a valuable alternative for obtaining uniform, high quality and disease-free planting material [1; 19].

The overall aims of this study were to establish the ability of *in vitro* rooting of the shoots multiplied in three successive subcultures, according to the concentration of auxin, and determining the *ex vitro* acclimatization capacity of the species *Aronia melanocarpa* cv. 'Nero'.

Material and Method

The biological material consisted of shoots micropropagated from the *in vitro* meristem-derived plantlets of *Aronia melanocarpa* cv. 'Nero'.

Establishing culture for in vitro rooting.

For rooting experiments, all microshoots of 1-2 cm length developed from meristem-derived plantlets were individually placed into glass jars of 350 ml containing 30 ml of culture medium composed of MS macroelements, LF microelements and MS vitamins, and supplemented with GA_3 and different concentrations of IBA (0.2 - 0.7 mg/L^{-1}).

Iron was added to the medium as separate stock solution of ferric sodium salt EDTA (32 mg/L^{-1}), and dextrose was used as carbon source in the culture media (40 g/L^{-1}). For all experiments, the pH of the culture medium was adjusted to 5.7 with 0.1 N KOH before autoclaving for 20 minutes at 121°C.

For a correct statistical interpretation of the rooting results, 20 microshoots were inoculated in each glass jars, in three repetitions. Observations regarding rooting of shoots were made every four weeks.

The main indicators of the influence of auxin concentration on the rooting capacity of microshoots were considered the percentage of rooted shoots, and the number and length of the roots/plantlet, before transferring the seedlings under greenhouse conditions.

Acclimatization.

In order to determine the most appropriate substrate for the acclimatization to *in vivo* conditions of the *in vitro* rooted shoots of *Aronia melanocarpa* cv. 'Nero', four variants of substrates were tested, as follows: perlite, cocopeat jiffy, mineral wool, and mixture of peat, manure and sand (1:2:1 v/v). The rooted seedlings were removed from the culture medium, rinsed with sterile distilled water, dried on sterile paper towels, and transferred to alveolar trays with diameter of 10 cm, filled with different types of substrate. Alveolar trays were transferred to the greenhouse and covered with clear polyethylene sheets for a high relative humidity. Every day, spraying with water was carried out under the plastic sheets and the substrate irrigation occurred 2-3 times per week. Developed plants were cultivated under the same conditions of the establishment stage. The data recorded for establishing the most adequate conditions for the acclimatization of rooted shoots were: (i) the survival percentage; (ii) the average number of leaves / plant; (iii) the average length of shoots/substrate variant.

Results and Discussions

In vitro rooting.

After four weeks in culture, the parameters studied (percentage of rooted microshoots, average number of roots per shoot, and average length of roots) were influenced by different concentrations of IBA added in the rooting expression media and hormonal composition of the basal media used for explants micropropagation (Figure 1).

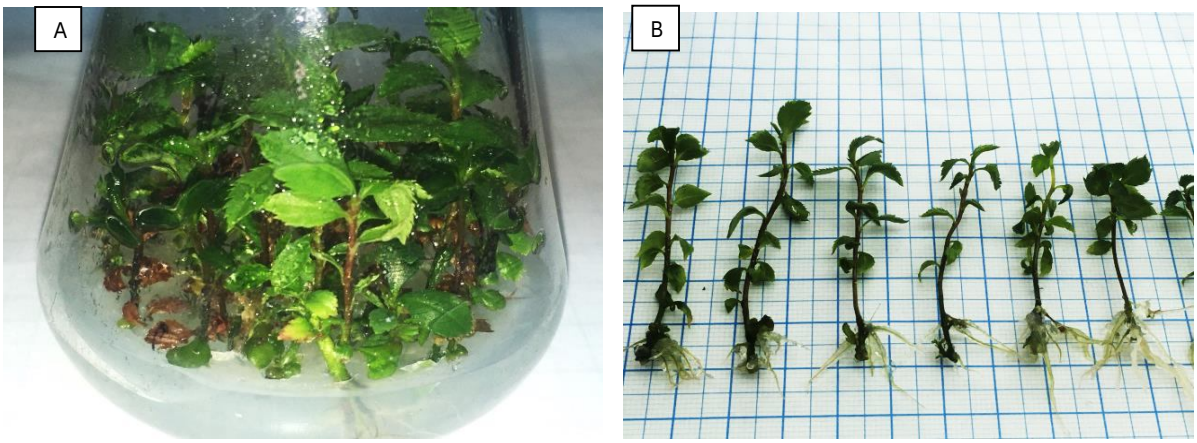

Figure 1. A, B: Root formation of regenerated shoots from meristem-derived plantlets in cv. 'Nero'.

