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RESEARCH PAPER

A Study on Clonal Propagation by Cutting of Native Boxwood Species *Buxus balearica*

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Abstract

The aim of the study was to determine the reproductive parameters of the endangered *Buxus balearica* species, which is naturally distributed in Türkiye, under in vivo conditions. The effect of 0, 2000, 4000, and 8000 ppm doses of indole acetic acid (IAA) and indole butyric acid (IBA) on the rooting of semi-wood cuttings of *B. balearica* was investigated. As a result, it was the 4000 ppm IAA application that increased the root length (5.4 cm) and root width (5.6 cm) the most compared to the control. In both hormone applications, 8000 ppm was the most effective application on root number (6 per plant) and root quality (3.1 and 3.0). In addition, the most effective application on shoot length (1.8 cm) and the number of leaves on the shoot (7.5 per shoot) was found to be 8000 ppm IBA application. Regarding the callus rate, 4000 ppm (21%) was the most effective application in both hormone applications. In terms of rooting rate, 8000 ppm IBA provided a 98.5% higher rooting rate compared to the control. Although hormone applications had different effects on root morphologies, IBA showed a better performance than IAA on rooting rate. Additionally, phytohormone applications had a positive effect on rooting. The data obtained can be used to propagate existing boxwoods in landscape areas and for the rehabilitation of disappearing forests.

1. Introduction

Buxaceae is a small family of mostly monoecious evergreen shrub or tree plants. It consists of six genera and distributed on all continents except Australia and Antarctica (Köhler, 2007). The genus *Buxus* can grow in a wide ecological range and even above 3000 m altitude. Europe, the Mediterranean basin and the Middle East, China, Japan, Korea, Malaysia and the Philippines, Africa, the Caribbean Islands, Mexico and South America, India, the northwestern Himalayas and the former Soviet Union regions are the regions where boxwood is most common. *B. balearica* is naturally distributed in the region between the Canary Islands and the Eastern Mediterranean (Larson, 1996; Decocq et al., 2004). It is found in the Balerian Islands and southern Spain, and a single population is found in Sardinia. It is also widespread in Morocco, Algeria, and Türkiye (Tutin et al., 1968). The distribution areas of *B. balearica* in Türkiye is distributed only in the provinces of Adana, Hatay and Antalya in the Mediterranean Region (Sarı et al., 2023).

Boxwoods are widely recognized for their versatility and aesthetic appeal in garden and landscape design. Historically, they were first used as ornamental hedge plants in Egyptian gardens around 4000 BC. Today, boxwoods serve various purposes, including single and mass plantings, hedges, potted plants, topiary, and cut greens (Larson, 1999; Batdorf, 1997; Batdorf, 2004; Van Trier et al., 2005; Sarı et al., 2023). Boxwood has significant economic value in the United States as a popular ornamental shrub, has significant economic value in the United States as a popular ornamental shrub. The economic impact of boxwood is significant, with annual sales exceeding 11 million plants and a market value estimated at approximately $126 to $140 million (Kramer et al., 2020; Dhakal et al., 2022). This makes boxwood one of the leading woody plants sold in the United States, often outpacing other popular species such as azaleas. Apart from ornamental plants, boxwoods were also used to make musical instruments, writing tablets, combs, carved ornaments, paintings and sculptures. Its branches with elegant and durable leaves were used in religious and festive ceremonies. For these reasons, it has been grown for centuries both in its natural distribution areas and outside its distribution area. Again, the high density of its wood and its ability to be shaped easily make it unique (Larson, 1996; Köhler, 2007; Van Trier and Hermans, 2007). In the 18th and 19th centuries, boxwood was used by engravers to print images. Boxwood also provides good sound projection as it is free from the veins produced by its growth rings due to its slow growth (Mitchell et al., 2018).

Again, boxwood is used in cosmetics to strengthen hair and in folk medicine to treat fever, rheumatism, arthritis, biliary tract infections, diarrhea and skin ulcers (Ait-Mohamed et al., 2011; Tuniyev, 2016). It has been reported that *B. balearica* is used against diabetes in Morocco (Benkhnigue et al., 2014). *Buxus* spp. is also used in cancer research (Ait-Mohamed et al., 2011). An alkaloid isolated from *B. sempervirens* has antimycobacterial properties that have been proven effective against *Mycobacterium tuberculosis* (Tosun et al., 2004).

