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PLANT TISSUE CULTURE STRATEGIES FOR SUSTAINABLE MULTIPLICATION OF BLACK CHOKEBERRY (*ARONIA MELANOCARPA*) GERMPLASM

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Abstract

Plants are essential for ecosystem stability, providing oxygen, food, and valuable bioactive compounds. However, urbanization, industrial expansion, habitat loss, and climate change are accelerating biodiversity decline. Plant tissue culture and micropropagation offer efficient methods for large-scale production of uniform, disease-free planting material independent of seasonal and environmental constraints. Aronia melanocarpa is a valuable berry shrub with high nutraceutical potential. This study aimed to develop an effcient micropropagation protocol by evaluating the effect of different cytokinins on in vitro shoot development. Explants were cultured on MS medium supplemented with meta-topolin, zeatin, kinetin, 2iP, or TDZ, combined with GA_3 and IAA. Meta-topolin resulted in the highest shoot proliferation, while kinetin and 2iP enhanced shoot elongation and rooting. TDZ induced hyperhydric and compact tissue formation. Rooting was successfully achieved on half-strength MS medium supplemented with activated charcoal and auxins, leading to effective plant acclimatization. The results demonstrate that meta-topolin is the most efficient cytokinin for rapid micropropagation of A. melanocarpa, providing a reliable system for large-scale propagation and conservation of valuable plant material.

Key words*: 2iP, kinetin, meta-topolin, thidiazuron, zeatin.*

INTRODUCTION

Aronia melanocarpa, commonly known as black chokeberry, is a valuable shrub species recognized for its fruits that have a high content of bioactive compounds and growing potential in horticulture and nutraceutical production. Native to North America, this species has attracted significant attention in recent decades for its nutritional and medicinal potential, leading to its widespread use in the food, cosmetic, and pharmaceutical industries (Piras et al., 2024). Berries are rich in phenolic compounds, as flavonoids: anthocyanins, proanthocyanidins, flavanols, flavonols; phenolic acids (chlorogenic, neochlorogenic, caffeic), tannins (Sidor & Gramza-Michałowska, 2019; Lee et al., 2021; Petre et al., 2021; Dufour et al., 2022; Popa et al., 2023). They also contain carbohydrates (sorbitol, fructose, glucose, and sucrose), organic acids (quinic, malic, citric, shikimic, oxalic and succinic acids), amino acids, proteins and minerals (K, Ca, P, Mg, Na, Fe, Se, and Zn), vitamins (ascorbic acid, vitamins C, E, B, and K), aromatic compounds, fibers, fatty acids, and essential oils (Catană et al., 2017; Lazăr et al., 2020; Wójtowicz et al., 2023; Piras et al., 2024; Pop et al., 2025). Due to their pronounced astringent flavor and distinctive bitter-almond scent, the fruits are primarily utilized in processed products and are less frequently eaten fresh. The fruits can be processed into a diverse array of products, including syrups, juices, jams, and jellies due to their high pectin content, as well as beverages such as wines, schnapps, and fruit infusions (Scott & Skirvin, 2007; Kulling & Rawel, 2008; Catană et al., 2021; Toma et al., 2022). They are also used in baked goods, desserts, and powdered dietary supplements (Catană et al., 2018; Sidor & Gramza-Michałowska, 2019). *Aronia melanocarpa* is

highly adaptable to a diverse range of soil types and pH levels (Brand, 2010; Dragomir et al., 2022), but it is sensitive in high salinity conditions (Nas et al., 2023). It also exhibits resistance to pests and fungal diseases (Scott & Skirvin, 2007; Palancean et al., 2015; Golache et al., 2024). Since 2020, *Aronia melanocarpa* micropropagation has advanced to support disease-free, genetically uniform plantlet production for cultivars like 'Nero' (Borsai et al., 2021; Rusea et al., 2022; Bayhan & Yücesan, 2024; Kaya & Özatay, 2024; Ekinci et al., 2024), 'Viking' (Nas et al., 2023; Yaman et al., 2025) and on other cultivars, such as 'Melrom' and 'Galicjanka' (Jakab et al., 2022; Isac & Plopa, 2022) and 'Eastland' (Celebi-Toprak & Alan, 2020). Effective micropropagation protocols employing shoot tips and nodal explants cultured on MS medium supplemented with BAP and IBA have resulted in high rates of shoot multiplication and root formation (Rusea et al., 2018; 2022). Common physiological problems, such as hyperhydricity, can be reduced through adjustments of agar and sucrose concentrations, as well as improved ventilation inside the growing vessels (Bayhan & Yücesan, 2024). Studies have also advanced techniques for callus formation (Calalb et al., 2014; Szopa et al., 2018), adventitious shoot regeneration (Rusea et al., 2018), and the establishment of cell cultures for the production of secondary metabolites (Shishkova et al., 2026). These advances contribute to the development of reliable propagation protocols, preservation of elite germplasm, and scalable production valuable *Aronia melanocarpa* cultivars.