Statistical analysis of data revealed that MS culture media supplemented with 0.7 mg/L^{-1} IBA was optimal for root induction, the percentage of multiplied shoots which developed roots *in vitro* reaching 94.99% for those developed within the third subculture (Figure 2). In this variant of rooting medium the average number of roots per rooted shoot was 6.06 (Figure 3).

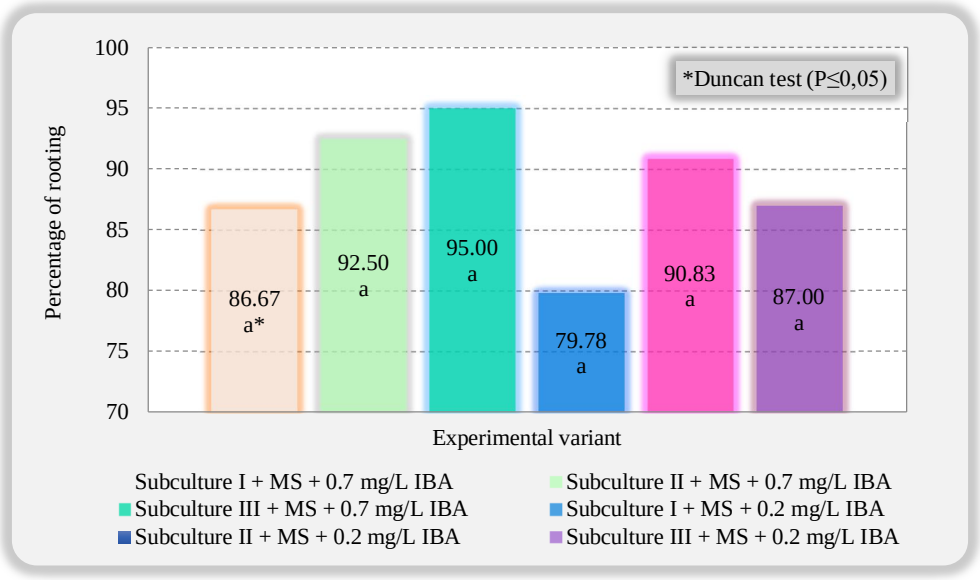

Figure 2. The influence of auxin concentration on the rooting percentage of shoots multiplied over the three subcultures.

However, as shown by statistical interpretation of the data, there were no significant differences between values of rooting percentage on MS media containing different concentrations of IBA (0.2 mg/L^{-1} and 0.7 mg/L^{-1}, respectively) for shoots of *Aronia* cv. 'Nero' multiplied within the three subcultures.

The average length of the roots was significantly influenced by the concentration of auxin in the rooting medium (Figure 4), the highest value (3.19 cm) corresponding to the shoots from the third subculture, rooted on the MS basal media supplemented with 0.2 mg/L^{-1} IBA.

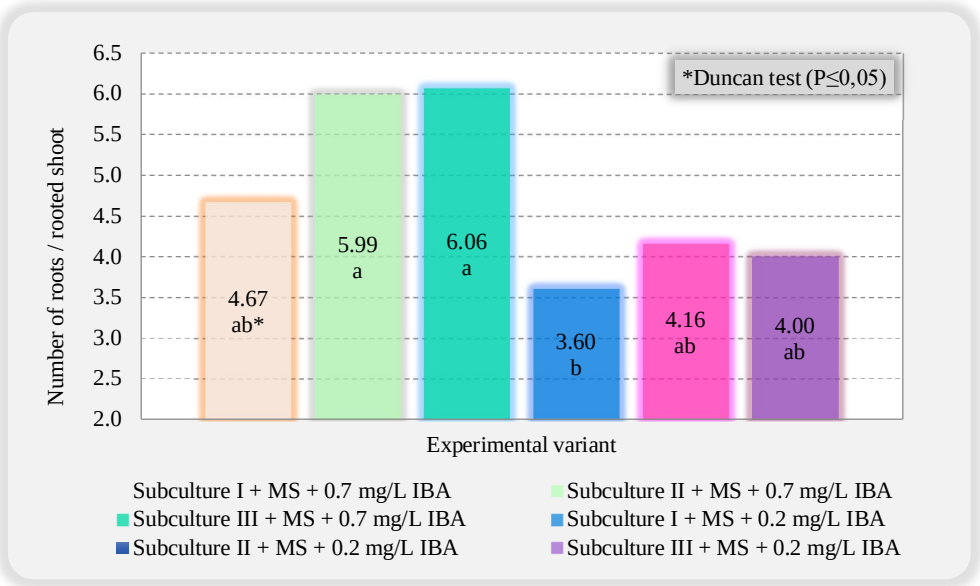

Figure 3. The influence of auxin concentration on the number of roots/rooted shoot for shoots multiplied over the three subcultures.

