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The Effects of GA_3 and AsA Applications on Vegetative Growth During Acclimatization

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HIGHLIGHTS

- GA_3 and AsA combination improved acclimatization success.
- Highest survival rate obtained with 25 ppm GA_3 + 300 mg L^{-1} AsA.
- 25 ppm GA_3 + 300 mg L^{-1} AsA produced the longest shoots.

Abstract

In vitro micropropagation enables the rapid and season-independent production of healthy plantlets, yet its long-term success largely depends on minimizing losses during the acclimatization phase. Because the culture vessels contain high humidity and free surface moisture, plantlets develop insufficient cuticles, which makes them highly vulnerable to desiccation when transferred to external conditions. Therefore, implementing supportive exogenous treatments during acclimatization is essential to enhance plantlet tolerance and reduce mortality. This study was conducted to evaluate the effects of gibberellic acid (GA_3) and ascorbic acid (AsA) applications on reducing losses encountered during the acclimatization process and improving the adaptation success of micropropagated plantlets under *in vitro* conditions. Survival rate (%), plant height (cm), shoot diameter (mm), number of leaves (pieces/plantlet), number of nodes (pieces/plantlet), and chlorophyll content (SPAD) were measured. The results revealed that the highest survival rate (51.33%) was obtained from 25 ppm GA_3 + 300 mg L^{-1} AsA, the longest shoots (18.40 cm) from 25 ppm GA_3 + 300 mg L^{-1} AsA, the thickest shoot diameter from 25 ppm GA_3 + 300 mg L^{-1} AsA (1.88 mm), the highest number of leaves (15.00 pieces/plantlet) from 25 ppm GA_3 + 150 mg L^{-1} AsA and 50 ppm GA_3 + 300 mg L^{-1} AsA, the highest number of nodes (14 pieces/plantlet) from 50 ppm GA_3 + 300 mg L^{-1} AsA, and the highest chlorophyll content (43.49 SPAD) from 25 ppm GA_3. The study demonstrates that combinations of GA_3 and AsA can be effective in reducing acclimatization losses.

Keywords: Acclimatization; Healing agent; *In vitro*

1. Introduction

Aronia is a plant belonging to the *Rosaceae* family. Aronia is a berry with high antioxidant content. There are three species: black chokeberry (*Aronia melanocarpa* (Michx.) Elliot), purple chokeberry (*Aronia prunifolia* (Marsh)), and red chokeberry (*Aronia arbutifolia* (L.) Elliot) (Šnebergrová et al. 2014). Its native range is North

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America. Aronia was introduced to Russia in the early 1900s, and its commercial cultivation began in Eastern European countries in the 1950s (Kokotkiewicz et al. 2010). It is a perennial, deciduous shrub. Since aronia flowers in late spring and is tolerant to cold, it is not adversely affected by late spring frosts (Ochmian, 2012; Sidor and Gramza-Michałowska, 2019). Owing to its high antioxidant, vitamin, and mineral content, it is referred to as a "superfruit" (Cacak-Pietrzak et al. 2023).

Aronia can also be propagated by seed. However, due to its genetic variability, it is not preferred. In addition to late fruiting, vigorous and irregular growth, and unsuitability for mechanical harvesting, seed propagation is therefore not recommended (Brand et al. 2022; Duman et al. 2025). Vegetative propagation can also be achieved through rooting of cuttings (Nas et al. 2025). In addition to these techniques, *in vitro* micropropagation allows the production of a large number of healthy and uniform plant materials regardless of the season (George and Debergh, 2008).

In micropropagation, the most critical factor affecting success is the acclimatization of plantlets to *ex vitro* conditions. During this stage, high mortality rates may occur due to factors such as light intensity, temperature, and water stress (Kumar and Rao, 2012). Substantial plant losses following transfer are associated with water loss, transplant shock, pathogen attack, and weak photosynthesis (Krishna et al. 2005). These adverse conditions negatively affect plantlets and, in severe cases, result in their death. To improve acclimatization success, applications of certain plant growth regulators are employed (Dias et al. 2014; Kara et al. 2022).

Among these, ascorbic acid (AsA), naturally found in plants, is an important antioxidant. It increases the plant's photosynthesis rate and improves photosynthetic pigments. It is known to increase tolerance to abiotic stress by enhancing oxidative defense capacity. Additionally, it plays a role in cell division and hormone synthesis, which play crucial roles in plant growth and development (Akram et al. 2017; Smirnoff, 2018; Zheng et al. 2022; Celi et al. 2023). Gibberellic acid (GA_3) is a plant growth regulator that plays a role in plant development. It plays a role in various plant processes, such as seed germination, leaf formation, the transition from the vegetative to the generative phase, and fruit formation (Fahad et al. 2015; Dinler and Çetinkaya, 2020; Korkmaz et al. 2020; Shah et al. 2023). Moreover, GA_3 has been reported to enhance tolerance to various abiotic stresses (Hasan et al. 2020).

AsA and GA_3 are known to support plant growth by enhancing antioxidant defenses, stimulating cell division, and improving ion balance and stress tolerance. Although their positive effects on photosynthesis and oxidative damage reduction under various stress conditions have been documented, there is no direct evidence explaining how these compounds help maintain the vitality of tissue-culture-derived plantlets during acclimatization (Weiss and Ori, 2007; Banerjee and Roychoudhury, 2019; Shaki et al. 2019; Emamverdian et al. 2020; Nagar et al. 2021; Abbas et al. 2022; Thakur and Singh, 2024). The acclimatization phase is a complex transition in which plantlets face multiple challenges, including excessive water loss, weak cuticle formation, irregular stomatal behavior, and oxidative stress. How AsA and GA_3 function together under these conditions remains unclear in the existing literature. Therefore, investigating the potential of these two compounds to enhance plantlet survival during acclimatization is essential to address this gap. This study provides initial insights into this mechanism and contributes new information to the field.