In vitro tissue culture techniques play and important role in the propagation this woody species, as they enable the production of disease-free and genetically uniform planting material. Woody species are generally recalcitrant to *in vitro* propagation (Grzelak et al., 2024), and we face problems such as: polyphenols oxidation, endophytic bacterial contamination (Quambusch & Winkelmann, 2018; Permadi et al., 2025), hyperhydricity of tissues, formation of basal callus, somaclonal variation, unsuccessful rooting, dormancy during acclimatization (Jain & Häggman, 2007; Nowakowska et al., 2023).

Phytohormones (plant hormones) are naturally occurring organic molecules found within plant tissues that function as signaling agents, regulating a wide range of physiological activities (Davies, 1995). Although present in very small amounts - typically below 1 nM to 1 μM - they exert significant effects on plant growth and development. These compounds are frequently synthesized in particular plant organs and then transported to other regions, where they trigger physiological, biochemical, and structural responses (Salisbury & Ross, 1992). Cytokinins, a group of phytohormones, play a fundamental role in plant biology, regulating numerous developmental and physiological processes. They are key contributors to cell division and differentiation, stimulate the formation and expansion of vegetative tissues, promote chloroplast development, and influence overall plant growth and metabolism. Through these functions, cytokinins help coordinate plant development and maintain proper physiological activity (Krikorian et al., 1987). In plant tissue culture, BAP is the most commonly used cytokinin, but it can lead to hyperhydricity, shoot tip necrosis, inhibit rooting during the acclimatization and cause early senescence (Werbrouck, 2021). Nevertheless, while higher concentrations of BAP can enhance the rate of shoot regeneration, they may also increase the likelihood of vitrification. During the acclimatization phase, plants affected by vitrification showed impaired development, characterized by reduced shoot elongation, smaller and narrower leaves, fewer and shorter roots, and lower survival percentages. These adverse effects were especially pronounced when plants were exposed to environments with lower humidity levels (Bayhan & Yücesan, 2024). Meta-Topolin (mT, 6-(3-hydroxybenzylamino) purine) is an exceptionally potent natural aromatic cytokinin that was first extracted by Strnad (1997) from the mature foliage of *Populus* x *canadensis* and has since been recognized for its biological activity. Can be used as an alternative to BAP, as it promotes rapid, high multiplication rates, improve rooting and *ex vitro* adaptation, while reducing structural abnormalities and somaclonal variations (Amoo et al., 2011; Cardoso, 2021; Zaytseva et al., 2021). Moreover, it can lead to improve both rooting success and acclimatization in plants (Nowakowska & Pacholczak, 2020). The

present study aims to evaluate the *in vitro* response of 'Alexandrina', a Moldavian cultivar, to 5 different types of cytokinins, others than BAP: 6-(γ, γ-dimethylallylamino) purine (2iP), kinetin, meta-Topolin, thidiazuron and zeatin.

MATERIALS AND METHODS

'Alexandrina' is a Moldavian cultivar bred by the researchers at the National Botanical Garden (Institut) 'Alexandru Ciubotaru' of the State University of Moldova (Palancean et al., 2015). The initial plant material - *in vitro* cultures (Figure 1) was obtained from the National Botanical Garden 'Alexandru Ciubotaru' of Chișinău. It was subsequently multiplied *in vitro* at the University of Agronomic Sciences and Veterinary Medicine of Bucharest, Romania, as part of the BioMedPlant project (*Valorization of medicinal spontaneous and cultivated plants through the use of advanced micropropagation techniques as sources of bioactive compounds*).



Figure 1. Initial *in vitro* cultures obtained from the National Botanical Garden 'Alexandru Ciubotaru' of the State University of Moldova

I. Multiplication phase

In vitro multiplication of *Aronia melanocarpa* 'Alexandrina' was performed out on Murashige and Skoog (MS) medium, supplemented with five different cytokinins, each at a concentration of 1 mg/L: meta-topolin (mT), zeatin (Z), kinetin (kin), 6-(γ, γ-Dimethylallylamino) purine (2iP) and thidiazuron (TDZ). To ensure proper multiplication, all media were supplemented with 0.5 mg/L IAA and 0.2 mg/L GA_3, as shown in Table 1.

