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The effects of different rooting temperatures and phytohormones on the propagation of boxwood cuttings

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Abstract

Due to its wide range of utilization possibilities, boxwood has undergone severe damage, like all over the world, caused by the excessive forest use in Türkiye from the past to the present. Therefore, it is important to propagate it to ensure the continuity of the species. In this study, the effects of different rooting table temperatures and phytohormones on rooting were investigated in the propagation of *Buxus sempervirens* L. subsp. *sempervirens* by softwood cutting. First callus and root formation dates, rooting percentage, callus percentage, root length and the number of roots were determined on the cuttings. As a result, the highest rooting percentages were obtained as 100% in IBA 3,000, IBA 5,000, IAA 3,000, NAA 3,000 and NAA 5,000 ppm treatments in Greenhouse-1 medium and IBA 3,000 and IAA 3,000 ppm treatments in Greenhouse-2 medium. In terms of root length and the number of roots, the highest values were determined as 2.01 cm and 7.83, respectively, in IBA 3,000 ppm treatment in Greenhouse-2 medium. With the high success obtained, it has been concluded that the propagation of the species can be made by using softwood cutting and the genetic infrastructure of the species can be preserved and transferred to future generations.

Keywords: *Buxus sempervirens*, ornamental plant, cutting propagation, auxin

Introduction

Buxus sempervirens L. (boxwood) is a perennial woody plant in the form of an evergreen shrub or tree of the Buxaceae family, that grows well on humic and calcareous soils (Anşin and Özkan 1993, Lorenzi and de Souza 2001, Acartürk 2006). Boxwood is common in Bulgaria, Germany, France, Georgia, Portugal, Spain, China, on the island of Sardinia (Lorenzi and de Souza 2001, Lehtijärvi et al. 2014), and in Türkiye, where it grows in Adana, Antalya, Bolu, Denizli, Hatay, Karabük, Kastamonu, Kahramanmaraş, Kocaeli, Muğla and Osmaniye provinces, being especially numerous in the Black Sea and Marmara regions (Ateş et al. 2010, Altunışık et al. 2017). Total area of boxwood stands in Türkiye is over 20,424 hectares (Anonymous 2021). The multipurpose use of forests, apart from timber in the standard sense, is increasing day by day, and the importance of non-timber forest products is now beginning to be better understood (Vurdu 1978, 1983). Boxwood, which grows naturally in Turkish forests, offers services with its valuable wood used in many different areas such as toys, mortars, spoons, combs, plates, backgammon stamps, mouthpieces, machine beds and shuttle besides its landscape uses (Türkyılmaz and Vurdu 2003, Türkyılmaz et al. 2006). Since there is a high amount of lignification in boxwood stems, they contain much more lignin and guaiacyl formation than normal wood (Yoshizawa et al. 1993, Baillères et al. 1997). In this way, with its more durable wood, it becomes an important raw material for sectors that need lignin in industrial production (Naneli et al. 2020). The species is a slow-growing (7 to 15 cm per year), perennial, compact and symmetric plant, and also suitable for pruning. Thanks to its ornamental value, it is used for topiary and the other various landscaping implementations (Niemiera 2012). Although it is generally used in landscape areas as an ornamental plant in Türkiye, it is also used for medical purposes in pharmacy, especially its extractions obtained from leaf and shell parts (Leporatti et al. 1985, Neves et al. 2009). In addition, some active substances of this species are used in the treatment of some important diseases of liver, stomach, intestines and cardiovascular system, hypercholesterolaemia, rheumatism, and diabetes (Durant et al. 1998, Uzun et al. 2008, Mohamed et al. 2011, Gül 2014, Ajebli and Eddouks 2018).

Both due to human intervention, disease and insect damages, boxwood areas in Türkiye have been exposed to great destruction and it has become a tree species that grows in small groups (Symmes 1984). Such biotic factors as parasitism in the form of fungal disease (boxwood blight) caused by *Cylindrocladium buxicola* (Henricot and Culham 2002) and *Cylindrocladium pseudonaviculatum* (Crous et al. 2002) and phytophagy by caterpillars of *Cydalima perspectalis* (Walker 1859, Lehtijärvi et al. 2014) have the most negative effects on boxwood trees. *C. perspectalis* (Lepidoptera: Crambidae) is an invasive species, by means of it, pathogenic fungi were transported to areas that they could not reach before. Therefore, it is considered as one of the most important pests of all boxwood areas in the world (Leuthardt et al. 2010, Hızal et al. 2012, Göktürk and Aksu 2014, Göktürk 2017, Burjanadze et al. 2019). Fungal pathogen *Cylindrocladium pseudonaviculatum* that was detected in the boxwood areas near Trabzon and Artvin for the first time in 2011 in Türkiye has spread to 90% of boxwood areas in a short time (Lehtijärvi et al. 2014). It is reported that this pathogen, which only needs high humidity and wetness of the plant to cause infection and can complete its development in the plant in a short time like a week, is the most important disease-producing factor that causes brownish circular spots, severe defoliation and dieback of trees in the boxwood areas in Artvin (Henricot et al. 2000, Henricot et al. 2008, Ivors and LeBude 2011, Akıllı et al. 2019).