Plants produced through tissue culture may experience high mortality rates during the final stage of micropropagation, as they are sensitive to sudden changes in environmental conditions (Bhojwani and Dantu, 2013). The main reason for this is that the growth conditions within culture vessels induce abnormal morphological and physiological characteristics in the plants (Sutter, 1984). Understanding these abnormalities is essential for developing effective transplantation protocols. Since plantlets transferred to *ex vitro* conditions can easily be damaged by abrupt environmental changes, a period of acclimatization is required to correct these abnormalities.

However, studies on the acclimatization of *Aronia melanocarpa* under *ex vitro* conditions are limited. Previous research has emphasized the importance of gradual humidity adjustment and the use of suitable growing substrates such as peat or perlite to achieve high survival rates (Sivanesan et al. 2016; Borsai et al. 2017; Çelebi-Toprak and Alan, 2018; Bayhan and Yücesan, 2024; Yaman et al., 2025). Nevertheless, most of

these studies have focused on the physical conditions of acclimatization, while the effects of biochemical regulators that may enhance stress tolerance and the success of plant adaptation have not been investigated.

In the present study, exogenous applications were conducted during the transition from the *in vitro* rooting medium to a peat–perlite substrate, aiming to promote faster and more effective adaptation of plantlets to external conditions. In this context, the effects of GA_3 and AsA treatments on survival rate and growth performance during acclimatization were examined. The objective was to establish an approach that could shorten the acclimatization period and reduce plant losses. The findings are expected to contribute not only to the optimization of *Aronia* micropropagation but also to improving acclimatization efficiency in other plant species propagated through tissue culture.

2. Materials and Methods

The study was conducted in the Plant Tissue Culture Laboratory of Harran University, Department of Horticulture. Single-node microcuttings of *Aronia melanocarpa* cv. Nero were used as plant material. The microcuttings were first soaked in a detergent solution for 10 minutes and then rinsed with tap water. Then, in a laminar flow sterile cabinet, the microcuttings were soaked in 70% ethanol for 2 minutes and then in 10% sodium hypochlorite solution for 10 minutes. Finally, they were rinsed three times with sterile distilled water to complete the sterilization process (Karakoyun et al. 2024). The microcuttings were transferred to a shoot propagation medium containing MS (Murashige and Skoog, 1962) supplemented with 3% sucrose, 1 mg L^{-1} GA_3, and 8 g L^{-1} agar. Plants grown in propagation medium were then transferred to a propagation medium containing 3% sucrose, 3 mg L^{-1} BAP, 1 mg L^{-1} kinetin, and 7 g L^{-1} agar (Almokar and Pırlak, 2018). Approximately four weeks later, plants taken from the propagation medium were transferred to a rooting medium (MS) containing 3% sucrose, 2 mg L^{-1} IBA, 0.5 mg L^{-1} NAA, and 7 g L^{-1} agar (Almokar and Pırlak, 2018) (Figure 1). The pH value of all culture media was adjusted to 5.8. 1 ml L^{-1} PPM (Plant Protector Mixture) was added to the medium to prevent contamination (Babu et al. 2022). Plantlets transferred to culture medium were maintained in a growth chamber for four weeks under white fluorescent light, 16:8 h photoperiod and 25 ± 1°C. After rooting, the acclimatization experiment was initiated. During the acclimatization stage, plantlets were irrigated regularly to maintain adequate humidity and prevent water stress. *Aronia melanocarpa* cv. Nero plantlets were gently removed from the culture vessels and transferred into plastic pots containing a 1:1 (v/v) mixture of sterilized peat and perlite. Immediately after transplantation, the plantlets were lightly watered with distilled water. During the acclimatization stage, high humidity conditions were maintained by covering the plantlets transferred to the peat–perlite substrate with transparent plastic cups. The cups were gradually opened over the following days. For the first two weeks, irrigation was performed every 5–7 days, and subsequently every 2–3 days depending on the increasing water requirements of the developing plants (Figure 1). Foliar spray treatments of 25 ppm GA_3, 50 ppm GA_3, 150 mg L^{-1} AsA, 300 mg L^{-1} AsA, 25 ppm GA_3 + 150 mg L^{-1} AsA, 25 ppm GA_3 + 300 mg L^{-1} AsA, 50 ppm GA_3 + 150 mg L^{-1} AsA, and 50 ppm GA_3 + 300 mg L^{-1} AsA were applied at 5-day intervals for a total period of 60 days (Table 1). The control group of plants received a spray of distilled water. The GA_3 and AsA solutions were prepared separately for the treatments. The GA_3 stock solution was first dissolved completely in a few drops of 70% ethanol and then diluted to the desired concentration with distilled water. The AsA solutions were freshly prepared and dissolved directly in distilled water. For each treatment, 100 mL of working solution was prepared and applied to the leaf surfaces of the plantlets as a fine mist. The spraying was performed using hand-held pressurized spray bottles in the morning hours, ensuring that the leaves were uniformly wetted without causing runoff.

