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Improvement of *Ex vitro* Rooting and Acclimatization to Increase Micropropagation Efficiency of Aronia (*Aronia mitschurinii*)

Sema Coşkun[a], Hatice Dumanoğlu[b]*, Gölge Sarıkamış[b], Rabia Albayrak Delialioğlu[c], Zahide Kocabaş[c]

[a]*Graduate School of Natural and Applied Sciences, Ankara University, Ankara, TÜRKIYE*

[b]*Department of Horticulture, Faculty of Agriculture, Ankara University, Ankara, TÜRKIYE*

[c]*Biometry and Genetics Unit, Faculty of Agriculture, Ankara University, Ankara, TÜRKIYE*

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Corresponding Author: Hatice Dumanoğlu, E-mail: dmanoglu@agri.ankara.edu.tr
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ABSTRACT
In this study, the objective was to establish an easier and more effective rooting and acclimatization protocol for the micropropagation of aronia compared to common *in vitro* and *ex vitro* methods. The effects of indole-3-butyric acid (IBA) applied at concentrations of 0, 25, 50, 100, 200, 500, 1000 and 1500 mg/L on *ex vitro* rooting and acclimatization of 'Viking' and 'Nero' aronia cultivars (*Aronia mitschurinii*) were investigated. The microcuttings were quick-dipped in IBA solution and then placed in floating perlite. They were incubated for 4 weeks at 25±2 °C under a 16-hour light (35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 8-hour dark photoperiod. The survival percentage of microcuttings decreased significantly (43.0-70.2%) at high concentrations of IBA including 500 mg/L and above, particularly in the 'Viking' cultivar. A second experiment was performed at lower concentrations including 25, 50, 100 and 200 mg/L IBA treatments and the control. The survival percentage of microcuttings was 97.7% and 94.6% and the rooting and acclimatization percentages of surviving microcuttings were 99.7% and 99.3% for 'Viking' and 'Nero' cultivars respectively without any significant difference among IBA treatments. To summarise, the experiment yielded successful results in the *ex vitro* rooting and simultaneous acclimatization of aronia microcuttings. These processes were achieved through the utilisation of a floating perlite bed, with and without low concentration of indole-3-butyric acid (IBA).

Keywords: *Aronia* spp., Chokeberry, Plant tissue culture, Floating perlite bed, IBA

1. Introduction

Aronia berries (*Aronia mitschurinii*) contain high levels of flavonoids, polyphenols, and anthocyanins that exert high antioxidant activity. Berries rich in antioxidants are often associated with health benefits (Özdemir & Eroğlu Özkan 2020; Green et al. 2023; Shi et al. 2024). There is growing interest to aronia cultivation due to its health promoting properties as well as the advantages while growing such as early fruit bearing, low production costs and high return of the investment (Mahoney et al. 2022). Furthermore, it is advantageous for organic agriculture due to its resistance to pests and diseases. Fruits are highly marketable as fresh and processed products such as juice, jam, wine, syrup, tea and can be used for food coloring, and as food additives (Dragomir et al. 2023; Green et al. 2023).

A. mitschurinii A. K. Skvortsov et Maitul., which includes commercial aronia cultivars, is a tetraploid hybrid taxon that was bred in Russia in the early 20th century as the fourth aronia species, in addition to black aronia (*A. melanocarpa* (Michx.) Elliott), red aronia (*A. arbutifolia* (L.) Pers.) and purple aronia (*A. prunifolia* (Marshall) Rehder), native to North America (Leonard et al. 2013). The parental lines of this species are reported as 75% *A. melanocarpa* and 25% *Sorbus aucuparia* (Brand 2017).

Aronia can generally be propagated by seed, rhizome division, stem cutting, grafting and micropropagation. All tetraploid *Aronia* species produce plants very similar to the maternal parent when propagated by seed due to diplospory apomixes; therefore, seed propagation can be used as clonal propagation in tetraploid aronia species, including *A. mitschurinii* (Brand 2017). As with many plant species, micropropagation is widely preferred in aronia because it provides rapid mass production of plants compared to traditional propagation methods (Litwinczuk 2013). However, rooting and acclimatization have a major impact on the success of micropropagation. Rooting of microshoots can be performed *in vitro* or *ex vitro*, depending on the rooting ability of the genotype (Aygun & Dumanoglu 2015; Soumare et al. 2021). *In vitro* rooting has some difficulties since the roots of *in vitro* plantlets is delicate and fragile and can often be damaged during planting in the growing medium (McClelland et al. 1990; Hazarika et al. 2006; George & Debergh 2008; Bhojwani & Dantu 2013). In addition, *in vitro* rooted plantlets may suffer from significant losses during acclimatization due to rapid moisture loss of *in vitro* plantlets that leads to low survival percentages

(Hazarika et al. 2006; George & Debergh 2008; Bhojwani & Dantu 2013). However, *in vitro* rooting has been widely used even in plants that are easy to root, such as aronia (Brand & Cullina 1992; Petrovic & Jacimovic-Plavsic 1992; Staniene et al. 1999; Litwinczuk 2002; Kwak et al. 2015; Sivanesan et al. 2016; Brand 2017; Almokar & Pırlak 2018; Brandova et al. 2019; Petrova et al. 2019; Çelebi Toprak & Alan 2020; Borsai et al. 2021; Imrak et al. 2021; Ionela et al. 2022; Jakab et al. 2022; Polat & Eskimez 2022).