Murashige and Skoog (MS) medium is most frequently used for *in vitro* rooting, although some other culture media, with different mineral content, such as NRM, B_5, or WPM, were also used in various plant species [12; 16; 26; 6].

According to studies conducted by numerous researchers, it has been shown that IBA in high concentration has positively influenced the *in vitro* rooting process. Brand and Cullina [5], developed a protocol to culture shoot-tip explants of *A. melanocarpa*

taken from mature-phase tissue, where regenerated shoots were rooted in 1/2 MS medium containing 1.0 mg/L^{-1} IBA with 83% rooting success.

Other authors [21; 18; 1] also found that MS medium supplemented with IBA (1.0 - 1.5 mg/L^{-1}) allowed the best results in the *in vitro* rooting process. Similarly, Sivanesan *et al.* [23], obtained the highest rooting rate (100%) when using MS medium, with an average number of 10.6 roots / explant and a 5.4 cm average root length. Studies on the micropropagation of other species, such as strawberry, have shown also that the concentration of 1 mg/L^{-1} IBA is most suitable for induction root per explant and root length average [1; 20]. However, in *Aronia melanocarpa*, Litwinczuk [14], found that a low concentration of IBA (0.05 mg/L^{-1}) proved to be optimal for root formation (90%).

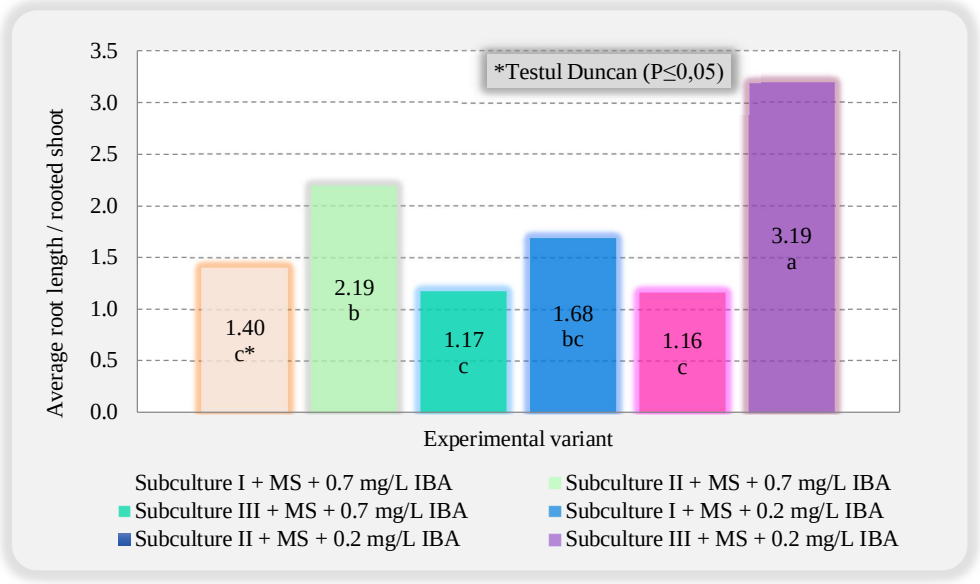

Figure 4. The influence of auxin concentration on the average root lenght/rooted shoot for shoots multiplied over the three subcultures

Acclimatization vitroplants.

The most efficient substrate for acclimatization of *in vitro* rooted shoots of *Aronia* cv. 'Nero' was perlite, which allowed a survival rate of 90% (Figure 5), as well as the highest value of plant height (5.74 cm on average) and number of leaves (10.1 on average) per rooted shoot (Figure 6).