Other media components included sucrose (3%), Kobe I agar (0.7%) and to reduce tissue oxidation, PVP (polyvinylpyrrolidone) was added in concentration of 100 mg/L. The pH was modified at 5.75 and the media was sterilized in the autoclave at 121°C for 20 minutes. indole-3-acetic acid (IAA) and gibberellic acid (GA_3) were co-autoclaved with the rest of the media components. All cytokinins were filter-sterilized after the media was autoclaved, using sterile polyethersulfone (PES) syringe filters (0.22 µm pore size).

Table 1. Types and concentrations of plant growth regulators (PGRs) supplemented to the multiplication media

Medium variant	Plant growth regulators		
	Cytokinins	Auxins	Gibberellins
V1	1 mg/L mT	0.5 mg/L IAA	0.2 mg/L GA_3
V2	1 mg/L Z	0.5 mg/L IAA	0.2 mg/L GA_3
V3	1 mg/L KIN	0.5 mg/L IAA	0.2 mg/L GA_3
V4	1 mg/L 2iP	0.5 mg/L IAA	0.2 mg/L GA_3
V5	1 mg/L TDZ	0.5 mg/L IAA	0.2 mg/L GA_3

II. Rooting phase

Shoots regenerated on media containing meta-topolin were subsequently cultured on half-strength MS medium supplemented with 1 g/L activated charcoal and different concentrations of either indole-3-butyric acid (IBA) or indole-3-acetic acid (IAA), as detailed in Table 2.

Table 2. Concentration of auxins (IBA and IAA) supplemented to the rooting media

Medium variant	Auxins	
	IAA (mg/L)	IBA (mg/L)
R1	0.5	-
R2	1	-
R3	-	0.5
R4	-	1

Sucrose, agar and PVP were added to the media in the same concentrations as in the multiplication media. Both auxins were sterilized with the rest of the media components, by autoclavation at 121°C for 20 minutes.

The concentration of $CuSO_4 \cdot 5H_2O$ was increased tenfold, to 0.125 mg/L, compared to the concentration present in the conventional half-strength MS formulation. This adjustment was made to stimulate root development and improve plantlet vigor, given the importance of copper in carbon dioxide fixation and ATP production (Marques et al., 2018).

The medium was dispensed into 720 mL glass jars at approximately 83 mL per vessel to ensure adequate air volume for shoot development.

The jars were fitted with lids containing air-permeable filters to facilitate gas exchange, thereby reducing ethylene accumulation and improving leaf adaptation to *ex vitro* conditions. Tamimi & Othman (2020) observed an increase in multiplication parameters (including number and length of shoots) and chlorophyll content on media with increased copper sulphate concentration on *Musa acuminata* shoots cultivated *in vitro*. Similarity, *Capsicum annum* cotyledons showed improved bud differentiation and elongation on media supplemented with higher concentrations of $CuSO_4$ x $5H_2O$ (Joshi & Kothari, 2007).

Total dry matter was determined by weighing 250 mg of finely cut and homogenized plant material (from 5 shoots/media variant), in crucibles, in 3 repetitions/variant, followed by drying for 14 hours at 105°C using a UN110 Memmert drying oven. This can be a good indicator of how the leaves will perform during the acclimatization phase.

III. Acclimatization phase

In vitro rooted shoots of 'Alexandrina' were placed in sowing trays with peat (sterilized in the autoclave at 121°C for 20 minutes) then covered with plastic lids to keep a high level of humidity (Figure 2). Prior to planting, the roots were washed of all agar traces and they were shortened at approximately 5 cm.



Figure 2. *In vitro* rooted shoots of *A. melanocarpa* 'Alexandrina' used for the acclimatization phase

The trays were kept in laboratory conditions at 20-25°C room temperature and illuminated using LED lamps composed of white, red and blue lights (Figure 3). The acclimatization of the shoots was carried out by periodically removing the lid and gradually exposing the shoots to the new humidity conditions, until the leaves were able to maintain their turgidity at normal laboratory humidity (approximately 40-50%).



