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Effect of Different Auxins on Root and Vegetative Development of *Aronia melanocarpa* cv. Nero Under *In Vitro* Conditions

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Abstract

Aronia is a berry-like fruit with high antioxidant content and can be propagated by generative and vegetative methods. Among vegetative propagation methods, plant tissue culture techniques are an important method due to their high propagation rate and the ability to produce disease and pest-free plant material. This study was conducted to determine the effects of different auxin types and concentrations used in the *in vitro* rooting stage of aronia plantlets on rooting and vegetative development. IBA and NAA at concentrations of 1 and 2 mg L^{-1}, and combinations of these concentrations, were applied to the culture medium during the rooting stage. Morphological parameters such as plant height, number of leaves, leaf area, rooting rate, root length, and number of roots were examined in the study. According to the results, the highest plant height was observed in the 2 mg L^{-1} IBA + 2 mg L^{-1} NAA application, and the highest number of leaves was observed in the 2 mg L^{-1} NAA and 1 mg L^{-1} NAA + 1 mg L^{-1} IBA applications. The highest rooting rate and number of roots were obtained with the 1 mg L^{-1} NAA + 1 mg L^{-1} IBA application, while the longest root was obtained with the 1 mg L^{-1} IBA application. In conclusion, it was determined that combinations of IBA and NAA are effective in increasing rooting success.

Keywords: Rooting, IBA, Micropropagation, NAA, Vegetative development

***In Vitro* Koşullarda *Aronia melanocarpa* cv. Nero'da Farklı Oksinlerin Kök ve Vejetatif Gelişime Etkisi**

Özet

Aronya, yüksek antioksidan içeriğine sahip üzümsü bir meyve olup generatif ve vejetatif yöntemlerle çoğaltılabilmektedir. Vejetatif çoğaltma yöntemleri arasında yer alan bitki doku kültürü teknikleri, yüksek çoğaltma oranı sağlaması ve hastalık ile zararlılardan ari bitkisel materyal üretimine olanak tanıması açısından önemli bir yöntemdir. Bu çalışma, aronya bitkiciklerinin *in vitro* köklenme aşamasında kullanılan farklı oksin tip ve konsantrasyonlarının köklenme ve vejetatif gelişim üzerine etkilerini belirlemek amacıyla yürütülmüştür. Köklenme aşamasında besi ortamına 1 ve 2 mg L^{-1} konsantrasyonlarında IBA ve NAA ile bu konsantrasyonların kombinasyonları uygulanmıştır. Araştırmada bitki boyu, yaprak sayısı, yaprak alanı, köklenme oranı, kök uzunluğu ve kök sayısı gibi morfolojik parametreler incelenmiştir. Sonuçlara göre en yüksek bitki boyu 2 mg L^{-1} IBA + 2 mg L^{-1} NAA uygulamasında, en fazla yaprak sayısı 2 mg L^{-1} NAA ile 1 mg L^{-1} NAA + 1 mg L^{-1} IBA uygulamalarında belirlenmiştir. En yüksek köklenme oranı ve kök sayısı 1 mg L^{-1} NAA + 1 mg L^{-1} IBA uygulamasında, en uzun kök ise 1 mg L^{-1} IBA uygulamasında elde edilmiştir. Sonuç olarak, IBA ve NAA kombinasyonlarının köklenme başarısını artırmada etkili olduğu belirlenmiştir.

Anahtar Kelimeler: Köklenme, IBA, Mikroçoğaltım, NAA, Vejetatif gelişim

Introduction

Aronia is a species belonging to the *Rosaceae* family and is native to eastern North America and Canada (Demirel et al., 2023). This fruit, rich in polyphenols and high antioxidant activity, is among the most preferred fruits today. It contains a high concentration of anthocyanins compared to other fruit types. In addition, aronia berries have a rich content of vitamins, minerals, and folic acid (Denev et al., 2019; Yılmaz et al., 2021).

Aronia melonocarpa is also used as an ornamental shrub due to its decorative color in the autumn. It is cultivated in Asian and European countries for both fruit production and as an ornamental plant (Hirvi and Honkanen, 1985; Sivanesan et al., 2016).