While Türkiye's boxwood forests experienced their first intense destruction in the 19th century with the intensive export of boxwood (Mitchell et al., 2018), the real damage was caused by *Cydalima perspectalis* Walker (Lepidoptera: Crambidae, Spilomelinae). Sarı et al. (2022) found that the boxwood moth dried out approximately 85% of the boxwood forests in Türkiye. One of these pests, *C. perspectalis*, is a species of Asian origin and was first detected in Europe in Germany and the Netherlands in 2007 (Billen, 2007; Van der Straten and Muus, 2010). In Türkiye, its presence was first detected in parks and gardens in Istanbul in 2011, in Düzce and Artvin in 2015, and in Bartın in 2016 (Hizal et al., 2012; Öztürk et al., 2016; Göktürk, 2017; Kaygın and Taşdeler, 2018; Yıldız et al., 2018). Later, Ak et al. (2021), found that *Cydalima perspectalis* was detected in the Mediterranean Region of Türkiye. Considering the diversity of species associated with boxwoods, serious ecological impacts may occur as a result of the destruction of natural boxwood stands in limited areas due to pests. For this reason, the extinction of boxwoods may indirectly cause the disappearance of many living creatures (Mitchell et al., 2018). Considering the conditions that boxwoods are exposed to, it is important to investigate propagation methods to ensure the continuity of the species. Although boxwoods can be propagated by seed, boxwood seeds ripen between June and July, with a high percentage (about 80%) of the ripened seeds being removed by rodents and ants. Seeds germinate under boxwoods in March, but the germination rate is low. Most germinating plants die during the hot and dry summer months (Lazaro and Traveset, 2005; Köhler, 2007). In addition, the slow development process from seed requires the use of vegetative production techniques. The rooting of boxwood is slow and uneven. It has also been reported that structural obstacles and time have no effect on rooting (Langé, 2014; Güney et al., 2023). However, it is very important to choose the most appropriate root hormone concentration for successful plant production, which provides great benefits to farmers in the production of ornamental plants with cuttings (Kaushik and Shukla, 2020). Due to the extreme decrease in boxwood trees in nature, it has become important to determine the most effective propagation methods and techniques. Because determining the most effective method will save time and money both in the reproduction of natural species and in the production of producers. Therefore, by using phytohormones in optimal concentrations, root cuttings are formed earlier and in greater numbers, callus formation is stimulated, and shoot growth of the cuttings increases significantly (Hartman, 2002). Singh et al. (2018) reported that IBA, IAA, and NAA are still the most commonly used auxins in rooting root cuttings.

This study aimed to determine the effects of different doses of IAA and IBA on the clonal propagation of *B. balearica*, which is distributed in very small areas in Türkiye and is endangered. In addition, this study aims to make recommendations to producers for rapid and effective propagation of the plant based on the results obtained.

2. Material and Methods

2.1. Plant material

This study was conducted in the Black Sea Agricultural Research Institute greenhouse in 2022 and 2023. In areas where boxwood grows naturally, plants that have no disease or harmful effects were selected and cuttings were taken. Cuttings belonging to the *Buxus balearica* species were obtained from Habib-i Neccar Mountain Nature Reserve (36°12’34.00”N, 36°10’58.14”E, 180-200 m) in Hatay province (Figure 1).

2.2. Experimental design

For *B. balearica*, cuttings were taken from plants that were 0.5-1.0 m tall. These cuttings were taken

Figure 1. *B. balearica* location view.

Figure 2. Rooting medium and appearance of rooted cuttings.

from one-year-old upright branches located on the upper part of the plant. To prevent drying during transportation from the collection area to the production unit, 10-15 cm-long shoots were cut, moistened and placed in plastic bags. The cuttings brought to the institute were kept in cold storage for one day and then taken into the planting process.

The basal end of each cutting were dipped in 0, 2000, 4000, and 8000 ppm doses of Indole-3-butyric acid (IBA) and Indole-3-acetic acid (IAA) for 10 seconds. The cuttings were then planted in the perlite. The planted vials were placed on rooting tables with bottom heating and top fogging. The ambient air temperature was maintained at 20 ± 2°C, root table temperature at 25 ± 2°C, and air humidity level at 75 ± 2% (Figure 2).

2.3. Measurements

After a 110-day rooting period, the following variables were recorded: root length (cm), root width (mm), number of roots, root quality (0-5), callus rate (%), rooting ratio (%), shoot length (mm) and number of leaves (per shoot) were measured. Root length, root width and shoot length (mm) were measured with a digital caliper. Number of roots, callus rate, rooting ratio and number of leaves were determined by counting. Root quality was assessed using a modified version of the (0-5) scoring method developed by Çelik (1982) for grapevine cuttings. This method was adapted for boxwood cuttings, and the root system of each cutting was categorized into six seperate groups, with scores ranging from 0 to 5. Each cutting's root system was then evaluated numerically based on these categories. In this evaluation, 0: there is no rooting, 1: Poor rooting (those with number of roots between 1-2), 2: There is a medium level of rooting (those with a number of roots between 3-4), 3: Good rooting (those with number of roots between 5-6), 4: Rooting is very good (those with number of roots between 7-8), and 5: It states that rooting is perfect (those with 9-10 or more roots).