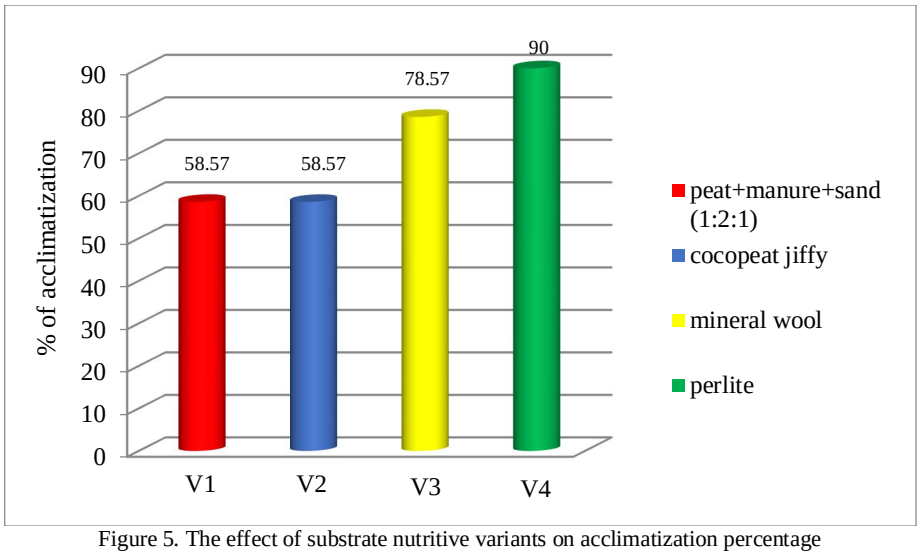

Figure 5. The effect of substrate nutritive variants on acclimatization percentage of *Aronia* vitroplants

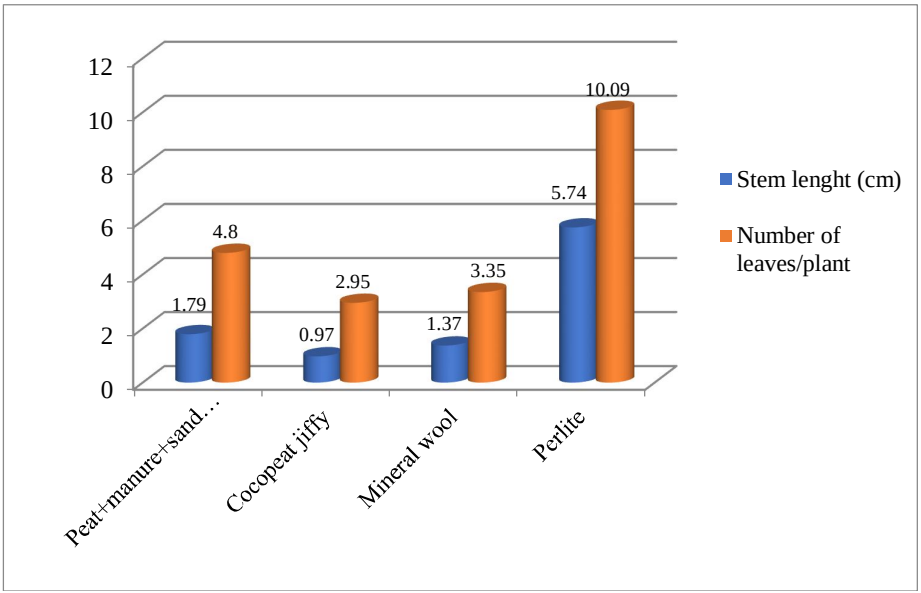

Figure 6. The effect of nutritive substrate variants in the acclimatization phase, on the growth characteristics of *Aronia* vitroplants.

Acclimatization of vitroplants to the greenhouse or field conditions is crucial in micro-propagation because there are major differences regarding abiotic factors between the artificial environment and the greenhouse or field environment [10]. The low percentage of plant acclimatization is a major problem in large-scale commercial *in vitro* propagation. Rigorous observance of the acclimatization process, which must include an efficient and quality substrate, but also an optimal relative humidity, is vital in obtaining healthy, uniform, vigorous and quality planting material.

The results obtained in *Aronia* seedling acclimatization are in good agreement with those previously reported by other authors [14; 6], who found that the perlite substrate is optimal for ensuring high survival rates. The use of perlite as a substrate in the acclimatization process has also led to very good results in many other plant species [7; 11; 20].

Conclusions

The microshoots of *Aronia melanocarpa* cv. 'Nero' multiplied *in vitro* in the third subculture, have the best rooting percentage and the highest values of roots number per rooted shoot in the MS culture medium supplemented with 0.7 mg/L^{-1} IBA.

Regarding the average root length of micropropagated shoots, the best results were obtained also for those developed during the third subculture, on rooting medium supplemented with 0.5 mg/L^{-1} IBA.

The vitroplants were successfully acclimatizated in perlite substrate.

Consequently, the results obtained in this study could contribute to the commercial production of black chokeberry plants by *in vitro* propagation.

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