Aronia can be propagated by generative and vegetative methods. This species is easy to propagate from seed. However, plants obtained by this method bear fruit late, grow strongly but irregularly, and do not possess characteristics suitable for mechanical harvesting. Therefore, propagation by seed is not recommended in the cultivation of this fruit species (Litwińczuk, 2012). This species can also be propagated by vegetative methods such as cuttings, layering, and grafting. However, these methods may have some limitations, such as the inability to obtain a sufficient number of cuttings from the parent plants and the failure to ensure homogeneous development in the propagated plants. In addition, the fact that the parent plants from which cuttings are taken are generally old can lead to a decrease in shoot development and fruit yield; it can also pose a risk in terms of diseases and pests. For these reasons, the use of these propagation methods is preferred to a limited extent (Şirin et al., 2025).

Plant tissue culture techniques, a type of vegetative propagation, have emerged as one of the most

preferred modern propagation methods in recent years, compared to traditional methods. Plant tissue culture techniques allow for year-round production regardless of the vegetation period, provide virus, disease, and pest-free plant material, and enable the production of a large number of plants in a limited area, thus saving land use. Furthermore, they contribute to the conservation of endangered species and genetic resources, and the production of plant species and varieties that are difficult to propagate can also be achieved with this method. These features are among the important advantages of plant tissue culture techniques (George et al., 2008; Şaşkın et al., 2022).

Micropropagation, one of the plant tissue culture techniques, consists of the following basic stages: explant sterilization, explant culture, shoot propagation, rooting, and acclimatization (Nagini and Karthik, 2025).

Among these stages, the rooting stage is of great importance in ensuring the adaptation of the plantlets obtained under *in vitro* conditions to the external environment and in the healthy development of the plantlets. Auxin group plant growth regulators are used during the rooting stage to enable successful root formation of the plantlets. The most commonly used auxins include IAA (indole-3-acetic acid), IBA (indole-3-butyric acid), NAA (naphthalene acetic acid), and 2,4-D (2,4-dichlorophenoxyacetic acid) (Farah et al., 2025). Auxins promote cell elongation, cell division, and root formation. While low concentrations promote root formation, high concentrations can lead to callus formation in plantlets. Auxin types and concentrations vary depending on the purpose of plant tissue culture studies (Şaşkın et al., 2022).

This study was conducted to investigate the effects of different auxin types and concentrations used during the rooting stage of plantlets propagated by micropropagation *in vitro* on the root and vegetative development of *Aronia melanocarpa* cv. Nero.

Material and Method

Plant material

Aronia is a woody plant, a perennial shrub. The bark of one-year-old and older branches is gray-brown, while annual shoots are glossy reddish-brown. Oval-shaped, white lenticels are found on the branches and shoots. The leaves are oval-shaped, with finely serrated edges and a pointed tip. The upper side of the leaf is dark green, while the underside is light green and hairy. The flowering period varies depending on temperature and lasts approximately 20 days (Engin et al., 2018).

Method

This study was conducted in the plant tissue culture laboratory and climate chamber of the Department of Horticulture, Faculty of Agriculture, Harran University, between 2024 and 2025. The plant material used was *Aronia melanocarpa* cv. Nero plantlets propagated *in vitro* using micropropagation methods.In order to obtain the plantlets used in the study, shoots were first taken from plants grown in pots in the greenhouse in May, during their active growth period, and divided into micro cuttings in a laboratory environment. The micro cuttings were first soaked in soapy water for 10 minutes, then rinsed under tap water until completely clean, undergoing a pre-sterilization process. Following this process, for surface sterilization, the micro cuttings were first kept in 70% ethyl alcohol for 2 minutes, then in 10% NaClO (sodium hypochlorite) solution for 10 minutes in a sterile cabinet (vertical airflow), and then rinsed three times with sterile distilled water. The micro cuttings, after surface sterilization, were transferred to a shoot medium prepared using MS (Murashige and Skoog, 1962) culture medium solidified with 3% sucrose, 1 mg L^{-1} GA_3, and 8 g L^{-1} agar (Nas et al., 2023). The pH of all culture media was adjusted to 5.8. In addition, 1 ml of L^{-1} PPM (Plant Preservative Mixture) was added to the culture media to prevent bacterial contamination (Ak et al., 2021; Kara et al., 2022; Ekinci et al., 2024). To obtain a sufficient number of plantlets, the plantlets growing in the shoot medium were transferred to a shoot propagation medium prepared using MS culture medium solidified with 3% sucrose, 3 mg L^{-1} BAP, 1 mg L^{-1} kinetin, and 7 g L^{-1} agar (Almokar and Pırlak, 2018; Ekinci et al., 2024a). The study was designed using rooting media containing different auxin types and concentrations (Table 1; Figure 1).