Figure 3. Acclimatization of *Aronia melanocarpa* 'Alexandrina' shoots in laboratory conditions

The trays were kept in laboratory conditions at 20-25°C room temperature and illuminated using LED lamps composed of white, red and blue lights (Figure 3). The acclimatization of the shoots was carried out by periodically removing the lid and gradually exposing the shoots to the new humidity conditions, until the leaves were able to maintain their turgidity at normal laboratory humidity (approximately 40-50%).

Acclimatization of *Aronia melanocarpa* can also be done in a growing chamber with high humidity (Figure 4). Both methods work as long as proper humidity, temperature and light can be ensured.



Figure 4. Acclimatization of *in vitro* obtained shoots in a Memmert growing chamber with high humidity

Statistical analysis

Statistical analyses were performed using Microsoft Excel 2021 with the Real Statistics Resource Pack (www.real-statistics.com). Descriptive statistics, including the mean and standard error, were calculated for all measured variables. Prior to inferential analysis, the assumption of homogeneity of variances was evaluated using Levene's test. Depending on whether this assumption was met, differences among treatments were analyzed using either one-way analysis of variance (ANOVA) or the non-parametric Kruskal-Wallis test. When statistically significant differences were detected ($p < 0.05$), multiple comparisons were performed using Tukey's honestly significant

difference (HSD) test following ANOVA or Dunn's post hoc test following the Kruskal–Wallis test. Results were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSIONS

I. Multiplication phase

Average number of shoots obtained from each explant

Meta-Topolin medium enhanced the multiplication rate (Figure 5), by generating highest number of shoots/ explant (20.7 ± 4.59) (Figure 6), followed by variant V2 with zeatin, with an average of 4.7 ± 1.36 shoots/ explant. Lowest results were obtained on V3 with kinetin (1 shoot/explant) and variant V4 with 2iP (with 1.16 shoots/explant), with no statistically significant differences between them, as confirmed by Dunn's test.

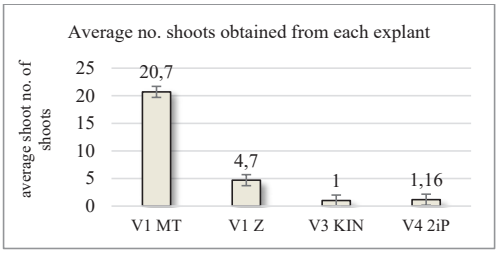


Figure 5. Average number of shoots obtained from each explant

Meta-Topolin has increased *in vitro* multiplication in several woody species, such as *Actinidia arguta* (Shakshi et al., 2026), *Daphne mezereum* (Nowakowska et al., 2023), *Salix tetrasperma* (Reshi et al., 2025), *Feronia limonia* (Anis et al., 2023) *Rubia cordifolia* (Kunal et al., 2025). Use of meta-Topolin was also shown to stimulate the development of the photosynthetic apparatus in *Musa* spp., which is a crucial aspect in the rooting and acclimatization phases, as it increases the survival rates of the plantlets (Aremu et al., 2012). Similarly, in *Momordica balsamina*, leaves that were obtained from shoots cultivated on media with meta-Topolin showed veins and trichomes that were more developed and an increase in the density of stomata and stomatal index, compared to the leaves obtained on shoots cultivated in the presence of BAP (Chouhan et al., 2025). In addition, in *Oxystelma esculentum*, the use of media with mT was shown to also enhance cutin deposition and the development of vascular and ground tissue systems (Jayaprakash et al., 2021).

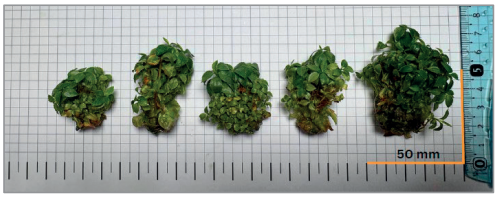


Figure 6. High shoot proliferation of explants cultivated on media with meta-Topolin

Rusea et al. (2018) obtained an average of 6.08-10.2 shoots per explant on media with 0.5-2.0 mg/L BAP combined with 0.1-1.0 mg/L IBA. By comparison in our previous study, we obtained averages between 8.3 and 13.3 shoots/explant in 'Alexandrina' and 5.21-8.37 shoots/explant in 'Nero' on media with 0.3-0.5 mg/L BAP and 0.2 mg/L GA_3 (Nicolae et al., 2025). Litwińczuk (2013) recommends the use of BAP (1 mg/L) and IBA (0.05 mg/L) supplemented to media with a 150% increase in magnesium and calcium salts to enhance axillary shoot proliferation.

