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Determination of Shoot Regeneration Protocol in Aronia (*Aronia melanocarpa* (Michx.) Elliott) with New Generation Temporary Immersion Bioreactor System

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ABSTRACT

Aronia (*Aronia melanocarpa*) is an industrially important species belonging to the *Rosaceae* family. Among the berries, its importance has been increasing in recent years, and it is called a "super fruit" due to its effects on health. The purpose of this study was to compare the *in vitro* propagation of the 'Viking' aronia cultivar via the PlantForm bioreactor system, known as the new generation, with that via the solid culture propagation method, a traditional tissue culture approach. MS nutrient medium and cytokinin (BAP, KIN) sources (1.0, 2.0, and 4.0 mgL^{-1}) were used in solid culture micropropagation and PlantForm bioreactor system trials. At the end of each subculture, the number of shoots (pcs/plantlet), plant height (cm), plant diameter (mm), number of leaves (pcs), plant fresh and dry weight (g) data, rooting rate (%), root number (pcs), root length (mm), plant height (cm), and root fresh and dry weight (g) data were examined in the micropropagation experiments in both systems. In terms of shoot vitality, the media containing KIN gave better results with 100% vitality rates in both systems. The PlantForm system yielded better plant height and fresh and dry weight results than the solid culture system. In fact, the highest value of 4.12 cm in plant height was obtained from MS+4.0 mgL^{-1} KIN+0.01 mgL^{-1} IAA medium. The best values in plant fresh and dry weight were determined in MS+2.0 mgL^{-1} BAP+0.01 mgL^{-1} IAA medium in the PlantForm system. In this study, the PlantForm bioreactor system was more effective for micropropagation than solid culture media.

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ÖZET

Aronya (*Aronia melanocarpa*), *Rosaceae* familyasına ait endüstriyel açıdan önemli bir türdür. Son yıllarda meyveler arasında önemi artmakta olup, sağlık üzerindeki etkileri nedeniyle "süper meyve" olarak adlandırılmaktadır. Bu çalışmanın amacı, 'Viking' aronya çeşidinin *in vitro* çoğaltımı, yeni nesil olarak bilinen PlantForm biyoreaktör sistemi ile geleneksel bir doku kültürü yaklaşımı olan katı kültür çoğaltım yöntemiyle karşılaştırmaktır. Katı kültür mikroçoğaltımı ve PlantForm biyoreaktör sistemi denemelerinde MS besin ortamı ve sitokinin (BAP, KIN) kaynakları (1.0, 2.0 ve 4.0 mgL^{-1}) kullanılmıştır. Her alt kültür dönemi sonunda her iki sistemde yapılan mikroçoğaltım deneylerinde sürgün sayısı (adet/fide), bitki boyu (cm), bitki çapı (mm), yaprak sayısı (adet), bitkinin yaş ve kuru ağırlığı (g), köklenme oranı (%), kök sayısı (adet), kök uzunluğu (mm), bitki boyu (cm) ve kökün yaş ve kuru ağırlığı (g) gibi veriler incelenmiştir. Sürgün canlılığında her iki ortamda da %100 canlılık oranları ile KIN kullanılan ortamlar daha iyi sonuç vermiştir. PlantForm sistemi, bitki boyu ile yaş ve kuru ağırlık açısından katı kültür sistemine kıyasla daha iyi sonuçlar vermiştir. Nitekim bitki boyunda 4.12cm ile en fazla değer MS+4.0 mgL^{-1} KIN+0.01 mgL^{-1} IAA

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PlantForm

ortamından elde edilmiştir. Bitki yaş ve kuru ağırlığında da en iyi değerler PlantForm sisteminde MS+2.0 mgL^{-1} BAP+0.01 mgL^{-1} IAA ortamında tespit edilmiştir. Bu çalışma, mikroçoğaltım açısından PlantForm biyoreaktör sisteminin katı kültür ortamına kıyasla daha etkili olduğunu ortaya koymuştur.

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INTRODUCTION

Plant tissue culture is the "*in vitro* culture" of plant cells, tissues, organs, seeds, protoplasts, or embryos on a nutrient medium under aseptic conditions. This involves providing an ideal controlled growth environment that includes temperature, photoperiod, humidity, light, and all medium components (Bridgen et al., 2018). Tissue culture techniques, referred to as "*in vitro* culture" in horticultural plants, are considered effective methods for determining the genetic homogeneity of plants (Hwang et al., 2022). In the *in vitro* clonal propagation of horticultural plants, providing all the necessary nutrients, energy, and water for plant or explant growth through basal media is achieved with inorganic and organic nutritional supplements. It has many benefits, such as obtaining disease-free new plants, facilitating breeding efforts, and preserving genetic resources. Although plant propagation in tissue culture is costly, more plants can be produced in a shorter time when labor-saving automation techniques are applied. (Mansuroglu, 2001). Although tissue culture has many advantages, there are several limiting factors, such as intensive labour, vitrification, the need for a large number of culture vessels, and semisolid to solid nutrient media (Rathore et al., 2004; Berthouly & Etienne, 2005). Additionally, in semisolid and solid media, there are issues related to the uneven distribution of plant growth regulators in the nutrient medium and problems arising from different degrees of sensitivity depending on the brands of agar used in the cultured tissues (Gupta & Prasad, 2006; Karakoyun et al., 2023).

In solid culture systems, subculturing is necessary for continuity in developing explants on solid nutrient media. This situation also increases the risk of contamination (Umarusman et al., 2020). Although the propagation of plants in tissue culture can be costly, when automation techniques that reduce labour are employed, it becomes possible to produce more plants in a shorter period. The cost of gelling agents such as phytagel, agarose, alginate, and agar used in the *in vitro* propagation of plants is the highest in solid cultures (Quiala et al., 2012). Therefore, liquid culture systems have been developed as alternatives to semisolid and solid media, thus making cost-effective micropropagation possible (Welander et al., 2016). However, in these liquid culture systems, the continuous exposure of plants to a liquid medium can cause them to be damaged or even die due to a lack of oxygen (Maurizio et al., 2015). Therefore, in the micropropagation of plants, temporary immersion systems (TIS), which eliminate the disadvantages of semi-solid, solid, and liquid culture media and have superior qualities compared to gelling agents, allow both more cost-effective *in vitro* propagation and the elimination of several limiting factors (Welander et al., 2016). Bioreactor systems, temporary immersion systems, are designed to improve quality in *in vitro* cultures and have many advantages. The micropropagation of plants with a bioreactor system ensures air renewal inside the culture containers through aeration, which occurs within the culture vessels. Thus, both the medium that provides nutrients to the plant and the air flow come into contact with the plant, transferring nutrients to the plant's tissues without causing harm (Chen et al., 2001; Chen et al., 2003).