Ex vitro rooting has several advantages compared to *in vitro* rooting. The vascular system between the root and the stem develops better forming healthy plantlets, resistant to stress conditions. Provides well developed root system, increase in root number, and higher survival percentage (Yan et al. 2010). *Ex vitro* rooting is more economical than in vitro rooting which comprises the 35–75% of the total costs of micropropagation (Debergh & Maene 1981). *Ex vitro* rooting can decrease the production costs up to 71% (Ranaweera et al. 2013). *Ex vitro* rooting of microshoots is conventionally performed using closed systems such as greenhouses providing fog or mist systems, plastic covers, plant growth cabinets, etc. that provide humidity control to prevent moisture loss from microshoots (McClelland et al. 1990; Hartmann et al. 2011; Brand 2017). These systems increase the cost of production and their success depends particularly on the control of the relative humidity of the environment. Several research studies report the rooting and acclimatization of aronia microshoots using these systems (Brand & Cullina 1992; Litwinczuk 2002; Brand 2017; Borsai et al. 2021). *Ex vitro* rooting of microshoots using floating perlite bed enable rooting as well as the acclimatization of the microshoots during culture. This approach provides an easy and cost effective and more successful way of rooting and acclimatization of microshoots without the need for the conventional systems (Clapa et al. 2013a). The method involves laying perlite gradually on the surface of the water in a tub, allowing it to float on the surface of the water. Since perlite retains water and air, it provides a suitable rooting environment for microshoots. The presence of high relative humidity above the perlite bed, prevents moisture loss from the microshoots. With this method, microshoots can successfully root and acclimatize under controlled conditions within a period of 4-6 weeks. The water in the tub is not replaced during this process, however the amount of water lost by evaporation is added underneath the perlite bed with the help of a funnel. There is no need to add fertilizers, plant growth regulators and biostimulants to the rooting medium and a large number of microshoots can be planted at the same time (Clapa et al. 2013a; Nawandish et al. 2024).

In this study, the effect of immersing 'Viking' and 'Nero' (*Aronia mitschurinii*) aronia microcuttings in different concentrations of IBA solutions on *ex vitro* rooting and acclimatization compared to a control group was investigated. The use of a floating perlite bed for this process was used for the first time to improve the efficiency of micropropagation in aronia.

2. Material and Methods

2.1. Plant material

Microshoots of aronia cultivars ‘Nero’ and ‘Viking’ (*A. mitschurinii* A. K. Skvortsov et Maitul.) were used as the plant material. They are the two most widely cultivated commercial aronia cultivars worldwide (Jurikova et al. 2017). While ‘Nero’ originated from Czechia ‘Viking’ originated from Finland (Ochmian et al. 2012).

2.2. Explant preparation

Microshoots were obtained from subcultures that were derived from shoot tip cultures subcultured at 4-week intervals during proliferation. Murashige & Skoog (1962), basic nutrient medium with 1/3 ammonium nitrate was used for both cultures. The medium contained 0.5 mg L^{-1} BA (6-benzylaminopurine) and 0.3 mg L^{-1} GA_3 (gibberellic acid), 30 g L^{-1} sucrose. The pH of the medium was adjusted to 5.7 and solidified with 0.8% agar. The cultures were incubated in a climate room at 25±2 °C with a photoperiod of 16 h light (35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 8 h dark. Microcuttings of 1.5-2.0 cm long carrying 4-5 leaves were excised from the microshoots obtained from the 6^{th} and 7^{th} subcultures in the laboratory under non-aseptic conditions (Figure 1 a, b).



(a) **(b)**

Figure 1- (a) Proliferated microshoots obtained from the subcultures and (b) microcuttings prepared from microshoots

2.3. Ex vitro rooting experiments

Ex vitro rooting experiments were carried out by planting microcuttings in a floating perlite bed (Clapa et al. 2013a). Before planting, different concentrations of IBA including 25, 50, 100, 200, 500, 1000 and 1500 mg/Lwere applied to microcuttings by quick-dip method for 10 seconds except for the control samples. According to the findings of the first experiment, lower IBA concentrations including 25, 50, 100 and 200 mg/L IBA were selected for further experiments due to a lower survival percentage of microcuttings at higher IBA concentrations. The floating perlite bed was prepared by pouring 3 liters of tap water into a square plastic tub of 25 cm wide and 11 cm deep with a volume of 6 liters. Three liters of agricultural perlite (3-6 mm diameter) was gradually and evenly laid over the surface of the water. Microcuttings were planted in the floating perlite bed in a vertical position, such that the half of the microcutting remained in the perlite bed. The cultures were incubated for 4 weeks at 25±2 °C under a 16-hour light (35 $\mu mol \cdot m^{-2} \cdot s^{-1}$) and 8-hour dark photoperiod. During this period, tap water was added to the tub using a funnel from one side of the floating perlite bed when needed due to the evaporation of water. There was no need to check relative humidity and no need to apply fertilizers to the cultures. After four weeks, microcuttings were removed from the floating perlite bed (Figure 2).



Figure 2- Aronia microcuttings 4 weeks after planting in a floating perlite bed

2.4. Survival and growth performance of ex vitro rooted microcuttings

Survival percentage of cultured microcuttings, rooting percentage of survived microcuttings, average number of roots, length of the longest root, fresh and dry root weights, rooting level (scaled as 1=weak, 2=medium, 3=good, 4=very good) of both aronia cultivars were determined. For the level of rooting, microcuttings were classified according to the location of the roots. Root formation was on one side of the microcutting in level 1, on two sides in level 2, on three sides in level 3, and on four sides in level 4 (Alizadeh & Dumanoğlu 2022). In addition, shoot length, number of leaves, fresh and dry shoot weights were measured in rooted microcuttings.