Average shoot length

Regarding shoot growth, explants grown on medium with KIN (Figure 7), even although showing limited proliferation, exhibited the greatest shoot elongation (44.94 ± 9.52 mm), followed by variants V4 (39.29 ± 9.24 mm) and V2 (33.09 ± 8.45 mm). The explants cultivated on the mT-supplemented medium produced shortest shoots (21.53 ± 5.17 mm).

Rusea et al. (2018), observed that 'Nero' explants cultivated on media with BAP and IBA can reach lights of 1.45-2.24 mm, however, this is highly dependent on the cultivar, as our past studies have also shown that 'Nero' grows smaller in comparison with 'Alexandrina' (Nicolae et al., 2025).

Additionally, in this experiment, *A. melanocarpa* 'Alexandrina' shoots that were obtained on KIN medium showed shoots with more lignified tissue and higher leaf surface area (data not shown). In micropropagation of *Quercus robur*, KIN promotes the formation of a more developed secondary xylem and

enhances cell wall lignification. Furthermore, the shoots cultured in the presence of KIN exhibit anatomical characteristics associated with increased cellular differentiation rather than vigorous proliferative growth (Martins et al., 2022). Moreover, the low shoot induction efficiency of cytokinins such as KIN or 2iP may be linked to fast cytokinin catabolism by cytokinin oxidase/dehydrogenase enzymes (Schmülling et al., 2003; Khablak et al., 2024).

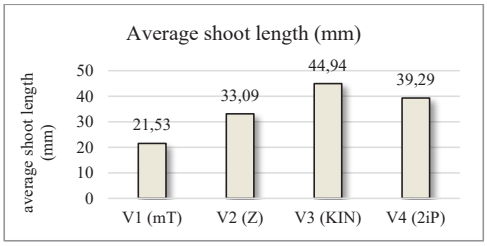


Figure 7. Average shoot length (mm) of explants cultivated on the multiplication media

Average biomass (g)

Highest biomass was accumulated on explants cultivated on V5 – TDZ (2.33 ± 1.07 g/explant) and V1 – mT (1.95 ± 0.72 g/explant), with no statistically significant differences between them, as it was pointed out by Dunn's test (Figure 8).

Even though variant V5 – TDZ generated the highest biomass/explant, the inclusion of TDZ resulted in the formation of compact, globular masses of shoots that were small, highly friable, and hyperhydrated rendering them unsuitable for further multiplication (Figure 9).

Thidiazuron (TDZ) is a is a non-purine phenylurea and possesses cytokinin-like properties; however, unlike adenine-based cytokinins such as BAP, KIN and Z, it lacks a purine ring in its molecular structure (Dewir et al., 2018; Nautiyal et al., 2022).

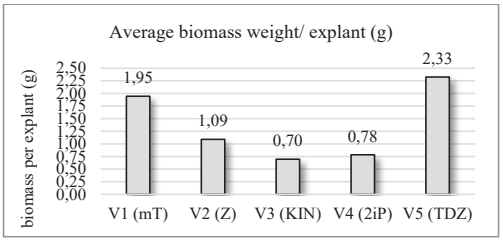


Figure 8. Average biomass (g) produced from explants cultivated on multiplication media variants

TDZ is extremely potent and effective in stimulating cell division and organogenesis (Manokari et al., 2026), which could be associated with its resistance to cytokinin/dehydrogenase-mediated breakdown and increased internal cytokinin concentrations (Brunoni et al., 2021). However, it is known to cause several anomalies in plant tissue culture (Dewir et al., 2018). Most common effects include fasciated and compact shoots, hyperhydricity, and reduced rooting capacity (Huetteman & Preece, 1993; Lu, 1993).



Figure 9. Explants obtained on multiplication media with TDZ

Average basal callus mass (mg)

Regarding the production of basal callus, figure 10 shows that variant V2 with Z produced the highest basal callus mass (327.42 ± 42.75 mg/explant), followed by V1 (mT) with 219.90 ± 70.67 mg/explant; however, Dunn's test indicated that the difference between these two variants was not statistically significant. Callus on V4 (2iP), which generated an average of only 1.16 shoots/ explant. By comparison, callus tissue obtained on V1 (mT) had strong organogenic potential, as the explants obtained generated a high number of shoots (20.7 ± 4.59 shoots/ explant), while the average on V2 (Z) was very low (4.7 ± 1.36 shoots/ explant). Variants V3 (KIN) and V4 (2iP) produced the lowest amounts of basal callus; nevertheless, the callus formed on V3 (KIN) showed no shoot regeneration capacity, as explants cultured on this medium developed solely via the apical

meristem, yielding only one shoot per explant. Similar observations can be made about the callus on V4 (2iP), which generated an average of only 1.16 shoots/ explant.