Aronia (*Aronia melanocarpa* (Michx.) Elliott), which has become increasingly important among berries in recent years, is called a "superfruit" and contains all vitamins except vitamin B12 and vitamin D. Currently, the cultivation of aronia is increasing due to its health benefits. Aronia, which is accepted as a valuable medicinal plant due to its polyphenols and anthocyanins, is grown as an important industrial product (Denev et al., 2019; Şahin & Erdoğan, 2022). Moreover, aronia, with its shrubby form, is a highly attractive species due to its leaves turning red in the fall. Therefore, cultivation as an ornamental plant, in addition to being grown for its fruit, has been practiced since the 1940s and still holds potential (Onay et al., 2012). The most widely cultivated aronia varieties in the world are 'Nero' and 'Viking'. 'Viking' is a highly vigorous variety that can grow up to 2.0 meters tall. It is highly productive. It has been determined that in the conditions of Yalova province of Türkiye, the 'Viking' aronia variety outperforms the 'Nero' variety in terms of first flowering and fruit set (Poyraz Engin & Mert, 2024). The establishment of orchards is becoming more common with the development of the berry fruit industry, leading to an increasing demand for saplings. With the growing demand for saplings, *in vitro* micropropagation methods that provide easy and economical propagation and virus-free, clean, and uniform sapling production have gained importance. The *in vitro* micropropagation technique has been used successfully for commercial clonal propagation of many plants (Onay et al., 2012). Unlike traditional *in vitro* micropropagation methods, temporary immersion bioreactor systems are both economical and fast, and easy to micropropagate. It is also a system designed to improve the quality of *in vitro* cultures. The temporary immersion bioreactor system has many advantages, including transparency, autoclavability, ease of carrying, gas exchange, and the presence of an air pump and time controls, making it different from other systems (Aragón et al., 2010; Welander et al., 2014; Martínez et al., 2019).

The temporary immersion bioreactor system was tested in this study for the *in vitro* micropropagation and rooting of the 'Viking' aronia variety, one of the berry fruits increasingly gaining popularity in the country. This study aimed to provide an alternative propagation strategy for *in vitro* culture to traditional solid nutrient media-based micropropagation and rooting approaches. This approach might contribute to the future as an economic and quick propagation strategy for the commercial production of saplings of diverse species.

MATERIAL and METHOD

Materials

In this research, the 'Viking' variety of aronia was utilized. The explants used in the study were obtained by micropropagation at the Selçuk University Department of Horticulture Tissue Culture Laboratory. In the stages of micropropagation and rooting, a solid medium and a new generation temporary immersion bioreactor system were used throughout the culture. The temporary immersion bioreactor system used is the PlantForm brand and has a 12-culture container (Figure 1) (Anonymous, 2024).

Figure 1. PlantForm Temporary Immersion System (Anonymous, 2024)
Şekil 1. PlantForm Geçici Daldırma Sistemi (Anonim, 2024)

Method

Transferring Shoots to Micropropagation Medium

In the study, micro-cuttings of the selected 'Viking' aronia cultivar were collected in the spring, when the plants began to emerge from dormancy. *In vitro* clonal propagation was carried out under sterile conditions in the tissue culture laboratory using shoot tip culture. The micro-cuttings taken were subjected to surface sterilization, firstly soaked in 70% ethanol for 2min and then soaked in a 15% (v/v) NaOCl (Sodium hypochlorite) solution with a few drops of Tween 20 for 10min, followed by sterile distilled water washed three times (Ozelçi & Yigit, 2022). After surface sterilization, the micro-cuttings for shoot development were transferred to basal MS medium (Murashige & Skoog, 1962), supplemented with 1 mgL^{-1} GA$_3$ (Gibberellic acid) and 3% sucrose, with the pH adjusted to 5.78. Shoots obtained under *in vitro* conditions were used for the study when they reached approximately 1 cm in length. The micropropagation of shoots from micro-cuttings was carried out in solid culture and via the PlantForm bioreactor system. In the micropropagation stage, two different cytokinins (BAP (6-Benzylaminopurine) and KIN (Kinetin)) were added at three different concentrations of 1.0, 2.0, and 4.0 mgL^{-1} along with 0.01 mgL^{-1} IAA (Indole 3-acetic acid) to the medium (Figure 2). According to these combinations, the study was planned as seven applications as follows.

1. Control (MS media)
2. MS media + 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA
3. MS media + 2.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA
4. MS media + 4.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA
5. MS media + 1.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA
6. MS media + 2.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA
7. MS media + 4.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA

Preparation of the Rooting Medium

After enough plantlets were obtained (after approximately 40-45 days), the plantlets were transferred to rooting

MS medium containing three different IBA concentrations of 1.0, 2.0, and 3.0 mgL^{-1}. The plantlets were transferred separately to rooting media for both solid medium and bioreactors. In both systems used in the study, the MS nutrient medium containing 3% sucrose was used during the rooting stages. When 7.4 gL^{-1} agar was added to the solid culture medium, it was not added to the PlantForm culture medium. The pH of all the nutrient media used in both systems' rooting treatments was adjusted to 5.78.