Considering these conditions that the boxwood is exposed to, it is important to study propagation methods to ensure the continuity of the species. The boxwood subject to the study is considered to be rooted easily because it does not contain anatomical obstacles that prevent the formation of adventitious roots (González and Diaz 1986). Therefore, boxwood, which also has a wide variety of forms, can be propagated by cuttings (Ürgenç 1998). The cutting propagation method, one of the vegetative propagation ones, includes a number of techniques for developing a new plant of a root, stem or leaf part taken from a parental plant to be propagated. In this method, cuttings are classified as softwood, semi-hardwood and hardwood cuttings according to their lignification status and the period they are obtained (Ürgenç 1998, Ermeydan et al. 2011). Boxwood can be propagated with softwood cuttings harvested in summer or as semi-hardwood cuttings harvested at the end of summer (Ürgenç 1998). There are many factors affecting rooting, including chemical (hormones, carbohydrates, etc.), connected with plant origin (time of cutting harvesting, cutting type, species, etc.), environmental (humidity control, additional lighting, floor heating, photoperiod, etc.) and other factors (medium, wounding, etc.), in the propagation of plants with cuttings (Genç 1995, Demirbaş 2010). In addition, phytohormones (plant growth regulators) are also very important in the process of cutting propagation. The proper hormone balance in the cuttings affects callus formation, initial root growth, root elongation, root hardening and subsequent development of rooting cuttings (Jaenicke and Beniest 2003). Indole-3-butyric acid (IBA), indole-3-acetic acid (IAA) and α-naphthalene acetic acid (NAA), which are widely used in cutting propagation, are phytohormones of the auxin group (Cooper 1935, Fogaça and Fett-Neto 2005), and these hormones play a central role in the root formation process and promote root growth (Davis et al. 1988, De Klerk et al. 1999, Grunewald et al. 2009). There is no universal rooting medium mix for cuttings, the suitable rooting medium varies depending on factors such as the species, type of cutting, and season (Hartmann et al. 2002). However, perlite is highly preferred in cutting propagation with its properties such as moisture retention, good ventilation, sterility and light weight (Şimşek 1993, Hartmann et al. 2002).

For boxwood, the losses suffered due to disease have reached very serious levels today and the species, which has become unusable as an ornamental plant in many countries, have started to disappear rapidly from its natural habitats (Altunışık et al. 2017). When habitat demands, root structure and regeneration ecology are evaluated together, boxwood, a K-strategist species, characterized by slow growth, low spreading ability, specific habitats, fixed population size, etc., has its place in the Red Data Book of Turkish Plants, due to insufficient knowledge and improper silvicultural operations (Çolak 2003). Boxwood, which is generally a forest tree in the form of a shrub, has been severely damaged by widespread use in the Turkish forests from the past to the present (Türkyılmaz et al. 2006), and has become a tree species that can be found in small micro-ecosystems that are highly unplanned and very difficult to reach even in forest management plans (Symmes 1984, Türkyılmaz and Vurdu 2004). From this point of view, boxwood, which is of great importance due to its many different areas of use both in Turkish forestry and the world one, should be reproduced and the sustainability of the plant should be ensured by planting in natural habitats and its distribution area should be expanded. Within the scope of the study, it was aimed to determine the best rooting conditions by investigating the effect of different rooting table temperatures and phytohormones on rooting in the propagation by softwood cutting of *Buxus sempervirens* L. subsp. *sempervirens*.

Materials and methods

The present study was carried out in The Research and Application Greenhouse within the Faculty of Forestry of Karadeniz Technical University (KTU) in May 2015. In the study conducted employing softwood cutting propagation, which is one of the vegetative propagation methods, cutting materials belonging to *Buxus sempervirens* L.

Figure 1. Planted and rooted cuttings

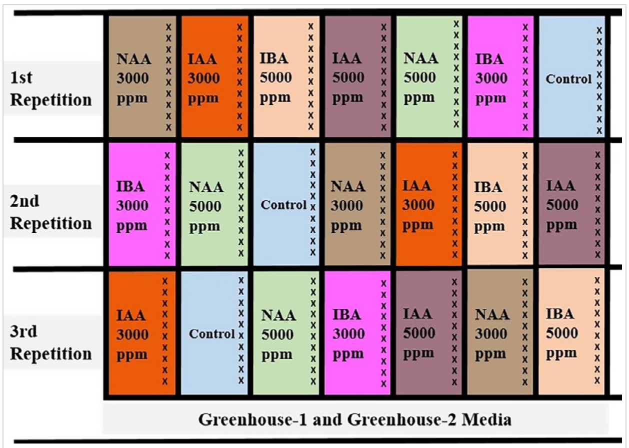

Figure 2. Trial design related to planted cuttings

subsp. *sempervirens* were obtained from 30-year-old stock plant located in the natural habitat of the species in Trabzon, Türkiye (Figure 1).