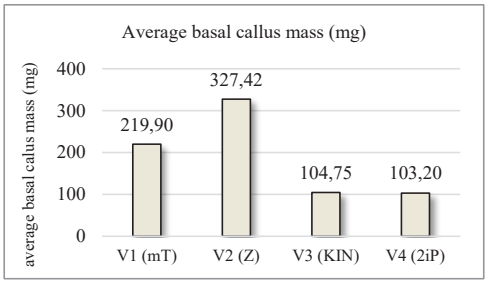


Figure 10. Average basal callus mass (mg) per explant

Average number of leaves/explant

Highest values regarding the number of leaves were observed on variant V3 (KIN) (Figure 11), with an average of 25.80 ± 8.05 leaves/explant, followed by V4 (2iP), with an average of 17.67 ± 4.26 leaves/explant, but with no statistically significant differences.

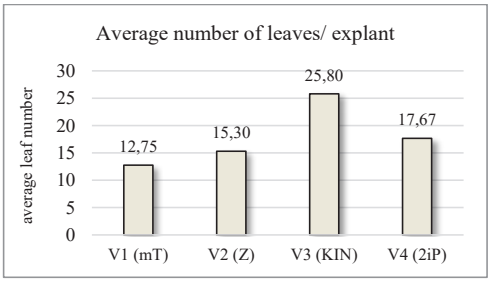


Figure 11. Average number of leaves/explant on the multiplication media variants

The lowest values were recorded for V1 (mT), with 12.75 ± 2.41 leaves per explant, and for V2 (Z), with 15.30 ± 3.24 leaves per explant; these variants also exhibited the lowest shoot heights. This parameter is an important indicator, as it is directly correlated with the number of axillary buds and, consequently, with the potential multiplication rate. In relation to shoot height, it can also provide information on internodal length and the spatial distribution of axillary buds.

Rhizogenesis during multiplication phase:

Rhizogenesis only occurred on variants V3 (KIN) and V4 (2iP), and, in a very low percentage, on V2 (Z) - only 10% (Figure 12). Variants V3 (KIN) and V4 (2iP) produced an average of 3.3 ± 2.21 roots/ explant and 3.1 ± 1.60 roots/ explant, respectively. Analysis of variance (Kruskal-Wallis Test) pointed out no significant differences between these values. Highest root length was obtained on V2 (Z): 92.67 ± 12.02 mm, followed by V3 (KIN): 79.47 ± 13.23 mm (Figure 13).

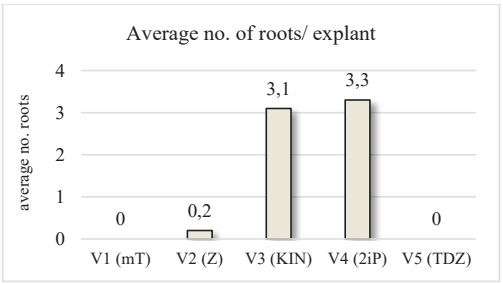


Figure 12. Average roots number of explants cultivated on multiplication medium variants

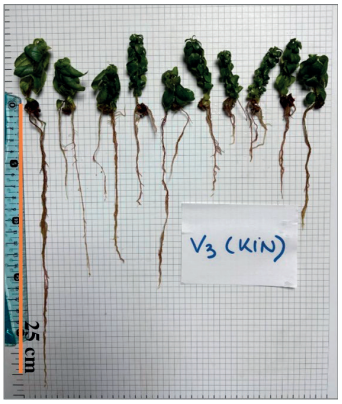


Figure 13. Rhizogenesis on explants cultivated on multiplication media with kinetin

The lowest values were observed on variant V4 (2iP) with 49.48 ± 9.21 mm; however, no statistically significant differences were identified regarding root length.

II. Rooting phase

Average number of roots and root length

Rooting was successfully achieved in 100% across all media variants. Differences between the growing parameters, such as shoot height, number of leaves were relatively minor, and statistically unconfirmed (using ANOVA in all cases), except for the average shoot height - R4 and R1 recorded significant differences.