2.4. Evaluation of data

In the clonal propagation experiments established with cuttings obtained from the shoot, all experiments were set up according to the randomized parcel divided parcel design in the evaluation of the data, with 3 replications and 30 cuttings in each replication. The data obtained were subjected to statistical analysis in the SPSS 20.0 package program. All analyses were statistically calculated within 5% and 1% error limits, and differences between applications were compared with the Duncan test.

3. Results and Discussion

3.1. Clonal propagation

It has been determined that different hormones significantly affect the rooting of the *B. balearica* species. At the end of a 110-day rooting process, the planted cuttings were removed from the rooting medium. This study examined the effects of various doses of IBA and IAA on rooting during the propagation of *B. balearica* using half-wood cuttings. The first root formations were observed at 55 days after planting with a 4000 ppm IBA application and at 62 days with an 8000 ppm IBA application. Full rooting was achieved after 110 days.

3.2. Root length

In the study, the effect of hormone, dose and hormone × dose interaction on average root length was found to be significant. While the highest average root length of 54.4 mm was obtained from 4000 ppm IAA application; The lowest average root length was obtained from the control application of IBA with 12.6 mm. In the hormone × dose interaction, root length was found to be 1.7% higher in IAA at 4000 ppm, while it was 187% higher in IBA at 8000 ppm, compared to the control. In dose application, the highest results were obtained from 42.0 mm and 41.9 mm and 4000 and 8000 ppm applications, and there was no difference between these two applications. Although there was no difference between these two doses, 4000 ppm increased root length by 26.9% and 8000 ppm by 26.6% compared to the control. In hormone application, the highest root length was found in IAA medium with 47.5 mm. Root length was found to be 45.2% higher in IAA than in IBA (Table 1, Figure 3).

3.3. Root width

In the study, the effect of hormone, dose and hormone × dose interaction on the average root width was found to be significant. In the hormone x dose interaction, the highest average root width of 55.8 mm was obtained from 4000 ppm IAA application; The lowest average root width was obtained from the control application of IBA with 13.6 mm. Compared to the control, 4000 ppm in IAA provided 17.4% wider roots, while 8000 ppm in IBA provided 166% wider roots. In dose application, the highest results (38.7 and 38.3 mm) were obtained from 4000 and 8000 ppm applications, and there was no difference between these two applications. In terms of dose averages, 4000 ppm increased root width by 26.5% and 8000 ppm increased root width by 25.2% compared to the control. In hormone application, the highest root length was found in IAA application with 41.9 mm. A 43.2% higher root width was detected in IAA application compared to IBA (Table 2, Figure 3).

3.4. Number of roots

The effect of hormone, dose and hormone × dose interaction on root number was found to be significant. While the highest average root number was obtained from 8000 ppm application with 6.0 in both IAA and IBA hormone applications; The lowest average root number was obtained from IBA's control application with 2.8. In the hormone × dose interaction, 33% and 114.3% higher root numbers were detected in both IAA and IBA applications at 8000 ppm, respectively, compared to the control. A similar effect was observed in terms of dose averages, and the highest value was determined at 8000 ppm with the number of 6 roots. A similar effect emerged in terms of dose averages; at 8000 ppm, the number of roots was 76.5% higher than the control. In hormone application, the highest root number was found in the IAA application with 4.8. A 14.2% higher number of roots was detected in IAA application compared to IBA (Table 3, Figure 3).