Table 1. Treatments with different GA_3 and AsA concentrations and their combinations.

Treatments	
Control	
25 ppm GA_3	25 ppm GA3 +150 mg L^{-1} AsA
50 ppm GA_3	25 ppm GA3 +300 mg L^{-1} AsA
150 mg L^{-1} AsA	50 ppm GA3 +150 mg L^{-1} AsA
300 mg L^{-1} AsA	50 ppm GA3 +300 mg L^{-1} AsA

2.1. *Statistical analysis*

At the end of the treatments, survival rate (%), shoot length (cm), shoot diameter (mm), number of leaves (leaves/plantlet), number of nodes (node/plantlet), and SPAD parameters were evaluated. The study was designed according to a completely randomized design with three replications, each replication consisting of 10 plantlets. Data obtained from the treatments were subjected to one-way analysis of variance (ANOVA) using the JMP Pro 17 statistical software in a randomized plot design, and means were compared using Tukey's test at a significance level of $p \leq 0.05$ (Gomez and Gomez, 1984). Hierarchical clustering analysis (HCA) and principal component analysis (PCA) were performed using R software (Version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria).

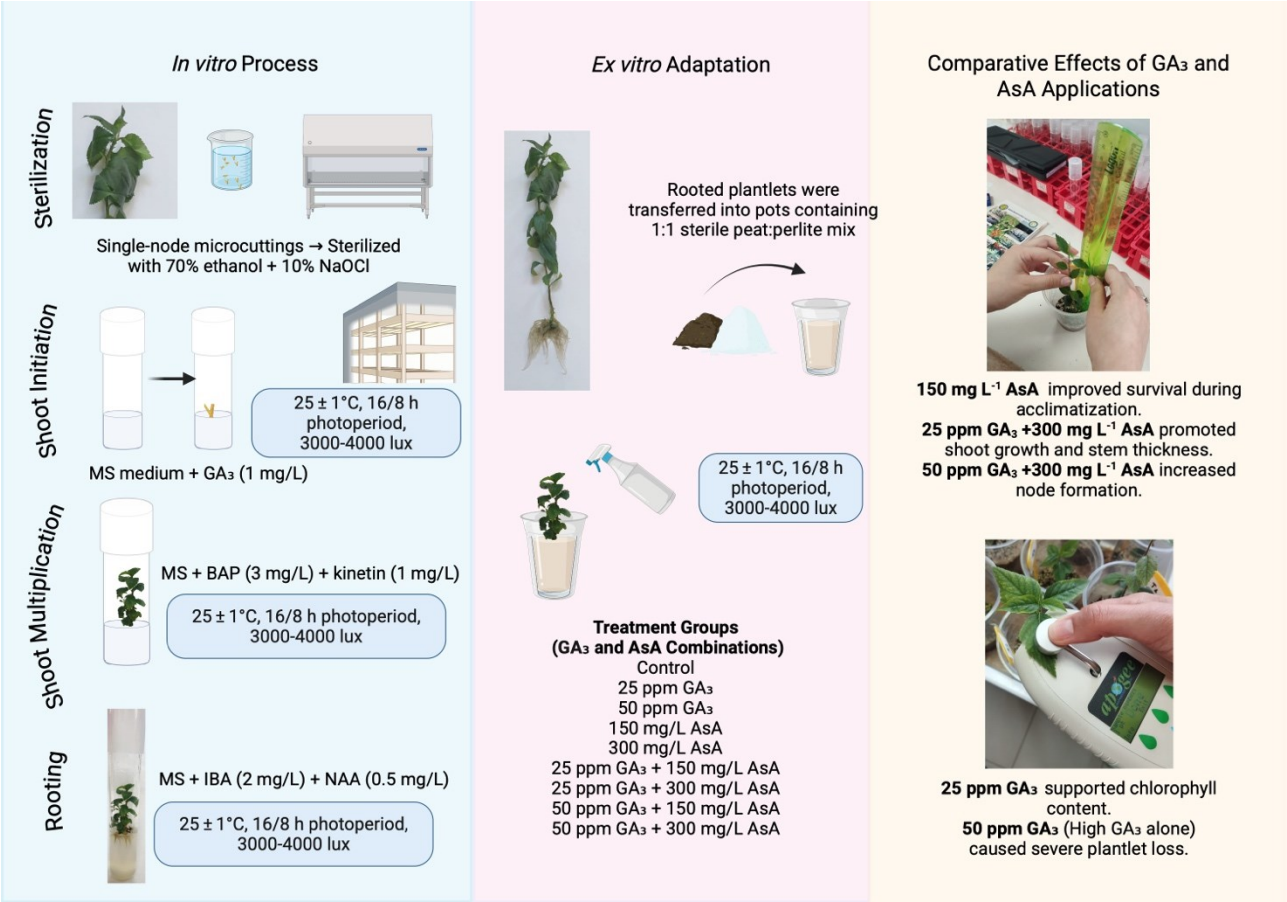

Figure 1. *In vitro* propagation, *ex vitro* adaptation, and effects of GA_3, and AsA on *Aronia melanocarpa* plantlets (https://www.biorender.com)