In the PlantForm bioreactor system, each culture container represented one treatment in the bioreactor, and 500 mL of liquid nutrient medium was added to each culture container. Each culture container, during the micropropagation stage, 15 shoots were transferred to while 50 plantlets were transferred during the rooting stage. During the micropropagation and rooting stages, the temporary immersion system was programmed to immerse for 15 minutes every 4 hours and aerate for 15 minutes every 4 hours (Aka Kaçar et al., 2020). This study planned a solid medium application with three replications, each replication consisting of five magenta boxes and each magenta box containing 5 magenta plants. 40 ml of solid nutrient medium was added to the magenta vessels. All solid culture media and bioreactors were autoclaved at 1.05 kgcm^{-2} and 121°C for 15min. The plantlets were cultivated under 16 hours of light, 8 hours of dark photoperiod illuminated by a cool-white, fluorescent light (50 µmol m^{-2}s^{-1}) and 25±1°C temperature during both systems' *in vitro* growth stages.

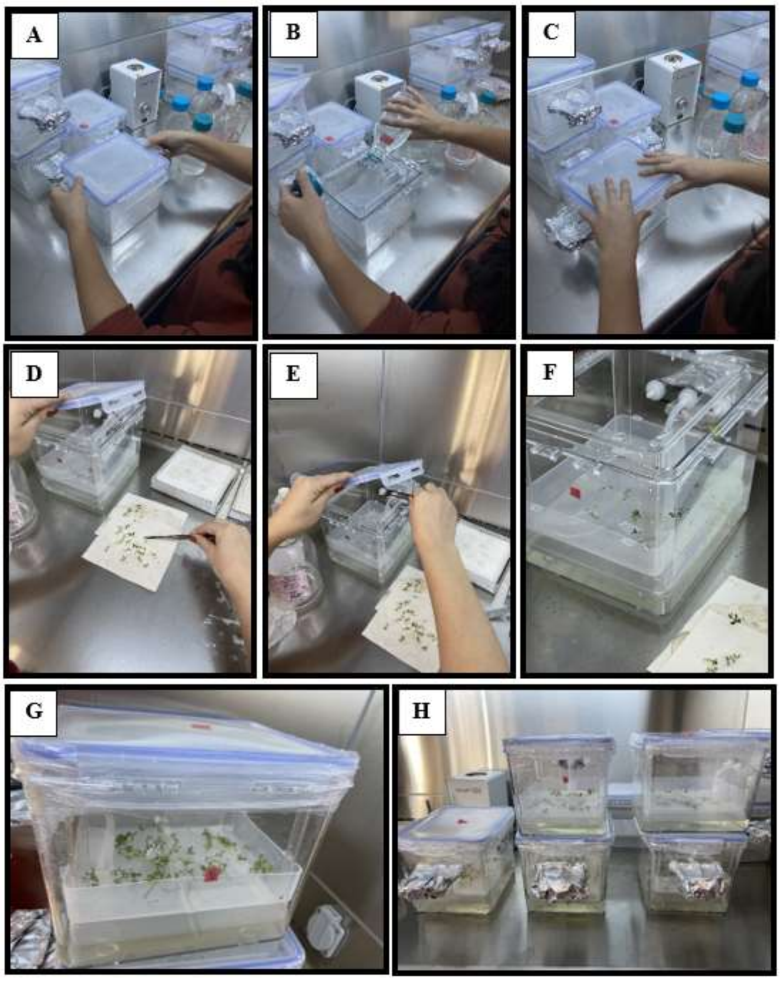


Figure 2. Transferring Shoots to Propagation Media in PlantForm Bioreactors, A, B, C: Placing the prepared propagation media into bioreactors D, E, F: Placing the prepared shoots into the bioreactors G, H: Plantlets transferred to the propagation media in bioreactors I, J: Plantlets propagated in the propagation media in the PlantForm bioreactor

Şekil 2. PlantForm Biyoreaktörlerinde Sürgünlerin Çoğaltım Ortamına Aktarılması, A, B, C: Hazırlanan çoğaltım ortamının biyoreaktörlere yerleştirilmesi D, E, F: Hazırlanan sürgünlerin biyoreaktörlere yerleştirilmesi, G, H: Biyoreaktörlerde çoğaltım ortamına aktarılan bitkiler, I, J: PlantForm biyoreaktöründe çoğaltım ortamında gelişen bitkicikler

Measurements and Analyses Conducted on Plants

In the solid culture and PlantForm bioreactor systems, shoot vitality (%), number of shoots per explant (pieces/plantlet), plant height (cm), plant diameter (mm), number of leaves (pieces), and plant fresh and dry weight

(g) were measured after each subculture. In the rooting experiments, parameters such as rooting percentage (%), number of roots (pieces), root length (cm), and root fresh and dry weight (g) were examined. Shoot viability was calculated as a percentage by taking the ratio of the number of viable explants to the total number of explants. The rooting rate was calculated as a percentage by dividing the number of rooted explants by the total number of explants (Demirkök, 2006). Plant and root fresh and dry weights were measured using a precision scale (İpek et al., 2014). Plant heights and root lengths were measured using a ruler, and plant diameters were measured using a calliper (Köksal et al., 2014).

Data analysis

All the data were subjected to analysis of variance (ANOVA) and Duncan's multiple range test using the statistical software SPSS 23.0 (SAS Inc.).

RESULTS and DISCUSSION

Previous studies on aronia have generally focused on its antioxidant content and cultivation techniques. While studies on aronia related to classical propagation methods are limited, none are related to next-generation propagation techniques. Many micropropagation studies have been conducted on plants using PlantForm temporary immersion bioreactor systems. In recent years, temporary immersion systems have gained increasing importance and have been developed. Studies on horticultural plants have generally been carried out by improving this system. Many temporary immersion systems (TIB, RITA, GIB, SETIS, and PlantForm) are currently used (Georgiev et al., 2014). The first studies were carried out on *Carex oshimensis* 'Evergreen', *Chrysanthemum morifolium*, *Ficus carica,* and *Ribes rubrum* plants. These studies revealed that temporary immersion made positive contributions to shoot quality, the proliferation coefficient, and the rooting rate (Maurizio et al., 2015; Biçen, 2017). In another study, it was determined that 'Meeker', 'Willamette', and 'Cacanska Bestrna' raspberry varieties grown in liquid media developed well in the bioreactors using a temporary or continuous immersion system and did not show hyperhydricity (Anđelić et al., 2025).