Table 1. Experimental design consisting of different auxin types and concentrations

Çizelge 1. Farklı oksin tipleri ve konsantrasyonlarından oluşan deneme düzeni

	Treatments
T0	Control
T1	1 mg L^{-1} NAA
T2	1 mg L^{-1} IBA
T3	2 mg L^{-1} NAA
T4	2 mg L^{-1} IBA
T5	1 mg L^{-1} NAA + 1 mg L^{-1} IBA
T6	2 mg L^{-1} IBA + 2 mg L^{-1} NAA

The plantlets, transferred to rooting media, were kept in a growth chamber illuminated with white fluorescent lamps under 16:8 hour photoperiod conditions, 65-70% air relative humidity and 25±1°C for 4 weeks (Şaşkın et al., 2022).

Morphological measurements

Plant height: Plant height of the plantlets measured in centimeters (cm) using a ruler (Ekinci et al., 2025).

Number of Leaves: The number of leaves of each plantlet was counted individually and recorded as one per plantlet (Ekinci et al., 2025).

Leaf Area (cm^2): For leaf area calculation, scanned leaf images were analyzed via Photoshop Portable Sfx. Program (Yazar, 2025)

Rooting Rate (%): The rooting rate was expressed as a percentage (%), calculated by dividing the number of plants that successfully rooted in the rooting medium by the number of plants transferred to the treatment and multiplying by 100 (Şaşkın et al., 2026).

Root Length: Root lengths of the plantlets were measured in centimeters (cm) using a ruler. (Şaşkın et al., 2026).

Number of Roots: The number of root of each plantlet was counted individually and recorded as one per plantlet (Şaşkın et al., 2026).

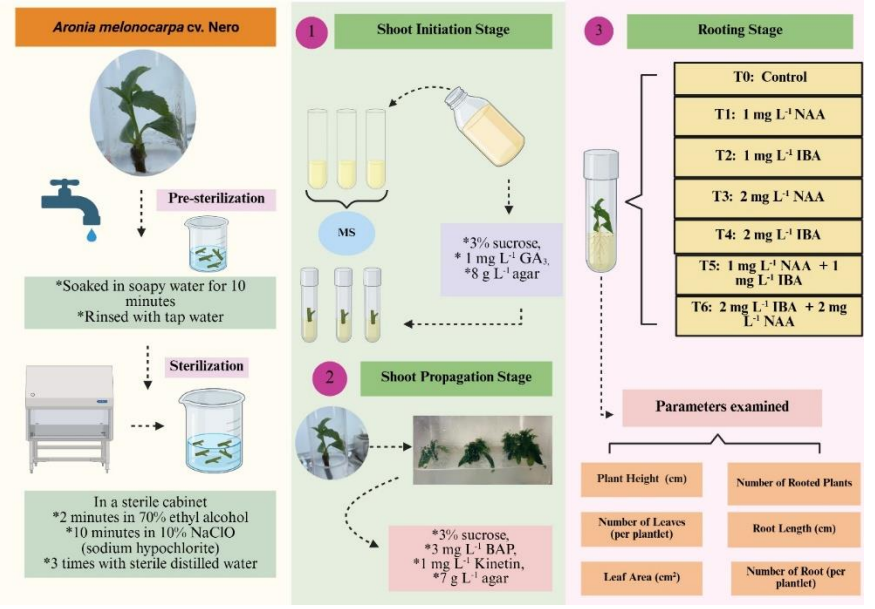

Figure 1. Schematic representation of the micropropagation steps of *Aronia melanocarpa* cv. 'Nero' plantlets *in vitro*, up to the rooting stage, involving different auxin types and concentrations.