3. Results and Discussion

3.1. *Survival Rate (%)*

The data on survival rate are presented in Table 2. Statistical analysis showed significant differences in survival rates among treatments ($p \leq 0.05$). The highest survival rate (51.33%) was recorded in 25 ppm GA_3+300 mg L^{-1} AsA, while the lowest (0.00%) occurred in Control and 50 ppm GA_3. The zero-survival rate in Control and 50 ppm GA_3 was due to plant mortality, which prevented any measurements from being taken. In vegetative plant production via micropropagation, one of the plant tissue culture techniques, the final stage of the process is acclimatization. In the plantlets transferred for the acclimatization process following rooting, sudden environmental changes during the transition to external conditions negatively affected the survival rate in the untreated control group. In addition, uncontrollable contamination during the acclimatization period prevented the sustainable development of the plantlets. During this stage, plant mortality can occur due to damage caused by high temperature, high light intensity, low relative humidity, and pathogens in the

external environment (Hazarika et al., 2006; Chandra et al., 2010; Grzelak et al., 2024). Based on this observation, the 0.00% survival rate in the control group can be explained. In the 50 ppm GA_3 treatment, the absence of surviving plantlets may be associated with adverse effects of a supra-optimal GA_3 concentration. Gibberellic acid is known to strongly promote cell elongation, stem and internode growth, and to modulate membrane properties and stress responses (Shah et al. 2023). Under such conditions, the abrupt changes in humidity and light during transfer to *ex vitro* conditions may have exacerbated GA_3-induced structural and physiological imbalances, ultimately resulting in complete mortality in this treatment by the end of the first week after transfer.

In the present study, the application of 25 ppm GA_3+300 mg L^{-1} AsA was found to improve the survival rate. AsA acts as a cofactor for many enzymes and plays an important role in mitigating the harmful effects of reactive oxygen species (Jalili et al. 2023). It also has a key role in regulating senescence processes (Barth et al. 2006). Our findings are supported by previous studies; for example, Vasar (2001) reported that foliar spraying of 0.5 mM AsA during the acclimatization stage of sweet cherry (*Prunus avium* L.) increased survival rate. Similarly, another study found that the application of 200 mg L^{-1} AsA to Garnem rootstock during acclimatization resulted in the highest survival rate (Ekinci et al. 2024). Ergin et al. (2014) reported that foliar application of AsA was highly effective in enhancing the growth of strawberry plants exposed to high temperature conditions. For instance, exogenous application of 3 mM AsA increased cell turgor in strawberry plants, thereby promoting their growth. Similarly, Ahmad et al. (2014) demonstrated that foliar or seed application of 20 and 40 mg L^{-1} AsA improved seedling development and yield production in maize under low-temperature stress. AsA is synthesized in the leaves, and its transport throughout the plant contributes to enhanced growth and improved physiological performance. During the adaptation to external conditions, exogenous application of AsA may help reinforce its endogenous synthesis in the leaves. Moreover, by promoting overall plant growth, AsA can positively influence survival rates (Parveen et al. 2025).

3.2. Plant Heigth (cm)

The data on plant height are presented in Table 2. Statistical analysis revealed a significant difference in plant height among treatments ($p \leq 0.05$). The longest shoots (18.40 cm) were obtained from 25 ppm GA_3 + 300 mg L^{-1} AsA, while the shortest plants (0.00 cm) occurred in control and 50 ppm GA_3, which was due to plant mortality in this treatment group, preventing measurements from being taken. The application of GA_3 at a low concentration in combination with AsA increased plant height. GA_3 plays a critical role in the morphological, physiological, and biochemical properties of plants. It promotes cell division, internode elongation, and shoot growth (Othman and Leskovar, 2022; Shah et al. 2023). AsA is a potent antioxidant compound that directly supports plant growth and development. It positively influences physiological development in plants by promoting cell division and elongation (Smirnoff and Wheeler, 2000; Çoban and Aras, 2022). Owing to these properties, the increase in plant height observed in this study is consistent with the elongation of internodes induced by AsA application. In a study on the vegetative growth of *Prunus amygdalus* var. Amara rootstock, the application of 300 mg L^{-1} AsA was found to significantly increase plant height (Al-Douri and Basheer, 2021). Similarly, in our study, the combinations of AsA and GA_3 were observed to positively influence vegetative growth.

3.3. Shoot Diameter (mm)

The data on shoot diameter are presented in Table 2. Statistical analysis showed a significant difference among treatments ($p \leq 0.05$). The greatest shoot diameter (1.88 mm; 1-82 mm) was recorded in 50 ppm GA_3+ 300 mg L^{-1} AsA; 25 ppm GA_3 + 300 mg L^{-1} AsA;, whereas the lowest (0.00 mm) occurred in control and 50 ppm GA_3, due to plant mortality in this treatment group, which prevented measurements from being taken. AsA is considered one of the most effective growth regulators in enhancing plant responses to abiotic stress (Conklin, 2001). Beyond its well-known antioxidant activity, cellular AsA levels are closely associated with the activation of complex biological defense mechanisms in plants (Conklin and Barth, 2004). In the study conducted by Farahat et al. (2013), foliar application of ascorbic acid to *Grevillea robusta* seedlings was reported to markedly enhance several vegetative growth traits, including plant height, stem diameter, leaf number, root development, and various biochemical constituents. The exogenous application of GA_3 at a low concentration

in combination with AsA increased shoot diameter. This effect can be attributed to the role of GA_3 and AsA in promoting cell division and cell expansion (Smirnoff and Wheeler, 2000; Shah et al. 2023). Our study showed that the combinations of GA_3 and AsA improved internode diameter, which may be attributed to the role of ascorbic acid in cell division and cell expansion (Smirnoff and Wheeler, 2000).