In this study, the micropropagation performances of the aronia plant were compared in solid culture media and the PlantForm temporary immersion bioreactor system under *in vitro* conditions. The shoot viability (%), plant height (cm), plant diameter (mm), and number of shoots per plantlet (shoot/plantlet) related to the trial results are presented in Table 1. The effect of plant growth regulators was statistically significant ($p<0.05$) in both growing systems for the examined parameters. When examining the viability rates of the microcuttings in culture, the lowest viability rates were 85.13% in the medium supplemented with 2 mgL^{-1} BAP + 0.01 mgL^{-1} IAA and 66.6% in the PlantForm bioreactor system supplemented with 2 mgL^{-1} BAP + 0.01 mgL^{-1} IAA. In solid culture, 100% viability was achieved with nutrient media containing 2 mgL^{-1} KIN + 0.01 mgL^{-1} IAA and 4 mgL^{-1} KIN + 0.01 mgL^{-1} IAA, while in the PlantForm bioreactor system, 100% viability was obtained with nutrient media containing 2 mgL^{-1} BAP + 0.01 mgL^{-1} IAA, 1 mgL^{-1} KIN + 0.01 mgL^{-1} IAA, and 4 mgL^{-1} KIN + 0.01 mgL^{-1} IAA. In the solid culture treatments, the plant heights ranged between 1.82 cm (4 mgL^{-1} BAP + 0.01 mgL^{-1} IAA) and 2.97 cm (1 mgL^{-1} KIN + 0.01 mgL^{-1} IAA). The greatest plant height (2.97 cm) was obtained from the nutrient medium containing 1 mgL^{-1} KIN + 0.01 mgL^{-1} IAA (Figure 3).

In the PlantForm bioreactor system, the greatest increase in plant height was achieved with the plant growth regulator combination of 4 mgL^{-1} KIN + 0.01 mgL^{-1} IAA (4.12 cm). This was followed by media supplementation with 1 mgL-1 KIN + 0.01 mgL^{-1} IAA (3.93 cm) and 2.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA (3.73 cm). The lowest plant height was obtained at 1.05 cm from the MS media supplemented with 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA (Table 1). In both systems used in the study, when comparing plant heights, it was observed that plants obtained from the PlantForm bioreactor system were longer. The different temporary immersion bioreactor systems can affect shoot growth and development differently. Indeed, Ramos-Castellá et al. (2014) reported that the RITA system in *Vanilla planifolia* produced a high number of shoots per explant. Still, the shoots produced by RITA were shorter in length.

A similar effect on shoot height was also noted in the study conducted by Ramos-Castellá et al. (2016), who used temporary immersion bioreactors (TIBs) for micropropagation of vanilla. However, it has been demonstrated that vanilla shoots exhibit greater shoot height in the SETIS bioreactor (Mosqueda et al., 2022). In our study, we also found that the plants in the PlantForm bioreactor system were taller than those in solid culture. In the micropropagation of the citrus rootstocks 'Tuzcu 3131' and 'C35 Citrange' using the PlantForm bioreactor system and solid nutrient media, plant height was found to be greater for the 'Tuzcu 3131' genotype in the PlantForm bioreactor system (Cengiz & Kaçar 2019). In another study, it was determined that *Vaccinium membranaceum* grown in semi-solid, stationary (Growtek) and RITA bioreactor systems had higher numbers of shoots per explant and shorter plants in the RITA bioreactor (Barua et al., 2025). The diameters of the plants obtained from solid culture and the PlantForm bioreactor system are presented in Table 1. When plant diameters were evaluated in solid culture treatments, it was determined that the control group without plant growth regulators had the greatest

Table 1. Micropropagation, proliferation, and plant height development rates of the 'Viking' aronia variety in solid culture and platform systems

Çizelge 1. 'Viking' aronya çeşidinin katı kültür ve platform sistemlerindeki mikroçoğaltımı bitki gelişim oranları

		Shoot Viability (%)		**Plant Height (cm)**		**Plant Diameter (mm)**		**Number of shoots (pcs/plantlet)**	
		Solid Culture	**PlantForm**	**Solid Culture**	**PlantForm**	**Solid Culture**	**PlantForm**	**Solid Culture**	**PlantForm**
Control (MS)		96.27 a ± 2.41	96.6 b ± 2.42	1.85 f ± 0.05	2.34 f ± 0.06	1.05 a ± 0.03	0.61 e ± 0.02	0.00 e ± 0.0	0.00 e ± 0.0
BAP + 0.01 mgL^{-1} IAA	**1 mgL^{-1}**	92.53 ab ± 2.31	86.6 d ± 2.16	1.93 e ± 0.05	1.05 g ± 0.03	0.92 c ± 0.02	0.83 a ± 0.02	17.37 c ± 0.43	0.73 e ± 0.02
	2 mgL^{-1}	85.13 b ± 2.13	100 a ± 2.5	2.18 d ± 0.05	3.73 c ± 0.09	0.80 e ± 0.02	0.75 b ± 0.02	20.77 a ± 0.52	7.60 a ± 0.19
	4 mgL^{-1}	85.90 b ± 2.15	93.3 c ± 2.33	1.82 f ± 0.05	3.01 d ± 0.08	0.98 b ± 0.02	0.71 c ± 0.02	19.58 b ± 0.49	6.33 b ± 0.16
KIN + 0.01 mgL^{-1} IAA	**1 mgL^{-1}**	93.33 ab ± 2.33	100 a ± 2.5	2.97 a ± 0.07	3.93 b ± 0.1	0.89 d ± 0.02	0.53 f ± 0.01	0.92 e ± 0.02	2.10 d ± 0.05
	2 mgL^{-1}	100.00 a ± 2.5	66.6 e ± 1.66	2.55 c ± 0.06	2.48 e ± 0.06	0.79 ef ± 0.02	0.61 e ± 0.02	0.85 e ± 0.02	3.23 c ± 0.08
	4 mgL^{-1}	100.00 a ± 2.5	100 a ± 2.5	2.91 b ± 0.07	4.12 a ± 0.1	0.77 f ± 0.02	0.62 d ± 0.02	2.23 d ± 0.06	5.58 b ± 0.14