2.5. Experimental design and statistical analysis

The experiment was performed according to the 'Randomized Plot Design' with three replications. While the first experiment included eight treatments (control, 25, 50, 100, 200, 500, 1000 and 1500 mg/L IBA), the second experiment included five treatments (control, 25, 50, 100 and 200 mg L^{-1} IBA). In the experiments, 25 microcuttings were used in each replication for the 'Viking' and 18 microcuttings for the 'Nero' aronia cultivars. Statistical analysis of the data were subjected to analysis of variance (ANOVA) by Minitab Package Program (MINITAB Inc., Trial Version). Significant differences were determined by Duncan's Test at $P \leq 0.05$. The percentage data were transformed into angle values prior to analysis.

The means of the variables by the IBA concentrations in both experiments were presented in the form of a heatmap using the 'pheatmap' package also in RStudio (Kolde 2019). To eliminate the differences in the variation ranges of the variables, the data were converted into Z-scores before generating heatmap plots. Additionally, to assess the effects of IBA concentrations on the variables and the relationships between them, Principal Component Analysis (PCA) was performed using the 'prcomp', ggplot2', 'ggfortify' packages, and the results were visualized using a biplot (Tang et al. 2016; Wickham 2016; R Core Team 2023).

3. Results

3.1. Effects of IBA concentrations on the ex vitro rooting of 'Viking' cultivar

In the first experiment including control and 25, 50, 100, 200, 500, 1000 and 1500 mg/L IBA, the differences among treatments were significant in all parameters except the rooting percentage and shoot length of microcuttings after 4 weeks in floating perlite

bed cultures (Tables 1 and 2). The survival percentage (%) of microcuttings was significantly lower at high IBA concentrations (500 mg/L and above) than at lower IBA concentrations (Table 1). In the second experiment conducted with lower IBA concentrations ranging from 25 mg/L to 200 mg/L in addition to the control, the difference among treatments in terms of survival percentage of microcuttings was not significant. The survival percentage was 97.7% as the average of the treatments (Table 1). Rooting percentage (%) was not statistically significant among treatments in both of the first and second experiments. However, the mean rooting percentage determined as the average of the treatments was higher in the second experiment (99.7%) than in the first experiment (82.5%) (Table 1, Figure 3).

Table 1- Effects of IBA treatments and the control on *ex vitro* rooting of 'Viking' microcuttings using floating perlite bed* (mean±SE)

Treatment	*Survival (%)*	*Rooting (%)*	*Root number/ microcutting*	*Longest root length (mm)*	*Rooting level (1-4)*	*Root fresh weight (mg)*	*Root dry weight (mg)*
First experiment							
Control	93.0±3.9 a (77.5±6.4)	91.9±4.8 (76.6±7.1)	7.5±0.4 ab	31.2±5.3 a	2.9±0.1 a	18.0±1.1 a	1.2±0.1 a
25 mg/L IBA	85.7±7.1 abc (71.6±9.2)	88.7±8.2 (74.5±9.1)	6.0±0.8 bcd	23.0±0.1 abc	2.4±0.2 ab	10.7±2.9 bc	0.8±0.1 bc
50 mg/L IBA	88.1±11.9 ab (77.8±12.2)	95.2±4.8 (82.6±7.4)	6.9±0.3 abc	27.5±1.7 ab	2.8±0.2 a	13.3±0.0 ab	1.0±0.1 ab
100 mg/L IBA	100.0±0.0 a (90.0±0.0)	74.4±1.6 (59.7±1.1)	5.4±0.7 cde	20.5±2.4 bc	2.8±0.1 a	10.6±2.6 bc	0.9±0.0 abc
200 mg/L IBA	85.7±7.1 abc (71.6±9.2)	76.5±8.8 (62.1±6.6)	8.0±0.5 a	28.3±2.8 ab	3.0±0.2 a	12.2±1.8 abc	0.9±0.1 abc
500 mg/L IBA	43.0±6.6 d (40.9±3.8)	80.0±20.0 (73.1±16.9)	4.1±0.8 de	18.7±1.2 bc	1.7±0.2 bc	6.4±1.0 cd	0.3±0.1 d
1000 mg/L IBA	70.2±8.7 bcd (57.5±5.8)	81.4±9.4 (68.8±10.7)	4.1±0.7 de	22.6±4.2 abc	2.2±0.4 ab	8.0±3.4 bcd	0.6±0.2 cd
1500 mg/L IBA	63.1±7.2 cd (52.8±4.3)	72.0±3.2 (58.11±2.1)	3.9±0.2 e	13.5±1.7 c	1.3±0.1 c	3.6±0.3 d	0.3±0.1 d
Average	-	**82.5±3.2 (69.4±3.1)**	-	-	-	-	-
P	0.005**	0.485 ns	0.001***	0.013*	0.002**	0.006**	0.001***
Second experiment							
Control	100.0±0.0 (90.0±0.0)	98.7±1.3 (86.1±3.8)	6.4±0.6	48.4±2.2	3.5±0.1	23.2±1.1	2.2±0.3
25 mg/L IBA	100.0±0.0 (90.0±0.0)	100.0±0.0 (90.0±0.0)	6.8±0.5	51.1±1.8	3.6±0.1	27.1±4.3	2.9±0.4
50 mg/L IBA	98.6±1.4 (86.1±3.9)	100.0±0.0 (90.0±0.0)	6.1±0.3	50.6±2.3	3.4±0.1	24.5±0.0	2.3±0.2
100 mg/L IBA	94.5±3.5 (79.2±5.9)	100.0±0.0 (90.0±0.0)	5.8±0.2	44.8±2.2	3.4±0.1	22.9±2.4	2.3±0.3
200 mg/L IBA	95.5±4.5 (82.8±7.2)	100.0±0.0 (90.0±0.0)	6.0±0.6	46.8±1.1	3.3±0.1	21.6±1.8	2.1±0.2
Average	**97.7±1.2 (85.6±2.0)**	**99.7±0.3 (89.2±0.8)**	**6.2±0.2**	**48.3±1.0**	**3.4±0.1**	**23.9±0.8**	**2.4±0.1**
P	0.420 ns	0.452 ns	0.569 ns	0.178 ns	0.337 ns	0.305 ns	0.429 ns

* IBA applied to the microcuttings for 10 s by quick-dip method. Rooting level, 1 = poor, 2 = medium, 3 = good, 4 = very good. Different letters in the same column indicate the statistical differences among treatments according to Duncan test (P≤0.05). *, **, *** P≤0.05, P≤0.01 and P≤0.001 statistically significant, ns statistically not significant. The numbers in parenthesis represent the angle value of percentages.