Table 2. The effects of GA_3 and AsA applications on survival rate, shoot length, and shoot diameter parameters.

Treatments	Survival Rate (%)	Plant Height (cm)	Shoot Diameter (mm)
Control	0.00±0.00b	0.00±0.00e	0.00±0.00c
25 ppm GA_3	50.00±0.00a	12.80±1.20bc	1.42±0.12ab
50 ppm GA_3	0.00±0.00b	0.00±0.00e	0.00±0.00c
150 mg L^{-1} AsA	50.00±0.00a	10.50±2.90cd	1.38±0.32ab
300 mg L^{-1} AsA	50.00±0.00a	8.37±0.61d	1.30±0.14ab
25 ppm GA_3 +150 mg L^{-1} AsA	44.44±9.62a	15.68±1.09ab	1.15±0.13ab
25 ppm GA_3 +300 mg L^{-1} AsA	51.33±8.08a	18.40±1.05a	1.88±0.51a
50 ppm GA_3 +150 mg L^{-1} AsA	44.44±9.62a	12.05±1.09bcd	0.84±0.06b
50 ppm GA_3 +300 mg L^{-1} AsA	43.33±11.55a	15.80±1.10ab	1.82±0.50a

Values are expressed as mean ± standard deviation. Different letters in each column indicate significant differences among treatments ($p \leq 0.05$).

3.3. Number of Leaves (pieces/plantlet)

The data on the number of leaves are presented in Table 3. Statistical analysis revealed a significant difference among treatments ($p \leq 0.05$). The highest leaf number (15.00 leaves/plant) was observed in 25 ppm GA_3 + 300 mg L^{-1} AsA; 50 ppm GA_3 + 300 mg L^{-1} AsA while the lowest leaf number (0.00 leaves/plant) was observed in control and 50 ppm GA_3 because plant losses prevented measurements in this treatment group. GA_3 plays a key role in critical stages of plant development, including leaf expansion, leaf initiation, leaf angle, leaf size, and leaf senescence (Li et al. 2013; Chen et al. 2014; Li et al. 2020; Ritonga et al. 2023). AsA also plays an important role in plant development by promoting cell division and expansion (Zhang et al. 2016). In this study, the combination of low concentrations of GA_3 and AsA is thought to have a synergistic effect, resulting in an increase in leaf number. Our findings are supported by previous research. It has been reported that AsA applications increased the number of leaves compared to the control in a study conducted on the 'Canino' apricot cultivar (El-Badawy, 2013). Similarly, Mayi et al. (2014) reported that treatments with ascorbic acid and humic acid substantially enhanced vegetative growth in olive seedlings. In their study, various concentrations of ascorbic acid (0–2000 mg L^{-1}) and humic acid (0–60 mg L^{-1}) were applied to the cultivars Khithairy and Sorany, and the results indicated that especially the application of 500 mg L^{-1} ascorbic acid led to notable improvements in plant height, leaf number, leaf area, and lateral shoot formation. These findings highlight the capacity of ascorbic acid to positively regulate growth-related physiological responses and improve overall plant vigor.

Table 3. The effects of GA_3 and AsA applications on the number of leaves, number of nodes, and SPAD parameters.

Treatments	Number of Leaves (pieces/plantlets)	Number of Nodes (pieces/plantlets)	SPAD
Control	0.00±0.00d	0.00±0.00d	0.00±0.00c
25 ppm GA_3	10.67±1.53bc	9.33±0.58bc	43.49±9.11a
50 ppm GA_3	0.00±0.00d	0.00±0.00d	0.00±0.00c
150 mg L^{-1} AsA	12.83±1.26ab	8.00±1.73bc	10.89±1.61b
300 mg L^{-1} AsA	13.00±1.00ab	7.67±1.15c	7.22±2.85bc
25 ppm GA_3 +150 mg L^{-1} AsA	14.00±2.65ab	11.33±2.08ab	7.03±3.67bc
25 ppm GA_3 +300 mg L^{-1} AsA	15.00±1.00a	13.00±1.00a	9.50±1.10bc
50 ppm GA_3 +150 mg L^{-1} AsA	7.50±1.32c	9.33±0.58bc	7.18±0.92bc
50 ppm GA_3 +300 mg L^{-1} AsA	15.00±1.00a	14.00±1.00a	16.83±1.01b

Values are expressed as mean ± standard deviation. Different letters in each column indicate significant differences among treatments ($p \leq 0.05$).

3.4. Number of Nodes (pieces/plantlets)

The data on the number of nodes are presented in Table 3. Statistical analysis showed a significant difference among treatments ($p \leq 0.05$). The highest node count (14 nodes per plant) was determined at 50 ppm GA_3 + 300 mg L^{-1} AsA, while the lowest node count (0.00 nodes per plant) was determined at control and 50 ppm GA_3. This is because the plant loss rate in this treatment group prevented measurements. Evaluation of the results revealed that combinations of 50 ppm GA_3 and AsA increased node number. GA_3 is an important phytohormone that affects internode length by promoting cell division and expansion. Studies have also reported that it increases plant tolerance to abiotic stresses by increasing antioxidant enzyme activities that scavenge harmful reactive oxygen species (ROS) (Shah et al. 2023). AsA positively affects plant vegetative development and has been reported to increase plant tolerance to abiotic stresses by enhancing oxidative defense potential (Akram et al. 2017). In this context, the combined application of AsA and GA_3 supported plant growth and increased the number of nodules, an indicator of plant development, in our study. The increase in nodule number is consistent with findings in the literature reporting the positive effects of AsA and GA_3 applications on plant vegetative development (Sajid and Aftab, 2009; Chowdhury et al. 2023; Ekinci et al. 2024).