Means separation within columns by Duncan's multiple range test. *$P<0.05$

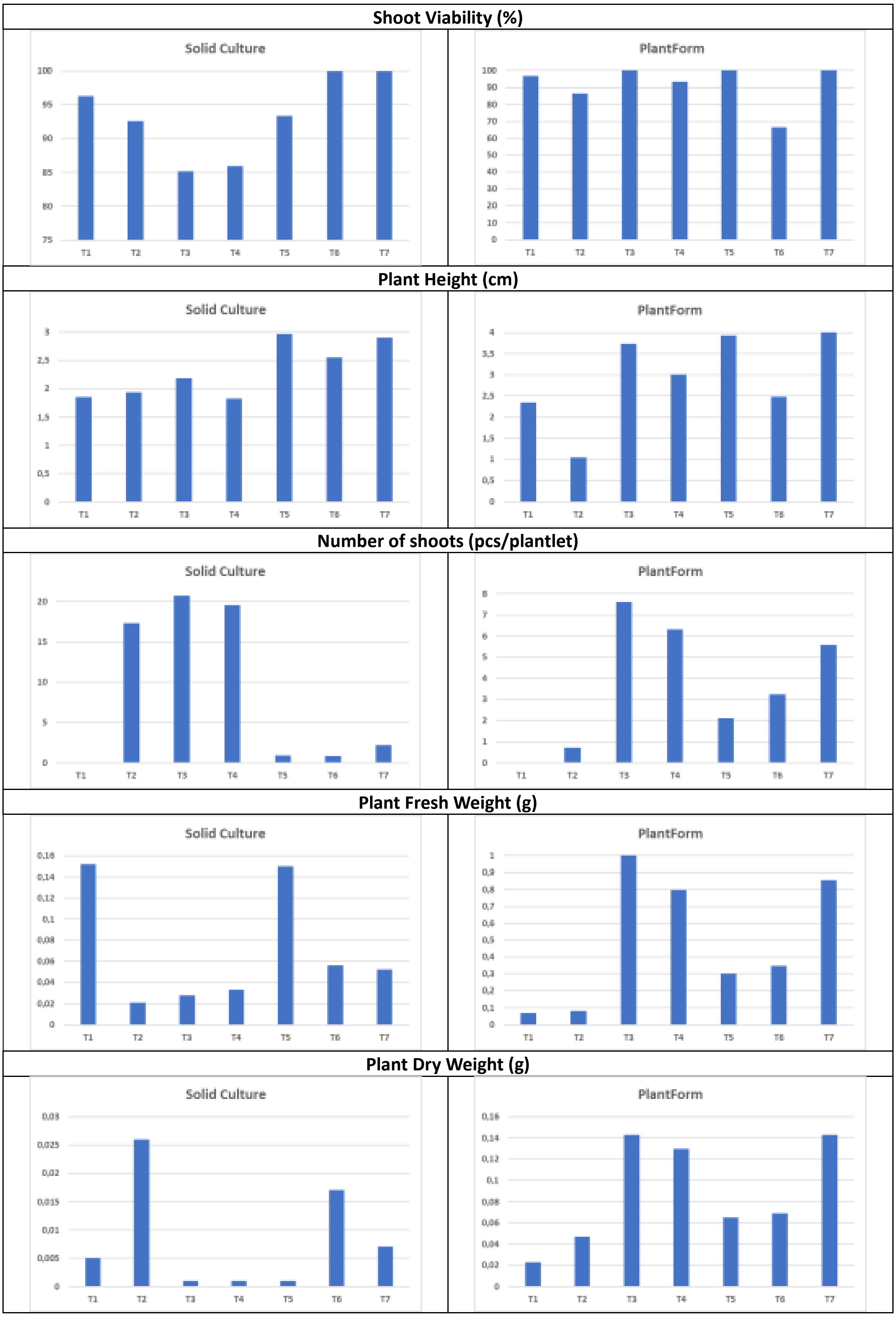
plant diameter (1.05 cm), followed by the combinations of 4 mgL^{-1} BAP + 0.01 mgL^{-1} IAA (0.98 cm) and 1 mgL^{-1} BAP + 0.01 mgL^{-1} IAA (0.92 cm). The plant diameters of the plants obtained in the PlantForm bioreactor system varied between 0.83 cm and 0.53 cm. The greatest plant diameter (0.83 cm) was observed in the MS media supplemented with 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA, followed by the combinations of 0.75 cm with 2.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA and 0.71 cm with 4.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA (Table 1). The plant diameters decreased as the concentrations of the two different plant growth regulators in both systems increased. The effects of various plant growth regulators on the number of shoots per plantlet in the solid culture and PlantForm bioreactor systems were determined for the 'Viking' aronia variety. The study revealed that the greatest number of shoots per plantlet in solid culture was obtained from MS nutrient media supplemented with 2.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA, with 20.77 shoots/plantlet. The ratios were 19.58 shoot/plantlet with 4.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA and 17.37 shoot/plantlet with 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA (Figure 3). Tissue culture yields different results depending on the type or concentration of plant growth regulator (Dagman, 2019). In a study on the *in vitro* propagation of aronia plants using different plant growth regulators, the best shoot development and maximum proliferation were obtained from plants treated with a combination of 2 mgL^{-1} BAP + 0.5 Kinetin + 0.1 mgL^{-1} IBA + 0.1 mgL^{-1} GA_3. In the PlantForm bioreactor system, the highest number of shoots per plantlet (7.60 shoots/plantlet) was obtained in MS nutrient media supplemented with 2.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA. This was followed by adding 4.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA to the MS nutrient media (Table 1). Sacco et al. (2013) reported that adding BA in the micropropagation of stevia resulted in 14 shoots per explant in the RITA and PlantForm systems. Ramírez-Mosqueda et al. (2016) and Bello-Bello et al. (2019) reported that using the SETIS bioreactor was more effective for the proliferation of vanilla shoots than other culture systems. Similarly, using the SETIS bioreactor has been reported to enhance shoot proliferation in species such as stevia, banana, and sugarcane (Rosales et al. 2018; Bello-Bello et al. 2019; da Silva 2020). In both systems, no proliferation occurred in the control group, whereas there was a greater incidence of proliferation in nutrient media supplemented with BAP than in those supplemented with Kinetin. Welander et al. (2014) compared the PlantForm bioreactor and agar media for the micropropagation of *Digitalis lutea× purpurea*, *Echinacea purpurea*, and *Rubus idaeus* species. According to the results, while the number of shoots was similar for both techniques in *Digitalis* and *Rubus*, it was observed that the number of shoots and quality were better in the bioreactor for *Echinacea*.