IBA is the most commonly used auxin in the rooting phase of *Aronia melanocarpa*, supplemented to a media recipe with a lower concentration of minerals (Almokar & Pırlak, 2018; Celebi-Toprak & Alan, 2020; Ekinci et al., 2024; Abduganiyeva et al., 2025). For varieties such as 'Melrom' and 'Nero', Isac & Plopa (2022) recommend the use of IBA in concentration of 0.7 mg/L. Other medias, such as half-strength Shrub Plant Medium (SPM) in combination with NAA and IBA, can also be used to obtain high rooting percentages in cultivars such as 'Viking' (Yaman et al., 2025). Concentrations over 3 mg/L IBA cand lead to an increase in callus formation and size (Abduganiyeva et al., 2025), which can be an undesirable aspect during the rooting stage. Jakab et al. (2022), recommend using IBA concentrations, as low as 0.5 mg/L. Litwińczuk (2013) recommends using IBA in concentrations as low as 0.05 mg/L.

Highest number of roots was obtained on the variant with 1 mg/L IBA (R4), 7.4 ± 1.74 roots/ shoot and on variant with 1 mg/L IAA (R2), 7 ± 2.41 roots/ shoot (Figure 14).

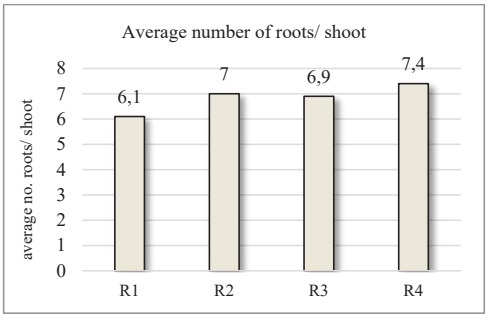


Figure 14. Average root number of explants cultivated on the rooting media variants

Both variants with lower concentrations of auxins generated a lesser number of roots: 6.9 ± 2.02 roots/ shoot on media with 0.5 mg/L IAA (R3) and 6.1 ± 3.14 roots/ shoot on media with 0.5 mg/L IBA (R1).

Average shoot height (mm)

Regarding the growth of these shoots, highest values were observed on variant with 1 mg/L IAA (R2) (Figure 15), with an average of 33.64 ± 5.12 mm and on the variant with 0.5 mg/L IBA (R3), with an average of 33.07 ± 3.30 mm. The Tukey HSD test indicated significantly lower shoot heights in R1 (0.5 mg/L IAA): 21.80 ± 2.48 mm and R4 (1 mg/L IBA): 26.34 ± 3.76 mm (Figure 16).



Figure 15. *In vitro* rooted shoots obtained on media with 1 mg/L IAA

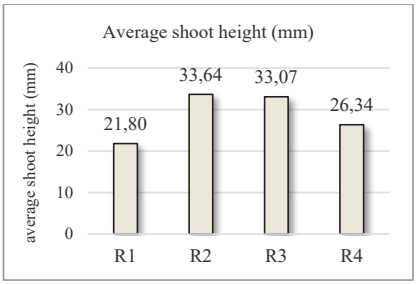


Figure 16. Average shoot height (mm) of explants

Average number of leaves

Proper growth and development of leaves is an important factor in the next phase, when the plantlets are acclimatized to *ex vitro* conditions. The subsequent growth of plantlets under *ex vitro* conditions depends both on the formation of new leaves and on the adaptation of existing leaves that were regenerated *in vitro*.

In regards with this aspect, variant with 1 mg/L IBA (R4) recorded the highest average number of leaves (8.5 ± 1.12 leaves/ explant). Middle values of 7.9 ± 1.81 leaves were obtained on variant with 0.5 mg/L IAA (R1) and 7.8 ± 0.60 on variant with 0.5 mg/L IBA (R3). Lowest values were recorded on media with 1 mg/L IAA (R2), only 7.4 ± 0.92 leaves/ explant (Figure 17). However, analysis of variance (ANOVA) recorded no statistically significant differences between the four variants of rooting media.