3.5. Root quality

The effect of hormone, dose and hormone × dose interaction on root quality was found to be significant. The highest average root quality was obtained from 8000 ppm application as 3.1 and 3.0, respectively, in both IAA and IBA two hormone applications. In the hormone × dose interaction, both IAA and IBA applications showed a higher root quality of 41% and 50%, respectively, compared to the 8000 ppm control. In terms of dose averages, it was again detected with 3.1 points in 8000 ppm applications, while root quality was 47.6% better than the control at 8000 ppm. In hormone

Table 1. Effects of IAA and IBA doses on root length (mm).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	53.5 ab	34.3 c	54.4 a	47.6 b	47.5 A
IBA	12.6 d	25.6 c	29.6 b	36.1 a	26.0 B
Dose average	33.1 C	30.0 C	42.0 A	41.9 A	
CV (%)	15				
Hormone (H)	**				
Dose (D)	*				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

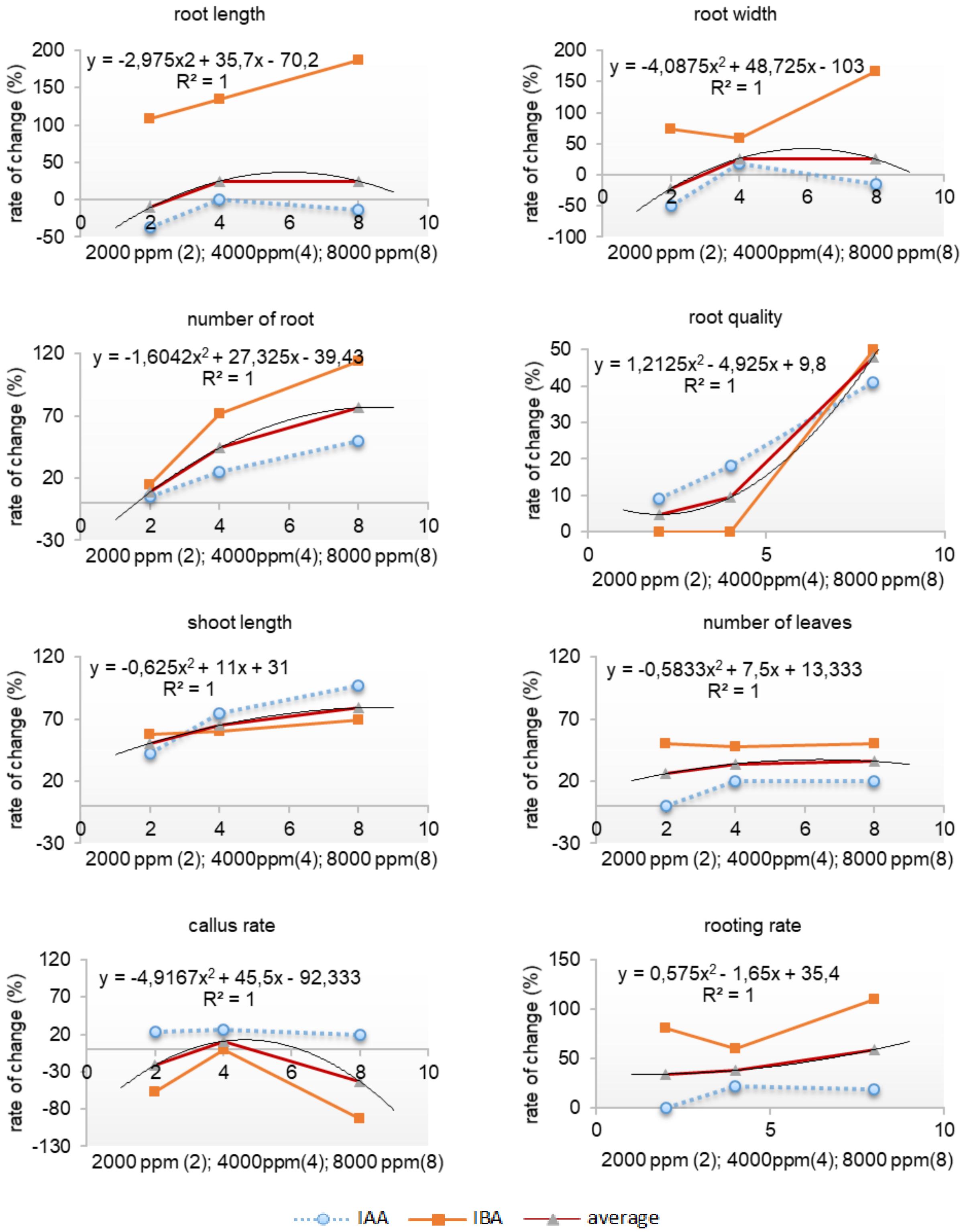

Figure 3. Change rates of rooting characteristics compared to control values after IBA and IAA applications.

Table 2. Effects of IAA and IBA doses on root width (mm).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	47.5 ab	24.0 c	55.8 a	40.4 b	41.9 A
IBA	13.6 c	23.7 b	21.5 b	36.2 a	23.8 B
Dose average	30.6 B	23.9 C	38.7 A	38.3 A	
CV (%)	17				
Hormone (H)	**				
Dose (D)	*				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

Table 3. Effects of IAA and IBA doses on root number.