The number of leaves (pcs/plant), leaf area (cm^2), plant fresh weight (cm), and dry weight (cm) of the plants obtained from both systems used in the study were significantly different ($p<0.05$). The data obtained are presented in Table 2. In the solid culture micropropagation treatments, the highest average leaf number, 9.11 pcs/plantlet, was obtained from the MS media supplemented with 4.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA. The lowest leaf numbers were obtained from the control group (7.16 pcs/plantlet) and the 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA group (7.20 pcs/plantlet). In the PlantForm bioreactor system, the highest average leaf number, 8.76 pcs/plantlet, was obtained from MS media supplemented with 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA. In contrast, the lowest leaf number, 4.26 pcs/plantlet, was obtained from MS media supplemented with 4.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA (Table 2). When the effect of solid culture on the leaf areas of the plants was examined, the greatest leaf areas were in the 2.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA (0.944 cm2), 1.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA (0.866 cm^2), and the control (0.847 cm^2) groups. The greatest leaf area in the PlantForm bioreactor system was detected only in the 2.0 mgL^{-1} BAP + 0.01

mgL^{-1} IAA group, with a leaf area of 1.051 cm^2. Previous studies have suggested that the improvement in photosynthetic activity during temporary immersion could be supported by changes in air exchange (Aragón et al. 2010; Bello-Bello et al. 2019). In the study, we may have correlated the increase in leaf number and leaf area in some plantlets in the PlantForm bioreactor system with the increase in photosynthesis. The fresh and dry weights of plants obtained from solid culture and the PlantForm bioreactor system were determined and are presented in Table 2. In the solid culture experiments, when the effect of plant growth regulators on fresh weight was examined, the highest fresh weight was observed in the control (0.152 g) and 1.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA (0.150 g) groups. The lowest fresh weight was obtained from the 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA (0.021 g) plant growth regulator combination group. In the PlantForm bioreactor system, the best fresh weight, 1.052 g, was obtained from MS media supplemented with 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA. Concerning the effect of plant growth regulators on dry weight in solid culture, it was determined that the control group (0.026 g) had the highest dry weight. The lowest plant dry weight was obtained from MS media supplemented with 2.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA, 4.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA, and 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA. When the effect of plant growth regulators on dry weight was examined in the PlantForm bioreactor system, media supplemented with 2.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA and 4.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA plant growth regulators had the greatest dry weight (0.143 g). In contrast, the lowest dry weight (0.023 g) was obtained from the control group (Figure 3). In a study aimed at enhancing the propagation potential of the carob plant under *in vitro* conditions using the PlantForm bioreactor system, it was determined that plants obtained from the PlantForm bioreactor system exhibited better growth than plants obtained from cultures using agar, with higher levels of both fresh and dry weights (Cavallaro et al., 2015). In a similar study, Cengiz and Kaçar (2019) compared solid and PlantForm bioreactor systems on several citrus genotypes. This agrees with the findings described by Martínez-Estrada et al. (2019), who observed an improvement in fresh and dry weights in the micropropagation of *Anthurium andreanum* in temporary immersion bioreactors. They demonstrated that plants grown in the PlantForm bioreactor system exhibited greater fresh and dry weights and developed better than those grown in solid systems. These results support the findings.

TIS can enhance physiological and morphological performance in plants by providing optimal aeration and intermittent feeding conditions (Zhang et al., 2018). In fact, in a study where intermittent immersion periods and different culture medium volumes were tested in raspberries grown in a temporary immersion bioreactor, it was determined that TIS with an immersion frequency of 2 min every 8 h and a volume of 25 mL of culture medium per explant had the best development parameters (Reyes-Beristain et al., 2025). Bioreactor systems have been used for large-scale micropropagation of various plant organs, such as shoots, shoot tips, buds, and somatic embryos, of different plant species. These systems offer benefits such as easy control of environmental conditions, high efficiency, and high-quality plant production. Some reported benefits of TIS include increased plant propagation and rooting rates, reduced labour, improved quality of micropropagation, improved leaf development, reduced hyperhydricity effects, and minimized plant drowning (Karakoyun et al., 2023). Indeed, it has been determined that the PlantForm bioreactor system exhibits a better proliferation rate, plant quality, plant height, and rooting than solid cultures in different blackberry varieties and carob genotypes (Umarusman & Kaçar, 2018; Umarusman et al., 2020). Additionally, PlantForm bioreactor systems, such as *Citrus* and *Prunus* rootstocks (Cengiz & Kaçar, 2019; Dagman, 2019), *Myrtus communis* and *Olea europaea* (Carla et al., 2015; Kaçar et al., 2020), *Rubus idaeus* (Welander et al., 2014), *Musa spp.* (Daungban et al., 2017) and ornamental plant species, including *Spathiphyllum* (Kaçar et al., 2020), *Pteridophyta* (Uğur & Mendi, 2020), *Quercus robur* (Gatti et al., 2015), *Phalaenopsis* (Masnoddin et al., 2016), *Guadua angustifolia* (Gutiérrez et al., 2016), and *Gerbera jamesonii* (Mosqueda Frómeta et al., 2017), have been successfully utilized in Türkiye and worldwide.