Şekil 1. *Aronia melanocarpa* cv. 'Nero' bitkiciklerinin *in vitro* koşullarda, farklı oksin tip ve konsantrasyonlarını içeren köklendirme aşamasına kadar olan mikro çoğaltım basamaklarının şematik gösterimi.

Statistical Analysis

The study was set up according to a randomized plot design with 3 replications, each containing 10 plantlets. The study was terminated after 4 weeks. The data obtained from the study were subjected to one-way analysis of variance using the JMP Pro 13 statistical program according to the randomized plot design and compared using the Tukey multiple comparison test at a significance level of $p \leq 0.05$. Hierarchical clustering analysis (HCA) was conducted via the Software R (Version 4.1.1, R Foundation for Statistical Computing, Vienna, Austria).

Results and Discussion

Plant height (cm)

The effects of different auxin types and concentrations applied to *Aronia melonocarpa* cv. Nero plantlets during the rooting stage on plant height were investigated. A statistically significant difference was found between the applications ($p \leq 0.05$). The highest plant height was observed in the T6 application (8.87 cm), and the lowest plant height was observed in the T1 application (2.75 cm) (Table 2).

The T6 application, containing a high concentration (2 mg L^{-1}) of IBA and NAA combination, produced the longest plantlets. *In vitro*, auxin types such as IBA and NAA are commonly used to promote root formation during the rooting stage. However, the effects of these auxins on the plant can vary depending on the concentration used, genotype, and environmental conditions (Jakhar and Choudhary, 2023; Mohusien et al., 2025). Previous studies on the *in vitro* propagation of aronia plants have yielded similar results to our research findings. The

literature reports that different IBA concentrations used in the rooting medium significantly affect plant development, and the highest shoot/plant length is obtained in nutrient media containing 2 mg L^{-1} IBA (Polat and Eskimez, 2022; Almokar and Pırlak, 2018).

Table 2. Effects of different auxin types and concentrations on plant height, number of leaves, and leaf area parameters
Çizelge 2. Farklı oksin tipleri ve konsantrasyonlarının bitki boyu, yaprak sayısı ve yaprak alanı parametreleri üzerindeki etkileri

Treat-ments	Plant Height (cm)	Number of Leaves (per plantlet)	Leaf Area (cm^2)
T0	2.75±0.50^{d}	11.67±1.26^{c}	1.63±0.08^{ab}
T1	4.70±0.92^{b-d}	14.67±2.52^{bc}	1.62±0.47^{ab}
T2	3.83±0.38^{cd}	19.17±0.76^{a}	1.29±0.17^{b}
T3	7.70±1.61^{ab}	19.50±0.50^{a}	1.49±0.12^{b}
T4	5.23±2.15^{b-d}	16.17±1.26^{ab}	1.48±0.22^{b}
T5	6.57±0.93^{a-c}	19.50±0.50^{a}	2.10±0.01^{a}
T6	8.87±0.66^{a}	19.00±1.32^{a}	1.41±0.15^{b}