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	4.0 b	4.2 b	5.0 ab	6.0 a	4.8 A
IBA	2.8 c	3.2 c	4.8 b	6.0 a	4.2 B
Dose average	3.4 C	3.7 C	4.9 B	6.0 A	
CV (%)	13				
Hormone (H)	**				
Dose (D)	**				
H × D	**				

There is a significant difference between the means with different letters (Duncan) within the error limits of $P< 0.05$ and $P< 0.01$.

Table 4. Effects of IAA and IBA doses on root quality.

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	2.2 b	2.4 b	2.6 b	3.1 a	3.0 A
IBA	2.0 b	2.0 b	2.0 b	3.0 a	2.0 B
Dose average	2.1 B	2.2 B	2.3 B	3.1 A	
CV (%)	18				
Hormone (H)	*				
Dose (D)	*				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of $P< 0.05$ and $P< 0.01$.

Table 5. Effects of IAA and IBA doses on shoot length (mm).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	7.2 c	10.2 bc	12.6 b	14.2 a	11.1 B
IBA	10.9 c	17.2 b	17.4 b	18.4 a	16.0 A
Dose average	9.1 C	13.7 BC	15.0 B	16.3 A	
CV (%)	16				
Hormone (H)	**				
Dose (D)	**				
H × D	**				

There is a significant difference between the means with different letters (Duncan) within the error limits of $P< 0.05$ and $P< 0.01$.

application, the highest root quality was found in the IAA application with 3.0 points. Root quality was found to be 12.5% better in IAA application compared to IBA (Table 4, Figure 3).

3.6. Shoot length

The effect of hormone, dose and hormone × dose interaction on shoot length was found to be significant. While the highest average shoot length of 18.4 mm was obtained from 8000 ppm IBA application; The lowest average shoot length was obtained from the control application of IAA with 7.2 mm. Although IAA and IBA applications provided different increases compared to the control in the hormone × dose interaction, higher shoot length was detected in both hormone applications at 8000 ppm, at the rates of 97.2% and 68.8%, respectively, compared to the control. In the dose application, it was detected in 16.3 mm and 8000 ppm applications, while a shoot length was found to be 79% higher than the control at 8000 ppm. In hormone application, the highest shoot length was found in IBA application with 16.0 mm. In IBA application, shoot length according to IAA was 44.1% higher than in the control (Table 5, Figure 3).

3.7. Number of leaves

The effect of hormone, dose and hormone × dose interaction on the number of leaves was found to be significant. Although IAA and IBA applications had different effects compared to the control in the hormone × dose interaction, 4000 ppm and 8000 ppm had the same effect in IAA, and 2000 ppm, 4000 ppm, and 8000 ppm had the same effect in IBA. The highest average number of leaves, 7.5, was detected in IBA's 2000 and 8000 ppm applications. In dosage application, it was determined that 4000 ppm (6.7 leaves per shoot) and 8000 ppm (6.8 leaves per shoot) applications had the same effect. Although there was no difference between 4000 ppm and 8000 ppm in terms of dose averages, 34% and 36% higher leaf numbers were obtained, respectively, compared to the control. In hormone application, the highest number of leaves was found in the IBA application with 6.9. The number of leaves was found to be 25.5% higher in IBA application compared to IAA (Table 6, Figure 3).

3.8. Callus rate

The effect of hormone, dose, and hormone × dose interaction on the callus rate was found to be significant. The highest average callus rate of 21.0% was observed with the 4000 ppm IAA and IBA applications, which showed similar effects. The lowest average callus rate of 1.5% was obtained from the 8000 ppm IBA application. In the hormone × dose interaction on the callus rate, IAA and IBA applications increased the callus rate by 25.7% and 10.5%, respectively, compared to the 4000 ppm

Table 6. Effects of IAA and IBA doses on number of leaves.

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	5.0 b	5.0 b	6.0 a	6.0 a	5.5 B
IBA	5.0 b	7.5 a	7.4 a	7.5 a	6.9 A
Dose average	5.0 B	6.3 AB	6.7 A	6.8 A	
CV (%)	18				
Hormone (H)	*				
Dose (D)	*				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

Table 7. Effects of IAA and IBA doses on callus rate (%).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	16.7 b	20.7 ab	21.0 a	20.0 ab	19.6 A
IBA	19.0 a	9.0 b	21.0 a	1.5 c	12.6 B
Dose average	17.9 B	14.9 C	21.0 A	10.8 D	
CV (%)	13				
Hormone (H)	**				
Dose (D)	**				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

Table 8. Effects of IAA and IBA doses on rooting rate (%).