After the micropropagation trials of the 'Viking' aronia variety, rooting trials were established in both solid culture and the PlantForm system. However, the desired success could not be achieved in the PlantForm system compared to the success obtained from solid culture. Similarly, no significant difference in rooting percentage was detected among the solid culture, RITA, and PlantForm bioreactors used for micropropagation of common medlar plants (San José et al., 2020). However, Sacco (2013) achieved the highest number of roots and rooting percentage (93%) in stevia shoots in the PlantForm system. When comparing the two systems, they observed that PlantForm had a larger area than the RITA bioreactor. In addition, Kaçar et al. (2020) reported that the PlantForm bioreactor system was much more successful for micropropagation and rooting of myrtle plants. In a study on the multiplication and rooting of 'Pircinque' strawberry variety explants in temporary immersion bioreactors at different immersion frequencies (5 and 8 times), it was found that immersion five times increased the growth of both the aboveground part and the root system (Camargo et al., 2019). According to the results, different bioreactors, plant growth regulators used in bioreactors, immersion frequencies, and plant species may yield different rooting percentages. The concentrations of the nutrient medium and plant growth regulators used in the bioreactor may not be suitable for root development in aronia and may have made root formation difficult. In addition, excessive immersion duration and frequency in the bioreactor system may have led to oxygen deficiency



T1: Control (MS media), **T2:** MS media + 1.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA, **T3:** MS media + 2.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA, **T4:** MS media + 4.0 mgL^{-1} BAP + 0.01 mgL^{-1} IAA, **T5:** MS media + 1.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA, **T6:** MS media + 2.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA, **T7:** MS media + 4.0 mgL^{-1} KIN + 0.01 mgL^{-1} IAA

Figure 3. The data from micropropagation trials of the 'Viking' aronia variety in solid culture and the PlantForm system

Şekil 3. Viking' aronya çeşidinin katı kültür ve PlantForm sisteminde mikroçoğaltım denemelerinden elde edilen veriler

Table 2. The data from micropropagation trials of the 'Viking' aronia variety in solid culture and the PlantForm system

Çizelge 2. 'Viking' aronya çeşidinin katı kültür ve PlantForm sisteminde mikroçoğaltım denemelerinden elde edilen veriler

		Number of Leaves (pcs/plant)		Leaf Area (cm^2)		Plant Fresh Weight (g)		Plant Dry Weight (g)	
		Solid Culture	PlantForm	Solid Culture	PlantForm	Solid Culture	PlantForm	Solid Culture	PlantForm
Control (MS)		7.16 d ± 0.18	7.23 b ± 0.18	0.847 a ± 0.02	0.070 g ± 0.0	0.152 a ± 0.0	0.070 g ± 0.0	0.005 c ± 0.0	0.023 f ± 0.0
BAP + 0.01 mgL^{-1} IAA	**1 mgL^{-1}**	7.20 d ± 0.18	8.76 a ± 0.22	0.108 c ± 0.0	0.081 f ± 0.0	0.021 f ± 0.0	0.081 f ± 0.0	0.026 a ± 0.0	0.047 e ± 0.0
	2 mgL^{-1}	8.77 b ± 0.22	7.00 c ± 0.18	0.337 b ± 0.01	1.051 a ± 0.03	0.028 e ± 0.0	1.052 a ± 0.03	0.001 d ± 0.0	0.143 a ± 0.0
	4 mgL^{-1}	7.72 c ± 0.19	4.26 g ± 0.11	0.211 bc ± 0.01	0.798 c ± 0.02	0.033 d ± 0.0	0.798 c ± 0.02	0.001 d ± 0.0	0.130 b ± 0.0
KIN + 0.01 mgL^{-1} IAA	**1 mgL^{-1}**	8.64 b ± 0.22	5.86 d ± 0.15	0.866 a ± 0.02	0.300 e ± 0.01	0.150 a ± 0.0	0.300 e ± 0.01	0.001 d ± 0.0	0.065 d ± 0.0
	2 mgL^{-1}	8.66 b ± 0.22	4.56 f ± 0.11	0.944 a ± 0.02	0.347 d ± 0.01	0.056 b ± 0.0	0.347 d ± 0.01	0.017 b ± 0.0	0.069 c ± 0.0
	4 mgL^{-1}	9.11 a ± 0.23	5.63 e ± 0.14	0.554 b ± 0.01	0.854 b ± 0.02	0.052 c ± 0.0	0.856 b ± 0.02	0.007 e ± 0.0	0.143 a ± 0.0

Means separation within column by Duncan's multiple range test. *P<0.05

due to constant liquid contact, thereby suppressing root development. Furthermore, aronia plants may not have responded positively to root development in liquid culture. As a result, to improve the success of bioreactor systems in the rooting stage, immersion durations and frequencies should be optimized, oxygenation should be increased, and appropriate plant growth regulator combinations should be determined. Alternatively, complementary strategies such as transferring plants propagated in the bioreactor system to solid culture for rooting could be developed. All parameters examined in the rooting trials in solid culture were statistically significant, and the rooting data in the solid culture system are presented in Table 3. Similar values were obtained for the IBA concentrations used when examining the rooting rates and root numbers of the plants placed in rooting media. The plants in the control group did not have roots, while 100% rooting was achieved by MS media supplemented with 3.0 mgL^{-1} IBA. In a study on the micropropagation of aronia plants, Imrak et al. (2021) achieved the highest rooting rate from ½ MS + 2 mgL^{-1} IBA + 0.5 mgL^{-1} NAA media, while Sivanesan et al. (2016) obtained the best rooting in aronia from ½ MS + 1.0 mgL^{-1} IBA media. These results are qualitatively similar to the findings. The number of roots of the plants varied between 7.35 and 7.81. The greatest number of roots, 7.81 pcs/plant, was obtained from the rooting media supplemented with 2.0 mgL^{-1} IBA. Similarly, the rooting media supplemented with 2.0 mgL^{-1} IBA (19.39 mm) obtained the maximum root length. In a different study, it was determined that the best nutrient medium for aronia plantlets was a root length of 23.26 mm and a root number of 13.03 when ½ MS + 1 mgL^{-1} IBA was used (Polat & Eskimez 2022). When the plants' root fresh and dry weights were examined, the best results were obtained from the rooting media supplemented with 2.0 mgL^{-1} IBA (Table 3). Significant differences were detected in the plant height and diameter of the rooted plants in the rooting media. The plant heights ranged from 5.49 to 6.43, and the greatest plant height was obtained from the rooting media supplemented with 2.0 mgL^{-1} IBA (6.43 cm). The maximum plant diameter of 1.12 mm was obtained from the control group plants, while a decrease was observed in the plants from the media containing IBA.