Number of leaves (per plantlet)
The effects of different auxin types and concentrations applied to *Aronia melonocarpa* cv. Nero plantlets during the rooting stage on number of leaves were investigated. A statistically significant difference was found between the applications ($p \leq 0.05$). The highest number of leaves occurred in applications T3 and T5 (19.50 per plantlet), while the lowest number of leaves occurred in application T0 (11.67 per plantlet) (Table 2). Auxins belong to the group of plant growth regulators that promote cell division and cell elongation (Şaşkın et al., 2022). Accordingly, the fundamental functions of auxins are supported by the morphological findings obtained in our study. In the study, the highest number of leaves occurred with a high concentration of NAA (2 mg L^{-1}) and with a combination of low concentration (1 mg L^{-1}) NAA and IBA. The effect of NAA on leaf formation has also been reported in the literature for different plant species. In particular, studies where NAA is applied in combination with cytokinins such as GA_3 and BAP have reported a significant increase in number of leaves. In a study conducted by Kumlay (2014), it was reported that in culture media containing GA_3-supplemented NAA, IAA, and IBA in three different potato varieties under *in vitro* conditions, the highest number of leaves occurred in culture media containing 0.25 mg L^{-1} GA_3 + 1 mg L^{-1} NAA and 0.25 mg L^{-1} GA_3 + 1 mg L^{-1} IAA. In another study, the effects of NAA applied at different concentrations on the development and number of leaves of *Dendrobium* orchid plantlets after 20, 40, and 60 days were examined. According to the findings, the highest number of leaves was reported to have occurred in nutrient media containing 0.1 and 0.2 mg L^{-1} NAA in measurements taken after 60 days (Parvin et al., 2009). In the study conducted by Nazir et al. (2022), it was reported that the highest number of leaves occurred in MS nutrient medium containing 2 mg L^{-1} BAP + 1 mg L^{-1} NAA in the *in vitro* regeneration of *Valeriana jatamansi* Jones plant.

Leaf area (cm^2)
The effects of different auxin types and concentrations applied to *Aronia melonocarpa* cv. Nero plantlets during the rooting stage on leaf area were investigated. A statistically significant difference was found between the applications ($p \leq 0.05$). The largest leaf area was observed in the T5 application (2.10 cm^2), and the smallest leaf area was observed in the T2 application (1.29 cm^2) (Table 2). Lakho et al. (2023) investigated the rooting ability of pineapple plants under *in vitro* conditions. The medium containing 1 mg L^{-1} IBA resulted in a higher leaf length compared to the control and other PGR applications. The largest leaf area was obtained from the 1 mg L^{-1} IBA + 1 mg L^{-1} NAA combination in our study. Determining the optimal growth regulator is important for successful tissue culture (Mansseri-Lamrioui et al., 2011). The type and concentration of auxin used vary depending on the species, variety, and even genotype. In T5 application, number of roots showed a positive correlation with leaf area. This suggests that root development supports shoot development by increasing the plant's water and nutrient uptake capacity. It is assessed that the increased root number contributes to the expansion of the photosynthetic surface area, especially by optimizing water and mineral transport, and that this is directly related to the increase in leaf area (Rahayu et al., 2005; Hermans et al., 2006; Sakakibara and Hirose, 2006; Puig et al., 2012).

Rooting rate (%)
The effects of different auxin types and concentrations applied to *Aronia melonocarpa* cv. Nero plantlets during the rooting stage on the rooting rate were investigated. A statistically significant difference was found between the applications ($p \leq 0.05$). The highest rooting rate occurred in the T5 (66.66%) application, and the lowest rooting rate occurred in the T0 (40.00%) application (Table 3). Rooting is one of the most critical stages of plant tissue culture techniques under *in vitro* conditions. Well-rooted plantlets directly affect the increased survival rate during the acclimatization process (Shukla et al., 2020; Lawson et al., 2023). Auxins, which are plant growth regulators, play an important role in the rooting stage. NAA, IAA, and IBA are the most commonly used auxins in promoting the growth and development of the root system (Poniewozik et al.,

2021). While auxins applied at low concentrations promote root formation, high concentrations lead to callus formation (Ekinci et al., 2025a). In our study, the combination of NAA and IBA applied at a low concentration (1 mg L^{-1}) resulted in the highest rooting rate. The literature shows that low concentrations of IBA and NAA result in high rooting rates, which supports the findings of our study. In a study by Yaman et al. (2025), it was reported that a combination of 0.5 mg L^{-1} NAA + 0.25 mg L^{-1} IBA resulted in 100% rooting in *Aronia melanocarpa* var. Nero under *in vitro* conditions**.**

Table 3. Effects of different auxin types and concentrations on rooting rate, root length, and number of roots parameters
Çizelge 3. Farklı oksin tipleri ve konsantrasyonlarının köklenme oranı, kök uzunluğu ve kök sayısı parametreleri üzerindeki etkileri