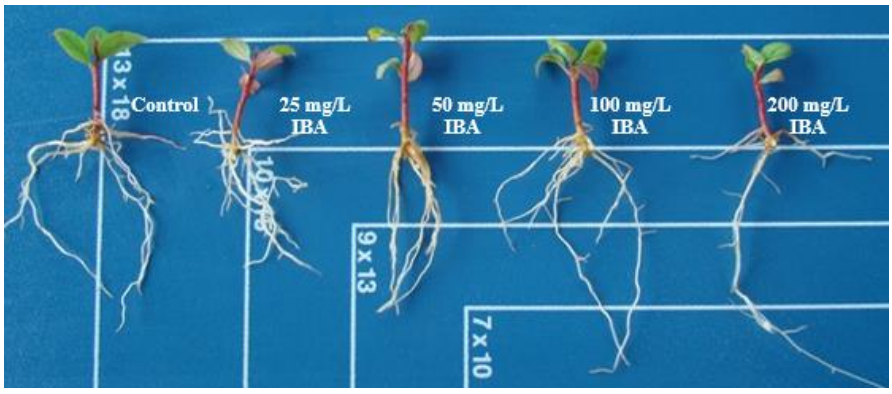
Table 2- Effects of IBA treatments and the control on shoot development of *ex vitro* rooted microcuttings using floating perlite bed in 'Viking' aronia cultivar * (mean±SE)

Treatment	*Shoot length (mm)*	*Number of leaves per plantlet*	*Shoot fresh weight (mg)*	*Shoot dry weight (mg)*
		First experiment		
Control	24.9±0.5	6.8±0.1 a	48.6±5.3 abc	9.3±0.9 a
25 mg/L IBA	23.8±0.4	6.2±0.2 ab	43.6±1.4 bc	6.8±0.1 c
50 mg/L IBA	24.9±1.1	5.9±0.1 abc	58.1±4.4 a	9.5±0.9 a
100 mg/L IBA	23.8±0.9	5.5±0.2 bc	42.7±1.7 bc	6.9±0.6 bc
200 mg/L IBA	25.6±1.2	7.0±0.3 a	50.8±2.1 ab	8.9±0.7 ab
500 mg/L IBA	22.2±2.5	4.9±0.4 c	44.1±4.1 bc	7.1±1.0 bc
1000 mg/L IBA	24.3±0.9	5.5±0.7 bc	45.8±4.2 bc	6.8±0.5 c
1500 mg/L IBA	24.8±1.3	5.3±0.1 bc	39.8±1.0 c	5.5±0.2 c
Average	**24.3±0.4**	-	-	-
P	0.721 ^{ns}	0.009**	0.028*	0.007**
		Second experiment		
Control	18.7±0.9	4.3±0.1 a	44.4±3.0	11.4±1.2
25 mg/L IBA	19.9±0.8	4.2±0.1 a	49.9±0.5	13.6±1.1
50 mg/L IBA	18.8±0.3	3.9±0.2 ab	43.4±3.1	11.4±0.6
100 mg/L IBA	19.0±0.4	3.5±0.1 b	46.7±4.2	12.1±1.0
200 mg/L IBA	18.5±0.4	4.0±0.2 ab	40.5±2.2	11.0±0.5
Average	**19.0±0.3**	**4.0±0.1**	**45.0±1.4**	**11.9±0.4**
P	0.578 ^{ns}	0.041*	0.274 ^{ns}	0.335 ^{ns}

* IBA applied to the microcuttings for 10 s by quick-dip method. Different letters in the same column indicate the statistical differences among treatments according to Duncan test (P≤0.05). *, ** P≤0.05 and P≤0.01 statistically significant, ^{ns} statistically not significant



(a)



(b)

Figure 3- Effects of different IBA concentrations and the control on *ex vitro* rooting of 'Viking' aronia microcuttings using floating perlite bed. (a) First experiment and (b) Second experiment

In the first experiment, rooting parameters of microcuttings such as root number, longest root length, rooting level, root fresh and dry weights were consistently higher in the control than in the IBA treatments. However, statistically significant differences were generally not found among the control and low IBA concentrations in terms of these parameters (Table 1). In the second

experiment, there was no statistically significant differences between control and IBA treatments in terms of rooting parameters. The number of roots per microcutting was 6.2, the longest root length was 48.3 mm, the rooting level was 3.4 out of 4, root fresh weight was 23.9 mg and dry weight was 2.4 mg as an average of the treatments (Table 1).

Shoot developmental parameters other than shoot length were generally higher in the control and low IBA concentrations, and high IBA concentrations negatively affected shoot development similar to rooting parameters (Table 2, Figure 3). In the second experiment, significant difference was found in terms of number of leaves per plantlet. In terms of shoot length the average of treatments was 19.0 mm, shoot fresh weight was 45.0 mg and shoot dry weight was 11.9 mg (P>0.05) (Table 2).

The heat map created using the standardized data from both experiments of Viking cultivar revealed that the parameters exhibited values above the average when 0-200 mg/L IBA concentrations were used. The increase in IBA concentrations generally had a negative impact on the parameters such as shoot length and number of leaves and partially on root number (Figure 5).