The study evaluated the *in vitro* micropropagation of the 'Viking' aronia variety using the PlantForm bioreactor system. While the system was found to be successful in terms of plant growth, it failed at the rooting stage. When assessing the efficiency of bioreactor systems in commercial production, factors such as propagation rate, cost-effectiveness, labor requirements, and process optimization should be considered. Temporary immersion systems (TIS), such as PlantForm bioreactors, emerge as an innovative method for commercial plant production through tissue culture. PlantForm bioreactors are designed to optimize growth conditions and resource utilization, which are critical factors in enhancing the scalability of plant propagation. The PlantForm bioreactor system requires less labor than solid culture, as the subculture frequency decreases and the need for manual handling is reduced.

Additionally, the absence of expensive gelling agents like agar provides a cost advantage. However, the initial investment in bioreactors is high, and their cost-effectiveness needs to be evaluated in the long term. The cost-effectiveness of PlantForm bioreactors in commercial applications is further emphasized by their ability to reduce manual labor in micropropagation processes. Utilizing a liquid culture approach, these bioreactors provide a liquid nutrient medium that requires less intensive handling, thereby reducing the labor costs typically associated with traditional semi-solid medium systems (Lyam et al., 2012). This reduction not only lowers operational costs but

Table 3. The rooting, root number, root length, and plant height development rates of the 'Viking' aronia variety in solid culture

Çizelge 3. 'Viking' aronya çeşidinin katı kültürde köklenme, kök sayısı, kök uzunluğu ve bitki boyu gelişim verileri

	Solid Culture					
	Rooting Rate(%)	**Number of Roots (pcs/plant)**	**Root Length (mm)**	**Root Fresh Weight(g)**	**Root Dry Weight(g)**	**Plant Height (cm)**
CONTROL	0.00 d ± 0.0	0.00 c ± 0.0	0.00 d ± 0.0	0.000 d ± 0.0	0.000 d ± 0.0	5.49 c ± 0.14
1 mgL^{-1} IBA	96.00 b ± 2.4	7.35 b ± 0.18	17.77 b ± 0.44	0.246 b ± 0.01	0.011 b ± 0.0	6.06 b ± 0.15
2 mgL^{-1} IBA	94.00 c ± 2.35	7.81 a ± 0.2	19.39 a ± 0.48	0.288 a ± 0.01	0.014 a ± 0.0	6.43 a ± 0.16
3 mgL^{-1} IBA	100.00 a ± 2.5	7.35 b ± 0.18	16.03 c ± 0.4	0.194 c ± 0.0	0.008 c ± 0.0	5.50 c ± 0.14

Means separation within column by Duncan's multiple range test. *$P<0.05$

also accelerates production cycles, which are key factors in the competitiveness of commercial farming. For example, experiments with strawberries have shown that optimal sucrose concentrations in PlantForm bioreactors lead to higher shoot and root numbers, indicating potential for specialized nutrient management approaches (Naser & Abdulhussein, 2023). A study demonstrating the broad applicability of PlantForm bioreactors in commercial plant production systems reported that bioreactors exhibited better growth than traditional systems. The effectiveness of bioreactors in propagating various species, such as *Olea europaea*, highlights their superior potential for producing high-quality planting material (Benelli & Carlo 2018). Despite these advantages, challenges remain, particularly in optimizing flooding and aeration parameters. Research indicates that achieving the right conditions is critical for successfully acclimating plantlets (Carlo et al., 2021). Therefore, operational protocols must be continuously improved to minimize variability and maximize output consistency. In conclusion, investigating the operational dynamics of the PlantForm system and integrating advanced technologies could make it a more commercially effective alternative in the agro-biotechnology sector.

CONCLUSION

This study investigated the effect of different plant growth regulator concentrations in the 'Viking' aronia variety on *in vitro* micropropagation and rooting using solid culture and one of the next-generation tissue culture techniques, temporary immersion PlantForm bioreactor systems. According to the data, the PlantForm system has been significantly more successful in plant growth, but has not achieved successful rooting. In the PlantForm system, it was determined that plant heights were greater for the Viking aronia variety. Similar values were obtained regarding the number of leaves and leaf area compared to those of the solid culture in the PlantForm system. In micropropagation experiments containing kinetin in solid culture, larger leaves were obtained than in media supplemented with BAP.

The immersion and aeration times are important among the technical parameters affecting success in temporary immersion bioreactor systems. These periods also directly affect plant development. The immersion time varies depending on the plant species. Additionally, the number of explants placed in the PlantForm bioreactor system's culture vessels can impact plant development and growth. Accordingly, the number of explants to be placed in culture vessels and the immersion duration may need to be separately optimized for each plant species. In this study's scope, using different combinations of aronia plants may lead to better results for future research. It is important to establish ideal protocols for *in vitro* micropropagation by comparing them with more traditional methods, such as temporary immersion bioreactor systems, semisolid culture, and liquid fixed cultures. Alternative hormone combinations, immersion frequency optimization, or staged hormone application can be used to increase rooting success in bioreactor systems. A two-stage system (bioreactor + short-term solid media) approach can be tried for rooting after PlantForm. Additionally, the absence of solidifying costs and the requirement for less labour in the PlantForm bioreactor system than solid culture may enable mass *in vitro* propagation of different plant species.

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Contribution Rate Statement Summary of Researchers

Şeyma ARIKAN: Planned the concept and design of the study, and statistical analysis of the study's data, and enabled the writing of the article with the study's data. **Merve KARAKOYUN MUTLUAY:** Ensured the propagation and preparation of plant material *in vitro* culture conditions, ensured the collection, and enabled the writing of the article with the study's data. All authors have read and agreed to the published version of the manuscript.

Conflict of Interest

The authors have no conflicts of interest in reporting.

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