3.5. Chlorophyll Content (SPAD)

The data on SPAD values are presented in Table 3. Statistical analysis revealed a significant difference among treatments ($p \leq 0.05$). The highest chlorophyll content (43.49) was recorded in 25 ppm GA_3, whereas the lowest (0.00) occurred in control and 50 ppm GA_3, due to plant mortality in this treatment group, which prevented measurements from being taken. The enhancement of SPAD values observed in this study can be explained by the well-known physiological mechanisms through which GA_3 and AsA influence chlorophyll biosynthesis and photosynthetic capacity. Exogenous GA_3 application has been reported to improve stomatal conductance, net photosynthetic rate, ion uptake, and hormonal balance, thereby promoting chlorophyll synthesis and increasing photosynthetic efficiency (Rady et al. 2021; Shah et al. 2023). GA_3 also stimulates cell elongation and chloroplast development, which can further support chlorophyll accumulation even under non-stress conditions (Iqbal and Ashraf, 2013). Similarly, ascorbic acid plays a critical role in protecting chloroplasts against oxidative damage. Halliwell (1987) reported that chloroplasts are rich in ascorbate, which serves as a key antioxidant. Exogenous AsA application has been shown to increase ascorbate levels within chloroplasts (Mozafar and Oertli, 1993), contributing to the maintenance of chloroplast membrane integrity and the prevention of chlorophyll degradation. Moreover, AsA participates in essential cellular processes such as cell metabolism, cell division, and cell expansion (Sabir et al. 2022), which may further support higher chlorophyll content in AsA-treated plants.

Therefore, the SPAD increase observed in this study can be attributed to these well-documented mechanisms: the ability of GA_3 to enhance chlorophyll biosynthesis and photosynthetic performance, and the role of AsA in stabilizing chlorophyll by protecting chloroplasts from oxidative deterioration. Siddiqui et al. (2020) reported that exogenous application of GA_3, either alone or in combination with melatonin, mitigated the negative effects of salinity stress on SPAD values in tomato seedlings. Similarly, El-Tohamy et al. (2023) reported that increasing concentrations of exogenous GA_3 application in gooseberry plants increased leaf chlorophyll content, which agrees with the findings of our study.

General Evaluation of Morphological and Physiological Responses

In this study, the effects of different GA_3 and AsA applications on the physiological and morphological responses of 'Nero' cultivar during acclimatization were evaluated through comprehensive analyses. The correlation matrix (Figure 2) showed strong positive relationships among the examined parameters, indicating that the characteristics related to plant growth progressed in a similar pattern during the acclimatization process. In particular, the high correlations observed between leaf number, shoot length, shoot diameter, and node number ($r > 0.90$) suggest that these parameters respond simultaneously to GA_3 and AsA treatments. This finding supports the idea that growth-promoting substances regulate multiple developmental processes in a coordinated manner. In contrast, the relatively low correlation between survival rate and other parameters

(r = 0.51) indicates that plant survival under ex situ conditions is influenced not only by morphological development but also by physiological and environmental factors.

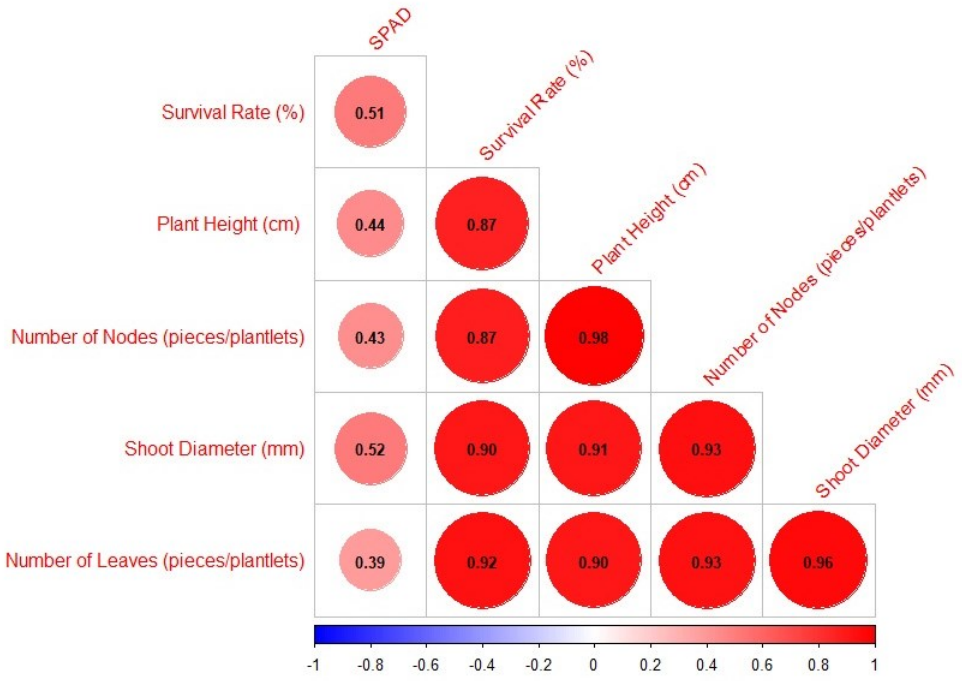

Figure 2. Correlation analysis of some morphological and physiological properties of *Aronia melanocarpa* cv. 'Nero' during the acclimatization phase. This figure shows the relationships among the evaluated parameters: Survival Rate, Shoot Length, Shoot Diameter, Number of Leaves, Number of Nodes, and SPAD (Soil Plant Analysis Development Value).