3.2. Effects of IBA concentrations on ex vitro rooting of 'Nero' cultivar

In the 'Nero' cultivar, it was determined that low or high IBA treatments did not have a significant impact on all parameters related to survival, rooting and shoot development in the first and second experiments assessed at the end of the 4-week rooting period in the floating perlite bed (P>0.05) (Table 3, Figure 4). However, high IBA concentrations applied in the first experiment caused damage and browning in some microcuttings in this cultivar (Figure 4).

Table 3- Effects of IBA treatments and the control on *ex vitro* rooting of 'Nero' aronia microcuttings using floating perlite bed* (mean±SE)

Treatment	*Survival (%)*	*Rooting (%)*	*Root number per microcutting*	*Longest root length (mm)*	*Rooting level (1-4)*	*Root fresh weight (mg)*	*Root dry weight (mg)*
First experiment							
Control	100.0±0.0 (90.0±0.0)	93.3±3.3 (77.7±6.1)	9.3±1.6	19.8±2.1	2.7±0.1	11.5±2.0	1.0±0.1
25 mg/L IBA	90.0±5.8 (75.0±7.9)	100.0±0.0 (90.0±0.0)	8.4±1.2	31.1±2.5	3.0±0.1	16.7±2.7	1.4±0.2
50 mg/L IBA	90.0±5.8 (75.0±7.9)	96.3±3.7 (83.5±6.5)	8.1±0.3	22.4±2.8	2.6±0.1	13.0±0.4	0.9±0.1
100 mg/L IBA	93.3±6.7 (81.1±8.9)	85.8±8.7 (72.0±9.7)	7.9±0.3	21.7±4.7	2.4±0.3	8.1±3.2	0.6±0.2
200 mg/L IBA	86.7±6.7 (72.3±8.9)	85.8±8.7 (72.0±9.7)	8.7±0.1	31.0±2.6	2.7±0.3	16.0±4.6	1.2±0.4
500 mg/L IBA	96.3±3.7 (83.5±6.5)	89.6±5.8 (74.7±7.9)	6.7±1.1	21.1±5.3	2.2±0.5	4.0±1.2	0.9±0.4
1000 mg/L IBA	90.0±10.0 (76.7±13.3)	93.3±6.7 (81.1±8.9)	8.6±2.0	22.9±2.5	2.3±0.2	9.3±3.1	0.7±0.2
1500 mg/L IBA	93.3±3.3 (77.7±6.1)	92.6±7.4 (80.6±9.4)	8.0±0.6	23.2±3.1	2.1±0.4	8.6±3.0	0.8±0.2
Average	**92.4±1.8 (79.0±2.5)**	**92.1±2.1 (78.9±2.6)**	**8.2±0.4**	**24.1±1.3**	**2.5±0.1**	**10.9±1.2**	**0.9±0.1**
P	0.759 ^{ns}	0.738 ^{ns}	0.845 ^{ns}	0.189 ^{ns}	0.336 ^{ns}	0.071 ^{ns}	0.390 ^{ns}
Second experiment							
Control	97.9±2.1 (85.1±4.8)	100.0±0.0 (90.0±0.0)	4.7±0.2	54.3±3.6	3.3±0.1	23.2±3.0	2.0±0.3
25 mg/L IBA	100.0±0.0 (90.0±0.0)	100.0±0.0 (90.0±0.0)	4.2±0.1	65.5±1.9	3.4±0.1	26.7±0.1	2.4±0.1
50 mg/L IBA	97.0±3.0 (84.2±5.8)	100.0±0.0 (90.0±0.0)	4.7±0.4	69.6±2.5	4.2±0.8	33.6±2.4	3.0±0.3
100 mg/L IBA	92.2±4.8 (77.0±7.0)	100.0±0.0 (90.0±0.0)	4.1±0.2	61.4±4.9	3.0±0.1	24.0±3.4	2.4±0.5
200 mg/L IBA	85.9±7.2 (71.8±9.2)	96.3±3.7 (83.5±6.5)	3.9±0.2	53.2±7.2	3.0±0.3	18.4±5.1	1.8±0.5
Average	**94.6±2.1 (81.6±2.9)**	**99.3±0.7 (88.7±1.3)**	**4.3±0.1**	**60.8±2.4**	**3.4±0.2**	**25.2±1.8**	**2.3±0.2**
P	0.313 ^{ns}	0.452 ^{ns}	0.134 ^{ns}	0.109 ^{ns}	0.271 ^{ns}	0.074 ^{ns}	0.264 ^{ns}

* IBA applied to the microcuttings for 10 s by quick-dip method. Rooting level, 1 = poor, 2 = medium, 3 = good, 4 = very good. Different letters in the same column indicate the statistical differences among treatments according to Duncan test (P≤0.05). ^{ns} statistically not significant. The numbers in parenthesis represent the angle value of percentages.