Hormone	Control	2000 ppm	4000 ppm	8000 ppm	Hormone average
IAA	63.3 b	63.3 b	77.0 a	75.0 ab	69.7 B
IBA	47.0 d	85.0 b	75.0 c	98.5 a	76.8 A
Dose average	55.2 C	74.2 B	76.0 B	87.5 A	
CV (%)	15				
Hormone (H)	**				
Dose (D)	**				
H × D	*				

There is a significant difference between the means with different letters (Duncan) within the error limits of P< 0.05 and P< 0.01.

a b c d

Figure 4. Root development by applications (a: Control, b: 2000 ppm, c: 4000 ppm, d: 8000 ppm).

control. In terms of dosage application, a callus rate of 21.0% was observed with the 4000 ppm application. Regarding dose averages, a 17.3% higher callus rate was found at 4000 ppm compared to the control. Among hormone applications, the highest callus rate was found with the IAA application at 19.6%. The callus rate was 55.6% higher in the IAA application than in the IBA application (Table 7, Figure 3).

3.9. Rooting rate

The effect of hormone, dose and hormone × dose interaction on the rooting rate was found to be significant. While the highest average rooting rate was detected in the 8000 ppm IBA application with 98.5%, the lowest average rooting rate was obtained from the control application of IBA with 47.0%. In the hormone × dose interaction, 4000 ppm in IAA increased the rooting rate by 21.6% compared to the control, while 8000 ppm in IBA increased it by 110%. In the dosage application, it was detected at 87.5% and 8000 ppm applications. At dose averages, 8000 ppm provided 58.5% more rooting than the control. In hormone application, the highest rooting rate was found in the IBA application with 76.8%. IBA application showed 10.2% more rooting than IAA (Table 8, Figures 3 and 4).

In this study, the effects of different doses of IBA and IAA on rooting in the propagation of *B. balearica* using half-wood cuttings were examined. The first root formations were observed at 55 days after planting with a 4000 ppm IBA application and at 62 days with an 8000 ppm IBA application. Full rooting occurred after 110 days. Vieira et al. (2018)

reported that approximately 116 days were needed for the rooting of semi-wood cuttings of *B. sempervirens*. In this study, where semi-wood cuttings were used, the full rooting time for both species was found to be similar to the duration reported by Vieira et al. (2018). Additionally, the period reported by Güney et al. (2023) was nearly twice the 57 days noted for *B. sempervirens*.

Three different doses of IAA and IBA were statistically effective on the rooting morphology of *B. balearica*. While IAA was the most effective application on root length, root width, number of roots, root quality and callus rate, IBA was more effective on shoot length, number of leaves and rooting rate. IAA application was more effective than IBA on root length, and almost twice as long roots were obtained in IAA. In hormone and dose interaction, 4000 ppm (5.4 cm) was the most effective application in IAA application, while 8000 ppm (3.6 cm) was the most effective application in IBA. However, in terms of dose averages, there was no statistical difference in the effect on root length between 4000 ppm and 8000 ppm applications. IAA and dose interaction on root length gave better results than other applications. A similar result was found for root width as for root length. Regarding the number of roots, 8000 ppm (6) IAA was the most effective application in all applications. Güney et al. (2023) in *B. sempervirens*, the highest values in terms of root length and number of roots were determined as 2.01 cm and 7.8, respectively, in 3000 ppm IBA application. Kaviani and Negahder (2017) in their study on *B. hyrcana* Pojark., they obtained a maximum root length of 7.3 cm and a number of roots of 8.7 with 1000 mg L^{-1} IBA and 1000 mg L^{-1} NAA. In the study conducted by Banko and Stefani (1986), as a result of IBA application, the number of roots and root length were as follows: 11.4 roots and 5.6 cm in *B. sempervirens*, 6.8 roots and 3.4 cm in *B. sempervirens* L. 'Suffruticosa', 8.6 roots and 9.8 cm in *B. microphylla* var. koreana Nakai, and 6.8 roots and 2.6 cm in *Buxus microphylla* var. japonica [(Mull. Arg.) Rehder & E.H. Wilson]. *Juniperus communis* 'Hibernica' the longest root length was 9.8 cm in the IBA 5000 ppm application, while the highest number of roots was determined as 8.1 roots in the IBA 3000 ppm application. Within the scope of this study, the longest roots of 23.2 cm were determined in the IAA 3000 ppm application in the *Juniperus chinensis* 'Stricta' variety (Güney et al., 2021). In fact, it is known that auxin stimulates root elongation (Davies, 2010). In addition, studies using green cuttings in cranberries have reported that the number of roots varies depending on the genetic structure (Balta et al., 2019) and that IBA application has a significant effect on the number of roots (Kalyoncu et al., 2008; Balta et al., 2019). As can be seen from previous studies, phytohormones have different effects on different species and varieties. While IBA was generally found to be more effective on root length and root number in studies conducted on boxwood, IAA was found to be more effective in this study. Additionally, unlike previous researchers, the effective dose in this study was found to be higher (8000 ppm). The reason for this difference was determined by Hartmann et al. (2011) and Azad and Matin (2015) reported that the effects on rooting in cuttings may vary depending on many internal (genetic structure, hormones, storage materials, etc.) and external (temperature, humidity, light, rooting mediums, cutting time) factors. These factors significantly affect the rooting rate, rooting quality and rooting properties of cuttings (Ağaoğlu et al. 2019).