Hierarchical clustering analysis (Figure 3) evaluated the effects of treatments on plant performance by grouping them into distinct clusters. The treatments were classified into four main clusters (I–IV), while the parameters were grouped into clusters A–D. Notably, the groups receiving combined GA_3 and AsA applications—particularly 25 ppm GA_3 + 300 mg L^{-1} AsA and 50 ppm GA_3 + 300 mg L^{-1} AsA—exhibited upward trends in most parameters and were distinguished by positive effect patterns. In contrast, the treatment with 50 ppm GA_3 alone was placed in Cluster IV with negative values across all parameters, highlighting its adverse impact. This finding suggests that high-dose GA_3 applied alone may suppress physiological and morphological development. On the other hand, GA_3 doses applied in combination with AsA appear to enhance stress tolerance and overall growth performance.

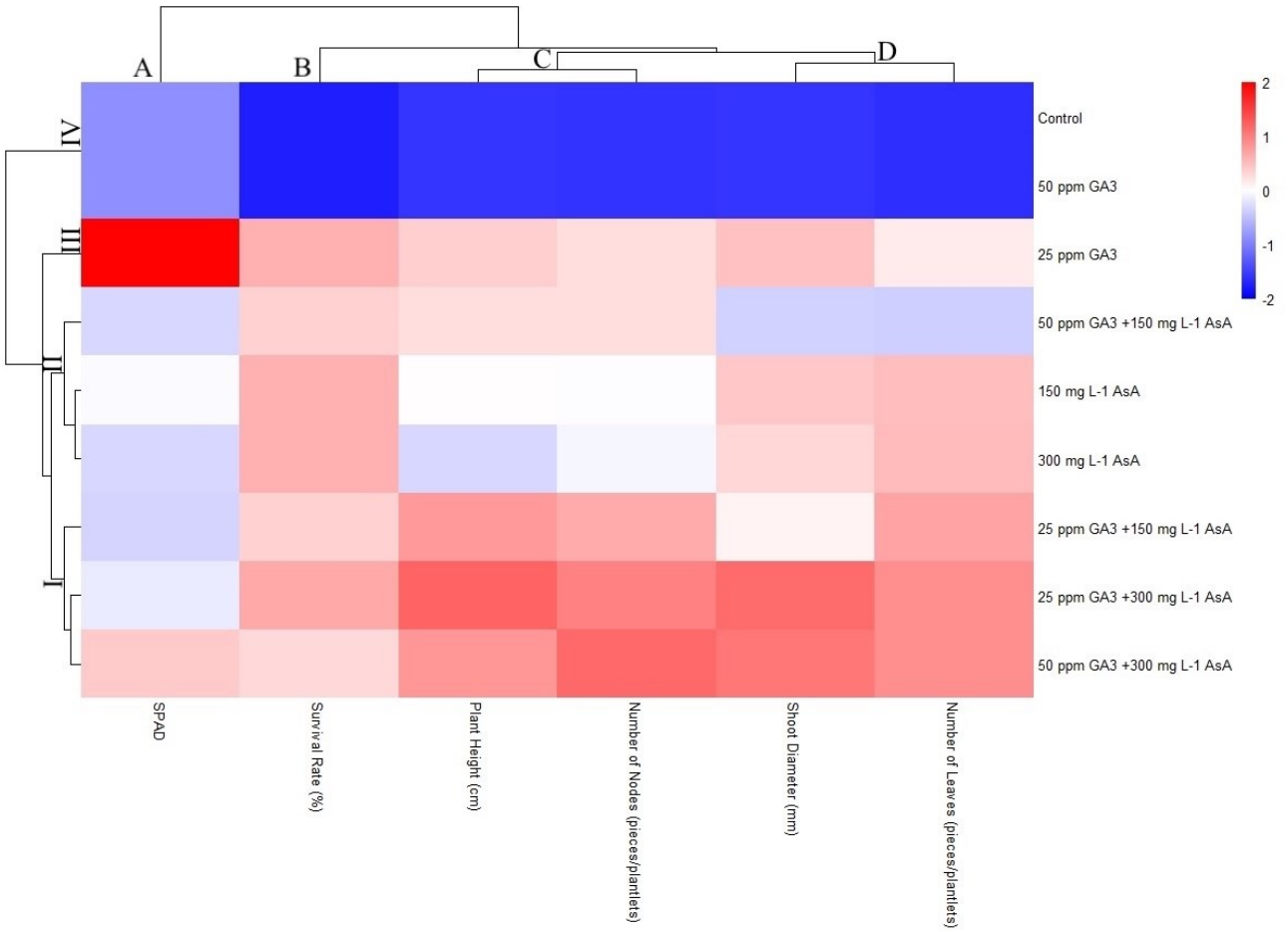

Figure 3. HCA for physiological and morphological parameters in *Aronia melanocarpa* cv. 'Nero' under different exogenous plant hormone treatments. Abbreviations: SPAD, GA_3, AsA. Treatments: hormone concentrations are given in ppm for GA_3 and mg L^{-1} for AsA.