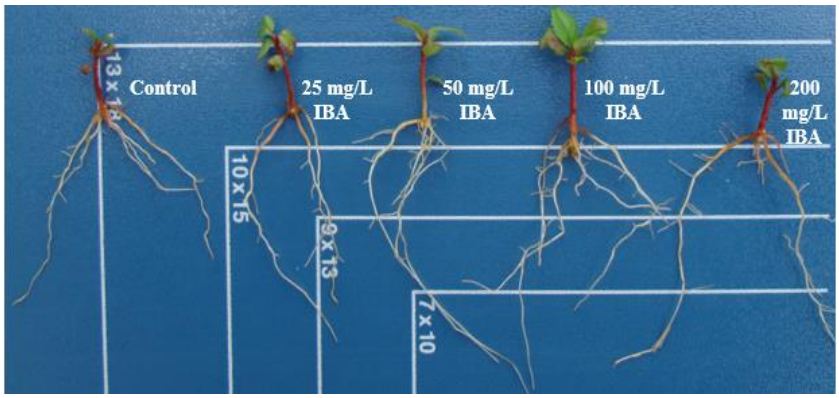
Table 4- Effects of control and IBA treatments on shoot development of *ex vitro* rooted microcuttings using floating perlite bed in 'Nero' aronia cultivar * (mean±SE)

Treatment	*Shoot length (mm)*	*Number of leaves per plantlet*	*Shoot fresh weight (mg)*	*Shoot dry weight (mg)*
First experiment				
Control	30.1±1.3	5.7±0.9	50.2±9.4	6.0±1.3
25 mg/L IBA	28.3±1.0	6.4±0.6	56.4±11.1	9.5±1.9
50 mg/L IBA	28.2±1.2	6.2±0.1	46.6±4.0	8.7±0.6
100 mg/L IBA	29.6±0.9	6.7±0.3	50.9±2.3	7.7±1.1
200 mg/L IBA	29.8±1.0	6.3±0.4	49.7±2.6	9.1±0.8
500 mg/L IBA	28.7±0.8	6.5±0.1	46.5±4.1	8.7±1.1
1000 mg/L IBA	28.6±1.2	7.2±0.7	41.8±0.6	8.4±0.9
1500 mg/L IBA	28.9±2.5	5.6±0.4	41.6±6.8	7.0±1.0
Average	**29.0±0.4**	**6.3±0.2**	**48.0±2.2**	**8.1±0.4**
P	0.951 $^{ns.}$	0.485 ^{ns}	0.809 ^{ns}	0.474 ^{ns}
Second experiment				
Control	17.3±0.2	4.6±0.2	45.4±3.1	10.7±0.7
25 mg/L IBA	16.2±0.5	4.3±0.1	46.1±1.6	11.4±0.6
50 mg/L IBA	17.4±0.7	4.7±0.2	59.0±1.7	15.3±0.4
100 mg/L IBA	17.3±0.4	4.0±0.3	45.6±3.6	12.8±1.9
200 mg/L IBA	17.6±0.1	4.0±0.2	48.0±4.8	12.8±1.5
Average	**17.2±0.2**	**4.3±0.1**	**48.8±1.8**	**12.6±0.6**
P	0.317 ^{ns}	0.216 ^{ns}	0.060 ^{ns}	0.132 ^{ns}

* IBA applied to the microcuttings for 10 s by quick-dip method. Different letters in the same column indicate the statistical difference between treatments according to Duncan test (P≤0.05). ^{ns} statistically not significant



(a)



(b)

Figure 4- Effects of low and high IBA concentrations as well as control on *ex vitro* rooting of 'Nero' aronia microcuttings using floating perlite bed. (a) First experiment and (b) Second experiment

As an average of the treatments, survival and rooting parameters except the root number were higher in the second experiment (Table 3) whereas shoot developmental parameters were higher in the first experiment (Table 4). In the second experiment where control and low concentrations of IBA were used, the average survival percentage was 94.6% and the rooting percentage was

99.3%. The root number per rooted microcutting was 4.3, the longest root length was 60.8 mm, the rooting level was 3.4, root fresh and dry weights were 25.2 mg and 2.3 mg, respectively (Tables 3 and 4, Figure 4).

The effects of the treatments on shoot development in rooted microcuttings of 'Nero' aronia cultivar were not significant. Shoot length and leaf number data of the first experiment were higher than those of the second experiment, but shoot fresh and dry weights were similar. Shoot fresh weight per rooted microcutting was 48.0 mg and 48.8 mg, and shoot dry weight was 8.1 mg and 12.6 mg in the first and second experiments, respectively (Table 4).

According to the heat map created using standardized data from both experiments of Nero cultivar, the mean values were generally below average at higher concentrations of IBA such as 500, 1000 and 1500 mg/L in most of the parameters. Nevertheless, a significant difference could not be observed between lower and higher concentrations in some parameters such as the longest root length, shoot length and number of leaves (Figure 5).