In the study, applications affected shoot length. In the hormone × dose interaction, the highest average shoot length of 1.8 cm was obtained from 8000 ppm IBA application; The lowest average shoot length was obtained from the control application of IAA with 0.7 cm. 8000 ppm was the most effective dose in both hormone applications. Yeshiwas et al. (2015) in their study on rose varieties, the shoot length was found to be 14.4 cm with 1000 ppm IBA application. Similarly, Akhtar et al. (2015) found the maximum shoot length to be 10.67 cm in the application of 450 ppm IBA on *Rosa centifolia*. In our study, IBA application increased shoot length. However, a lower shoot length was detected compared to previous studies on different species. The reason for this can be explained as boxwoods showing a low growth rate.

Applications also affected the number of leaves. Different results were obtained for the number of leaves in terms of hormone and dose interaction in both applications. The highest average number of leaves, 7.5 per plant, was observed in the 2000 ppm and 8000 ppm IBA applications. In terms of dose averages, the highest results were obtained at 4000 ppm (6.7 leaves per plant) and 8000 ppm (6.8 leaves per plant), with both doses showing similar effects. In the study conducted by Muhammad Shahab (2021) on the ornamental plant *Clerodendrum splendens*, it was observed that the maximum number of leaves per plant (14.0) was observed in cuttings treated with 20% IBA, followed by 10% IBA (12.3) and 0% IBA (12.0). The minimum number of leaves per plant (10.6) was determined for cuttings treated with 30% IBA. In their study on *Citrus medica* L., Al-Zebari and Al-Brifkany (2015) found that the 1000 ppm IBA application resulted in the highest number of leaves per shoot (12.72), while the control application produced the lowest value (8.36). In the 2000 ppm application, a lower number of leaves (11.42) was observed compared to the 1000 ppm application. The results of the researchers showed that IBA applications were effective in increasing the number of leaves on the shoot. In this study, IBA applications increased the number of leaves on the shoot compared to the control.

Rooting quality is an important feature that affects the seedling quality of cuttings in which root formation occurs (Yıldız et al., 2009). Significant differences were detected among the examined boxwood species and types in terms of rooting quality. Accordingly, root quality ranged from 2.0 to 3.1. The IAA application resulted in better root quality than the IBA application. The highest root quality was observed at a dose of 8000 ppm in both hormone applications. In terms of dose averages, 8000 ppm application increased root quality by 47.6% compared to the control. IAA (3) showed a 50% higher root quality than IBA (2). Compared to the control, rooting was high in all cultivars, including 'Winter Beauty,' 'Globosa,' and 'Rotundifolia,' meaning that most cuttings formed more than three roots (Salaš et al., 2012). Balta et al. (2019) found that rooting quality in cranberries increased with hormone application and varied significantly depending on the genotype. In our study, it was determined that the rooting quality of boxwoods varies according to species and variety, and improves as the dose of the applied hormone increases. Additionally, it was observed that boxwoods predominantly exhibit a medium level of rooting quality.

When it came to the interaction between hormone and dose on callus rate and dose averages, 4000 ppm was the most successful application in both cases. IAA and IBA treatments raised the callus rate by 25.7% and 10.5%, respectively, in the hormone and dosage interaction on the callus rate when compared to the 4000 ppm control. In terms of dose averages, a 17.3% higher callus rate was found at 4000 ppm compared to the control. The callus rate was 55.6% higher in the IAA application compared to the IBA application. In the study conducted on *Juniperus chinensis* 'Stricta', the highest callus formation (73.3%) was observed with the 3000 ppm IAA application (Güney et al., 2021). The results obtained in this study revealed that the callus rate also differs between species and varieties. In fact, the callus formation rate is an important factor that affects the rooting rate in cuttings (Kalyoncu et al., 2008). Propagation studies conducted with cranberries have reported that the callus rate varies significantly depending on the genotype (Yavuz, 2015; Balta et al., 2019).