Treat-ments	Rooting Rate (%)	Root Length (cm)	Number of Roots (per plantlet)
T0	40.00±0.00^{c}	1.85±0.13^{c}	2.50±0.50^{c}
T1	60.00±0.00^{a}	5.08±0.49^{b}	12.00±4.00^{a}
T2	60.00±0.00^{a}	6.92±0.37^{a}	11.00±1.00^{ab}
T3	47.73±1.96^{b}	5.02±0.60^{b}	12.33±2.08^{a}
T4	60.00±0.00^{a}	6.10±0.70^{ab}	7.00±1.00^{bc}
T5	66.66±6.67^{a}	6.12±0.80^{ab}	12.83±0.76^{a}
T6	60.00±0.00^{a}	5.12±0.55^{b}	12.50±0.50^{a}

Root length (cm)
The effects of different auxin types and concentrations applied to *Aronia melonocarpa* cv. Nero plantlets during the rooting stage on root length were investigated. A statistically significant difference was found between the applications ($p \leq 0.05$). The longest root length occurred in the T2 application (6.92 cm), and the shortest root length occurred in the T0 application (1.85 cm) (Table 3). In our study, it was determined that IBA applied at a low concentration (1 mg L^{-1}) resulted in the longest root formation. This effect is thought to be related to IBA stimulating the cellular division and elongation processes necessary for adventitious root development. Auxins regulate lateral root growth and development. IBA is a synthetic auxin widely used to promote root formation and accelerate root emergence (Estrella-Maldonado et al., 2022). Findings reported in the literature are consistent with the results obtained in our study. In a study by Güney (2019), it was reported that the longest roots were obtained from the combination of 1.0 mg L^{-1} IBA and 0.5 mg L^{-1} NAA applied to Myrobalan 29C rootstock during the rooting stage under *in vitro* conditions. In the study where potatoes were rooted under *in vitro* conditions, the fact that the application of 1 ml IBA resulted in the maximum root length supports our study (Jabeen et al., 2021).

Number of roots (per plantlet)
The effects of different auxin types and concentrations applied to *Aronia melonocarpa* cv. Nero plantlets during the rooting stage on number of roots were investigated. A statistically significant difference was found between the applications ($p \leq 0.05$). The highest number of roots occurred in the T5 application (12.83 per plantlet), and the lowest number of roots occurred in the T0 application (2.50 per plantlet) (Table 3). The highest number of roots obtained in the T5 application where low concentrations of IBA and NAA were applied in combination indicates that the combined use of these auxins promotes root formation. Findings reported in the literature are consistent with the results obtained in our study. In the study conducted by Yaman et al. (2025), it was reported that the highest number of roots in *Aronia melanocarpa* var. Nero *in vitro* was obtained from the combination of 0.5 mg L^{-1} NAA + 0.25 mg L^{-1} IBA, and this result demonstrated the synergistic effect of this combination of auxins on root formation and proliferation.

Hierarchical clustering analysis (HCA)
In this study, hierarchical clustering analysis was performed to determine the similarities and differences between treatments based on parameters such as plant height, number of leaves, leaf area, rooting rate, root length, and number of roots measured during the rooting stage. The dendrogram obtained from the HCA showed that the treatments were grouped according to their similarity levels in terms of growth and rooting parameters (Figure 2). The hierarchical clustering analysis revealed that the treatments generally clustered under two main groups. The first group included treatments showing lower values in terms of vegetative growth and rooting parameters, while the second group included treatments showing higher performance in terms of rooting and vegetative growth. The control treatment (T0), due to its low growth parameters, was separated from the other treatments and placed in a separate subgroup or clustered in the same group as low-performing treatments. This indicates that plant growth and rooting are more limited in the auxin-free medium. It was determined that treatments T1, T2, T3, and T4, which contain single auxin treatments, generally clustered in the same groups as treatments showing moderate growth. Among these treatments, applications containing IBA were found to be more closely associated with root length, whereas applications containing NAA were more strongly related to the number of leaves and the number of roots. It was determined that the combined auxin applications T5 (1 mg L^{-1} NAA + 1 mg L^{-1} IBA) and T6 (2 mg L^{-1} NAA + 2 mg L^{-1} IBA) were in the same cluster and showed similar and