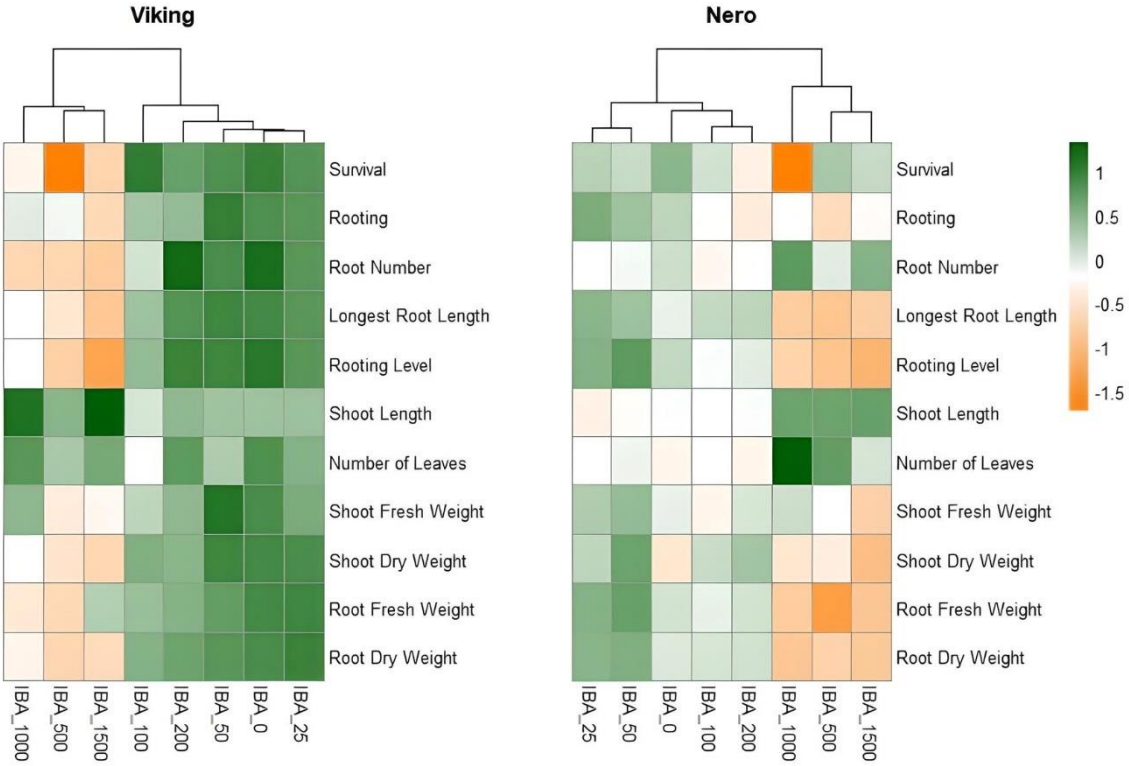


Figure 5- Heatmap plots for Viking and Nero aronia cultivars

4. Discussion

Ex vitro rooting and acclimatization of 'Viking' and 'Nero' aronia microcutings (*A. mitschurinii*) of the control and treated with 25, 50, 100, 200, 500, 1000 or 1500 mg/L IBA using a floating perlite bed were determined.

While *ex vitro* rooting, maintaining the humidity of the environment during the rooting and acclimatization is of great importance to avoid water loss from the microcuttings. The floating perlite bed method (Clapa et al. 2013a), can provide effective humidity control around microcuttings and therefore has been successfully used for rooting and simultaneous acclimatization during micropropagation of many plant species including *Rubus*, *Amelanchier*, *Vaccinium*, *Rosa* (Clapa et al. 2013a; Fira et al. 2014; Clapa et al. 2015), *Prunus* (Clapa et al. 2013b), *Paulownia* (Clapa et al. 2014), *Malus* (Fira et al. 2015; Clapa et al. 2016), *Lycium* (Fira et al. 2016), *Syringa* (Borsai et al. 2017) and *Pyrus* (Nawandish et al. 2024). To our knowledge, there is no scientific reports to date regarding the use of floating perlite bed in *Aronia* species. In the present study, survival, rooting and acclimatization percentages reaching to 100% were achieved in aronia cultivars with this method. The average rooting percentages of low IBA concentrations and the control, were 99.7% for the 'Viking' and 99.3% for the 'Nero' cultivars (Tables 1 and 3). The highest *ex vitro* rooting percentages in different aronia cultivars were reported as 70.4% (Litwinczuk 2002), 95% (Brand & Cullina 1992) or 94.0% (Borsai et al. 2021) using closed systems where the environmental humidity was controlled by gradually decreasing. In the present research, the rooting percentages obtained using floating perlite bed were higher than the above findings. In addition, rooting and acclimatization using the floating perlite bed could be achieved more effectively, since the relative humidity around the microcuttings resembles to natural conditions, and thus healthier and hardened plantlets can be obtained (Clapa et al. 2013a). During the propagation of plants using cuttings, the existence of suitable environmental conditions and the development of a useful method to stimulate shoot-root formation are the two main factors that affect the success of rooting (Özkaya & Çelik

1994). Steffens and Rasmussen (2016), report that adventitious roots in plants can occur during normal development as well as in response to stress conditions such as flooding, nutrient deficiencies, and wounding. They also state that the main hormone that stimulates adventitious root formation in submerged plants is ethylene, and that the ethylene biosynthesis is increased by the enzyme 1-aminocyclopropane-1-carboxylic acid, that stimulates rooting. It is also stated that gaseous ethylene is removed from plant tissues under normal environmental conditions, but that during submersion, water acts as a physical barrier, trapping ethylene in the plant, and that gibberellin increases the adventitious root growth promoted by ethylene (Steffens & Rasmussen 2016).

In previous studies, it was reported that in vitro rooting in aronia could be achieved with auxin treatments, IBA and NAA were the most commonly used auxins, however IBA was found to be more effective (Petrovic & Jacimovic-Plavsic 1992; Sivanesan et al. 2016; Petrova et al. 2019). In our study, in the first experiment, high IBA concentrations significantly decreased the survival percentage in microcuttings, especially in the 'Viking' cultivar (43.0-70.2%) (Table 1). Although the rooting percentages in surviving microcuttings in this cultivar were found to be statistically similar at low and high IBA concentrations, high IBA concentrations caused microcutting loss and ultimately reduced the number of plantlets obtained. Indeed, considering the average survival and rooting percentages, it could be estimated that at high IBA concentrations, only 45.7% of microcuttings became plantlets (Table 1). In the 'Nero' cultivar, the survival percentage in microcuttings was similar at high and low IBA concentrations. However, damage was observed in some microcuttings at high IBA concentrations (Figure 4).

In our study, rooting data showed that auxin treatments were not required for *ex vitro* rooting of aronia microcuttings using the floating perlite bed. Because, in the experiments, no statistically significant differences were found between control and IBA treatments in terms of rooting percentage of microcuttings in both aronia cultivars. Litwinczuk (2002), determined the *ex vitro* rooting percentages as 70.4%, 76.9% and 68.5% for 0, 1500 and 3000 mg/L IBA concentrations in aronia microcuttings, respectively. Furthermore, in accordance with our findings, Brand & Cullina (1992), reported *ex vitro* rooting percentages as 95% and Borsai et al. (2021) as 94% in aronia without auxin treatment. However, contrary to these findings, Brand (2017) reported that 1000 mg/L IBA treatment would increase rooting in *ex vitro* rooting of aronia. Rooting success is related to the genetic potential of plants and their physiology as a result of external treatments (Hartmann et al. 2011).