Principal Component Analysis (PCA) results (Figure 4) illustrate the distribution of treatments and their associations with the measured parameters. The first two principal components (PC1 and PC2) explained 82.3% and 12.6% of the total variance, respectively. Treatments containing both GA_3 and AsA clustered together and were positioned in the same direction as most growth-related variables (shoot length, shoot diameter, number of nodes, and number of leaves), indicating their positive influence on vegetative development. In contrast, the control and 50 ppm GA_3 treatments were positioned opposite to these parameters, suggesting weaker growth performance. The 25 ppm GA_3 treatment was separated along the SPAD vector, showing its specific association with chlorophyll content. Overall, the PCA clearly demonstrates that the combined GA_3 + AsA treatments—especially 25 ppm GA_3 + 300 mg L^{-1} AsA—are strongly related to improved growth and physiological performance during acclimatization. The findings clearly demonstrate that the application of AsA in combination with GA_3 during the acclimatization stage exerts positive effects on the survival rate and vegetative growth of *Aronia melanocarpa* plantlets.

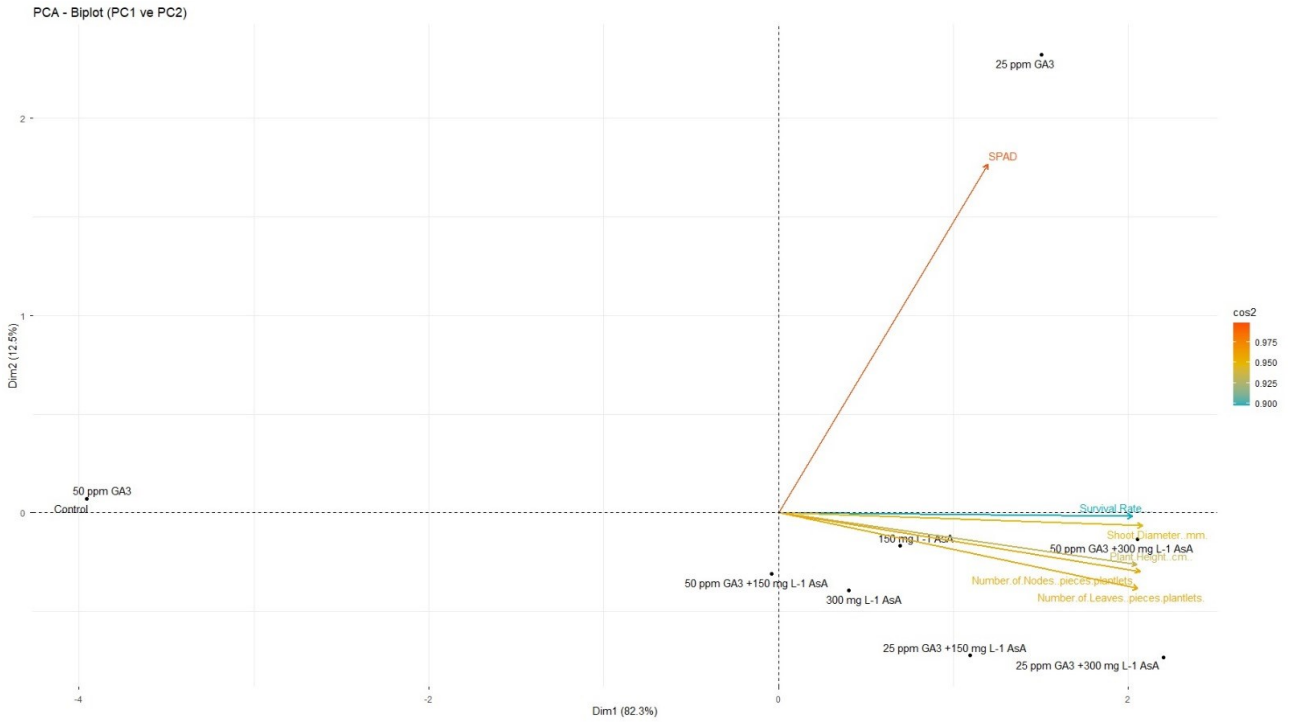

Figure 4. PCA biplot showing the relationship between different GA_3 and AsA combinations and morphological and physiological parameters in *Aronia melanocarpa* cv. 'Nero'.

4. Conclusions

The present study demonstrated that exogenous applications of GA_3 and AsA effectively influenced the acclimatization performance of *Aronia melanocarpa* cv. 'Nero' plantlets following *in vitro* micropropagation. The combination of 25 ppm GA_3 and 300 mg L^{-1} AsA resulted in the highest survival rate (51.33%) and promoted plant height, shoot diameter, leaf formation, and node development. These findings indicate that low GA_3 doses combined with high AsA concentrations can synergistically enhance plant growth and improve acclimatization success. During acclimatization, humidity hardening was implemented by gradually opening transparent covers placed over the plantlets to maintain high relative humidity, which facilitated their adaptation to external conditions. Despite this controlled transition, high GA_3 concentrations (50 ppm) negatively affected survival, likely due to excessive cell elongation and weakened tissue structure, increasing susceptibility to stress. In conclusion, the combined use of low-dose GA_3 and high-dose AsA is an effective approach to improving the physiological stability and growth performance of *Aronia melanocarpa* plantlets during acclimatization. These results provide a foundation for developing hormone-assisted acclimatization protocols for *in vitro* propagated plants under *ex vitro* conditions.

This study may serve as a model for improving acclimatization protocols in *Aronia* and other tissue-cultured plant species.

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