high performance in terms of general plant development and rooting parameters. This indicates that the combined application of auxins creates a synergistic effect on plant development and rooting. In particular, the T5 application was more strongly correlated with rooting rate and number of roots, while the T6 application was more strongly correlated with plant height. Overall, the results of the hierarchical clustering analysis support the results obtained from the analysis of variance and morphological measurements, revealing that auxin combinations yield more successful results in terms of both rooting and vegetative development compared to single auxin applications. Clustering analysis clearly demonstrated that combinations of IBA and NAA were the most effective treatments for *in vitro* rooting and vegetative development of *Aronia melanocarpa* cv. Nero plantlets.

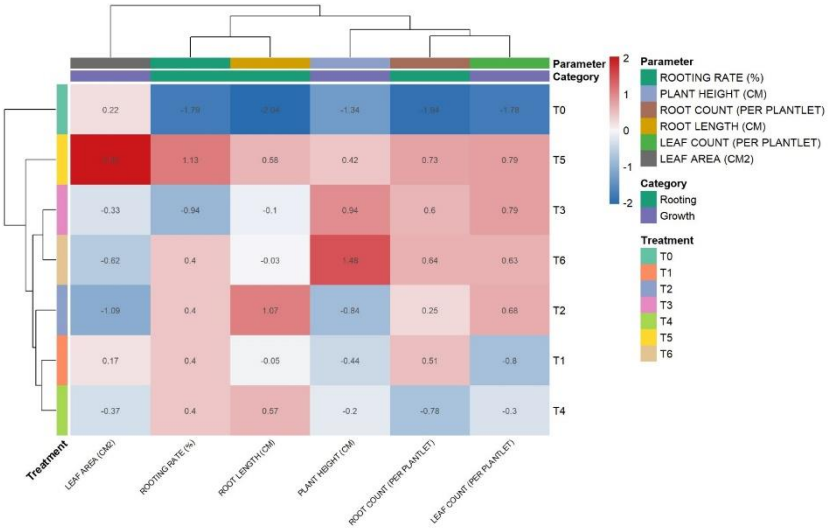

Figure 2. HCA graph showing the effects of different auxin types and concentrations on root and vegetative growth parameters in *Aronia melanocarpa* cv. Nero under *in vitro* conditions.
Şekil 2. *Aronia melanocarpa* cv. Nero'da *in vitro* koşullar altında farklı oksin tipleri ve konsantrasyonlarının kök ve vejetatif büyüme parametreleri üzerindeki etkilerini gösteren HCA grafiği.

Conclusion

This study revealed the effects of different auxin types and concentrations on root and vegetative development of *Aronia melanocarpa* cv. Nero plantlets during *in vitro* rooting. The study findings showed that the type and concentration of auxin used had a significant and decisive effect on both rooting success and vegetative development parameters. According to the findings, the high concentration (2 mg L^{-1}) combination of IBA and NAA (T6) yielded the best results in terms of plant height, while both high concentration NAA (T3) and low concentration combination of IBA and NAA (T5) applications were superior in terms of number of leaves. The T5 application significantly increased leaf area, and this increase is thought to be due to improved water and nutrient uptake provided by root development. When rooting parameters were evaluated, it was determined that the low concentration (1 mg L^{-1}) combination of IBA + NAA (T5) was the application that produced positive results in terms of both rooting rate and number of roots. In contrast, the longest root development was obtained with the 1 mg L^{-1} IBA application (T2). Overall, it was determined that the combined application of IBA and NAA, especially at low concentrations, showed a synergistic effect in increasing rooting success. In conclusion, the findings of this study are guiding not only for optimizing the *in vitro* rooting stage of *Aronia melanocarpa* cv. Nero, but also for developing micropropagation protocols in the future. Further studies, particularly considering the positive effects of low-concentration IBA and NAA combinations, are expected to contribute to improved acclimatization success, long-term growth performance, and commercial production efficiency.

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