Our findings showed that high concentrations of IBA generally affected not only the health of microcuttings but also the root number, root fresh and dry weights of rooted microcuttings. The root number per microcutting in 'Viking' cultivar varied between 3.9 and 4.1 at high IBA concentrations and between 5.4 and 8.0 at low concentrations (Table 1). It has been reported that high auxin concentrations inhibit the formation and development of root primordia (Carpenter & Cornell 1992). Also, the minimum longest root length value was found as 13.5 mm at 1500 mg/L IBA (Table 1). High concentrations of IBA generally decreased the leaf number, shoot fresh and dry weights (Table 2). Very high concentrations of auxin treatments can directly inhibit shoot growth (Thimann 1939). In our study, the effects of IBA treatments and the control on shoot length were found similar. The average shoot length was recorded as 19.0-24.3 mm in 'Viking' cultivar and 17.2-29.0 mm in 'Nero' cultivar (Tables 2 and 4). Shoot length of in vitro plantlets was reported as 9.4-10.6 mm by Kwak et al. (2015) and 22.63-28.54 mm by Polat and Eskimez (2022). Our findings are consistent with the findings of Polat & Eskimez (2022). The number of leaves, varied between 4.3 and 7.0 depending on the cultivar and our findings are lower than the 15.3 and 16.2 reported by Kwak et al. (2015). This was attributed to the influence of in vitro conditions used by Kwak et al. (2015). As a result of this study, it was observed that aronia microcuttings were successfully rooted *ex vitro* without IBA treatment and simultaneously acclimatized using the floating perlite bed. Our findings are supported by the PCA biplot graph in both cultivars (Figure 6). The length and direction of the vectors together indicated that IBA concentrations between 25-200 mg/L revealed similar findings to control. According to the direction of the vectors the concentrations below 200 mg/L and above 500 mg/L exhibited opposite response in both cultivars (Figure 6).

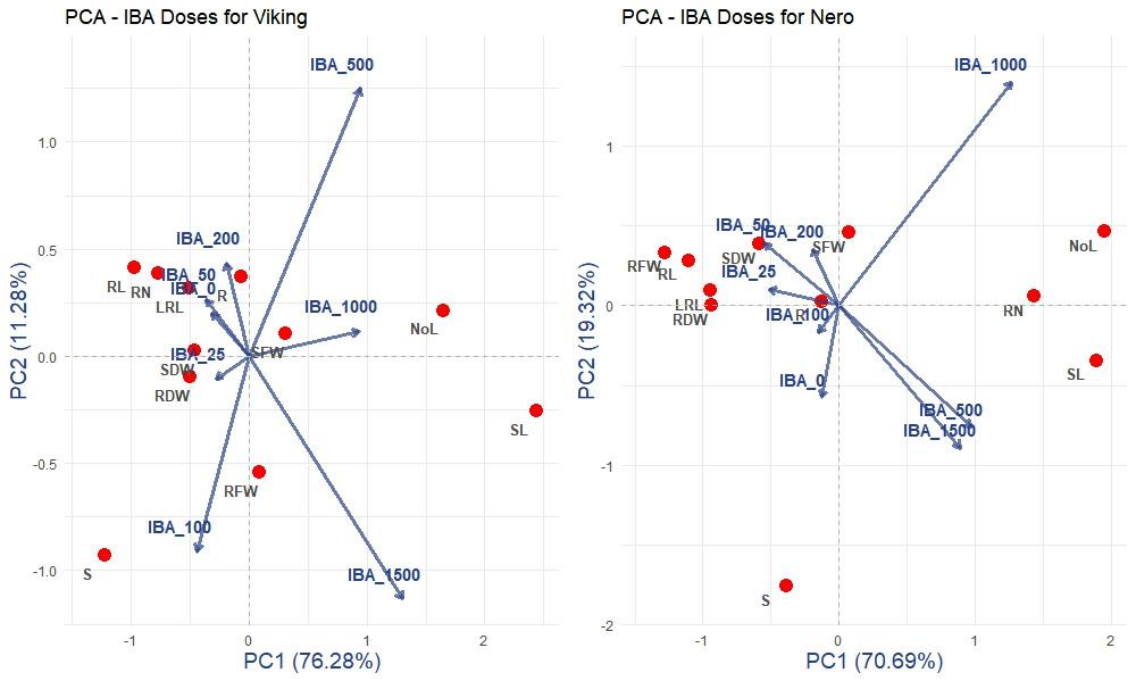


Figure 6- PCA and biplot graph for Viking and Nero genotypes

S: survival, R: rooting, RN: root number, LRL: longest root length, RL: rooting level, SL: shoot length, NoL: number of leaves, SFW: shoot fresh weight, SDW: shoot dry weight, RFW: root fresh Weight, RDW: root dry weight

5. Conclusions

The utilisation of this methodology is emphatically endorsed for the cultivation of aronia plants by micropropagation, owing to its capacity to enhance survival and rooting percentages in microcuttings while ensuring optimal development of plantlets. In comparison to other recognised methods, it offers a more accessible and economical alternative, whilst being a more straightforward process.

Author Declarations

Authors' contributions: Concept: H.D., Design: H.D.,, Data collection: S.C., H.D. Analysis: S.C., H.D., G.S., R.A.D., Z.K., Writing: S.C., H.D., G.S., R.A.D.

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Consent for publication: No need/ Not applicable.

Data availability: Data are available on request due to privacy or other restrictions.

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