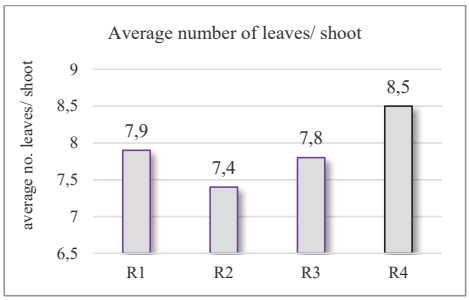


Figure 17. Average number of leaves/shoot of the explants cultivated on the rooting media variants

Dry matter of in vitro obtained leaves

Leaves that were regenerated from the shoots cultivated on media supplemented with IBA (0.5 and 1 mg/L) had an overall higher dry matter content, compared to the ones cultivated on media with IAA (Figure 18). Higher dry matter content (%) was obtained from the leaves from variant R4 (1 mg/L IBA): 34.16 ± 2.63% and R3 (0.5 mg/L IBA): 29.57 ± 1.81%.

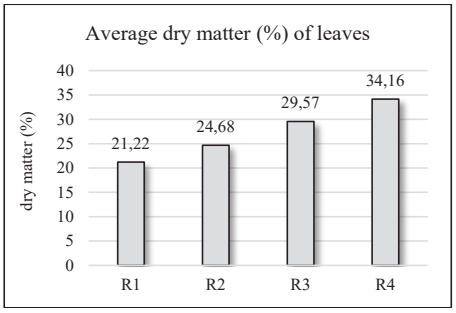


Figure 18. Average dry matter (%) of leaves obtained from shoots cultivated on rooting media variants

Leaves regenerated from the explants cultivated recorded lower values, of 24.68 ± 0.52% on R2 (1 mg/L IAA) and 21.22 ± 3.35% on R1 (0.5 mg/L IAA). For comparison, the average dry matter content was 15.11 ± 0.94% during the multiplication phase (on meta topolin medium) and 35.51 ± 0.92% in young greenhouse-grown plants of *Aronia melanocarpa* 'Alexandrina'

The dry matter can be a good indicator of how the leaves will perform during the acclimatization phase because it reflects how well the leaves are developed. Due to the culture environment of explants (high humidity, lower light intensity, controlled temperature, and heterotrophic or mixotrophic nutrition), leaves formed *in vitro* often have low dry matter due to high water content, thin cell walls, poor cuticle development, and limited lignification. Such leaves are fragile and poorly adapted to *ex vitro* conditions, making them highly susceptible to desiccation and transplant shock when humidity drops. Moreover, the stems of plantlets developed *in vitro* are often hypo lignified, their cell walls are thin, and the intercellular spaces are large, with limited development of vascular tissue (Ziv, 1995; Nowakowska et al., 2023).

For woody species, the rooting phase is not only important to generate a well-developed rooting system, but also to stimulate the proper development of leaves and stems (through using well-ventilated culture vessels, adapting culture media nutrients and growth regulators, use of plant elicitors) in order to ensure high survival rates during the acclimatization phase.

III. Acclimatization phase

All rooted shoots were successfully acclimatized. After 3 weeks of culture in laboratory conditions, they were planted individually in small pots and transferred to greenhouse conditions where they developed into healthy, uniform plants (Figure 19).



Figure 19. *In vitro* obtained plants of *Aronia melanocarpa* 'Alexandrina' grown in the greenhouse at USAMV Bucharest

Generally, if the rooting phase is carried on properly, acclimatization of *Aronia melanocarpa* can be achieved successfully. Several authors report good results (Celebi-Toprak & Alan, 2020; Borsai et al., 2021; Isac & Plopa, 2022; Rusea et al., 2022; Coşkun et al.,

2026), as long as the tissues are not vitrified, which can delay shoot and leaf growth, reduce the development of the rooting system and increase the risk of plant loss due to the dehydration of the tissues (Bayhan & Yücesan, 2024).

CONCLUSIONS

Meta-topolin (mT) is the most effective cytokinin for *in vitro* multiplication of *A. melanocarpa* 'Alexandrina', yielding the highest shoot proliferation (20.7 shoots/explant) and good biomass, without hyperhydricity. TDZ induced high biomass but led to compact, hyperhydric, non-viable shoot clusters — unsuitable for further multiplication. KIN and 2iP promoted shoot elongation and leaf formation but showed low multiplication rates and limited organogenic potential. IBA (especially 1 mg/L) is recommended for rooting to improve leaf dry matter content and acclimatization success. Leaf dry matter content is a reliable indicator of plantlet physiological quality prior to *ex vitro* transfer. Optimizing ventilation, light, and nutrient balance during rooting is critical for high survival rates during acclimatization.

The results demonstrate that meta-topolin is the most efficient cytokinin for rapid micropropagation of *A. melanocarpa* 'Alexandrina', providing a reliable system for large-scale propagation and conservation of valuable plant material.

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