In terms of hormone and dose interaction on rooting rate, 4000 ppm was the most effective dose in the IAA application, while 8000 ppm was the most effective dose in the IBA application. In terms of dose averages, 8000 ppm was the most effective dose in both applications. The highest average rooting rate was observed in the 8000 ppm IBA application, with 98.5%, while the lowest average rooting rate was obtained from the IBA control application, with 47%. It has also been reported that structural obstacles and time have no effect on rooting (Langé, 2014; Güney et al., 2023). However, in our study, it was determined that the rooting rate generally increased with the increase in hormone dose (control: 55.2%, 2000 ppm: 74.2%, 4000 ppm: 76.0% and 8000 ppm: 87.5%). In the study by Banko and Stefani (1986), rooting rates were found to be 92.5% in *B. sempervirens,* 51.3% in *B. sempervirens* L. 'Suffruticosa', 80.4% in *B. microphylla var. koreana Nakai*, and 72.8% in *Buxus microphylla var. japonica* [(Mull. Arg.) Rehder & E.H. Wilson]. Again, in the study conducted by Wang (1989), the rooting rate varied between 4% and 68%. In another study, for the control group, *B. sempervirens* semi-hardwood cuttings treated with IBA at 1500 mg L^{-1}, 3000 mg L^{-1}, and 6000 mg L^{-1} showed the highest rooting percentage of 97.5% (Vieira et al., 2018). Güney et al. (2023) again observed that on *B. sempervirens*, the highest rooting percentage (100%) was observed in GM-1 with IBA 3000, IBA 5000, IAA 3000, NAA 3000 and NAA 5000 ppm applications and in GM-2. Specified in IBA 3000 and IAA 3000 ppm applications. Unlike boxwoods, Güney et al. (2021) on junipers, the highest rooting percentage *for J. chinensis* 'Stricta' was obtained with 66.67% in the IAA 5000 ppm treatment. The highest rooting percentage for *J. chinensis* 'Stricta Variegata' was obtained as 60% at 3000 ppm application of IBA. This study clearly showed that two different hormones and different doses of these hormones may be effective in two different juniper varieties. Similarly, a study conducted with soft cuttings of *Berberis thunbergii* 'Atropurpurea Nana' showed that NAA (3000 ppm) and IAA (8000 ppm) hormones were more effective in promoting root formation and development in cuttings compared to the IBA hormone (Pulatkan et al., 2018). When the studies in the literature are generally evaluated, it is seen that IBA hormone doses are more successful in rooting cuttings (Pulatkan et al., 2018). However, as reported by Kaushik and Shukla (2020), rooting rates differed according to species and types. In this study, similar to the findings of the researchers, the effects varied between hormones and their doses. Specifically, the highest rooting rate was obtained from the 8000 ppm IBA application, while 4000 ppm was found to be the most effective dose in the IAA application, despite having a lower rooting rate compared to the 8000 ppm IBA application. Furthermore, Walter (1997), in research conducted on *Buxus*, found that high doses of IBA, such as 12000 ppm (1.2%), were the most effective application. These results indicate that there is no fixed hormone dose for rooting *Buxus* species and varieties.

4. Conclusion

In the study, it was determined that IBA and IAA applications had different effects on root morphology. While IAA was the most effective application on root length, root width, number of roots, root quality and callus rate, IBA was more effective on shoot length, number of leaves and rooting rate. As for the hormone dose, it was found that 8000 ppm, IBA application was more effective on the rooting and root morphology of boxwoods. In terms of root quality, boxwoods showed a medium level rooting quality. It is known that there are no internal or external inhibitors on the rooting of boxwoods. In other words, shrubs can be rooted without phytohormone application. However, it has been revealed by previous studies that phytohormone applications increase rooting and is supported by the results of this study. In addition, this study determined that rooting and root morphology development of boxwoods may differ depending on the applied hormone and dose. Approximately 85% of boxwood areas in Türkiye have disappeared due to the effect of the boxwood moth. Rehabilitating these destroyed areas will be possible by rapidly and effectively multiplying the remaining boxwood genetic resources. In addition, the results of this study, in which the best production conditions are investigated, provide guidance to producers in ensuring the sustainable use of boxwood and especially its use as ornamental plants.

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