The cutting materials were collected from the stock plant early in the morning and transported with a cooler to prevent moisture loss. While preparing 8–10 cm long cuttings, the basal ends of the cuttings were cut just below a node. To investigate the reactions of cuttings to rooting, two different media were adjusted with the automation system, belonging to the technological infrastructure of the Research and Application Greenhouse, that can regulate fogging, misting, irrigation and temperature settings. These two media are named and arranged as Greenhouse-1 medium (GM-1: air temperature at 20 ± 2°C, rooting table temperature at 25 ± 2°C and air humidity level at 70 ± 2%) and Greenhouse-2 medium (GM-2: air temperature at 20 ± 2°C, rooting table temperature at 20 ± 2°C and air humidity level at 70 ± 2%). On the one hand, perlite rooting medium in both greenhouse media was preferred due to high water retention and aeration capacity. On the other hand, to encourage rooting, 3,000 and 5,000 ppm concentrations of IBA, IAA and NAA phytohormones were prepared and used. In the study, which was established in three repetitions according to the '*randomized complete block design*', a total of 420 cuttings were planted for treatments (360 cuttings: 1 species × 1 rooting media × 2 greenhouse media × 2 concentrations × 3 phytohormones × 10 cuttings × 3 repetitions) and control cuttings in both greenhouse media (60 cuttings: 1 species × 1 rooting media × 2 greenhouse media × 10 cuttings × 3 repetitions). The cuttings were treated with 3,000 and 5,000 ppm of IBA, IAA, and NAA and the control cuttings were planted in GM-1 and GM-2 media which constitute the research treatments. The schematic representation of the planted cuttings according to the trial design is given in Figure 2.

After the rooting process was completed in greenhouse media, the first callus and root formation dates, rooting percentage (RP), callus percentage (CP), root length (RL) and the number of roots (RN) were determined in the cuttings removed from the rooting medium. The obtained data were statistically analyzed by using SPSS Statistics for Windows, version 23.0 (IBM 2015). In the study, an analysis of variance was performed to reveal the statistical significance of the effects of different greenhouse media (GM) and phytohormones (PH) on RP, CP, RL and RN. In addition, by evaluating the data in GM-1 and GM-2 together, the groups formed depending on PH were determined by Duncan's test.

Results

Planted cuttings were removed from the rooting medium at the end of the 57-day rooting process. Within the scope of the study, the first callus formation was obtained 15 days after planting in NAA 5,000 ppm treatment in GM-2, and the first root formation occurred 29 days after the planting in IBA 3,000 and NAA 5,000 ppm treatments in GM-2. The results of RP, CP, RL and RN obtained depending on different greenhouse media and phytohormones are given in Table 1.

When the results obtained as a result of the study were examined, the highest rooting percentages with 100% were determined in IBA 3,000, IBA 5,000, IAA 3,000, NAA 3,000 and NAA 5,000 ppm treatments in GM-1, and also in IBA 3,000 and IAA 3,000 ppm treatments in GM-2. While callus formation did not occur in any treat-

Table 1. The data obtained regarding the measured characteristics

GM	PH	RP (%)	CP (%)	RL (cm)	RN (root)
GM-1	IBA 3,000 ppm	100.00 ± 0.00	0.00 ± 0.00	1.26 ± 0.63	7.57 ± 3.13
	IBA 5,000 ppm	100.00 ± 0.00	0.00 ± 0.00	1.47 ± 0.85	7.23 ± 3.06
	IAA 3,000 ppm	100.00 ± 0.00	0.00 ± 0.00	1.31 ± 0.53	7.00 ± 3,35
	IAA 5,000 ppm	96.67 ± 5.77	0.00 ± 0.00	1.15 ± 0.61	5.76 ± 2.13
	NAA 3,000 ppm	100.00 ± 0.00	0.00 ± 0.00	1.38 ± 1.17	6.67 ± 4.14
	NAA 5,000 ppm	100.00 ± 0.00	0.00 ± 0.00	1.35 ± 0.93	7.60 ± 5.47
	Control	93.33 ± 11.55	0.00 ± 0.00	1.06 ± 0.61	4.82 ± 2.82
	Average	98.57 ± 4.78	0.00 ± 0.00	1.28 ± 0.79	6.69 ± 3.67
GM-2	IBA 3,000 ppm	100.00 ± 0.00	0.00 ± 0.00	2.01 ± 1.96	7.83 ± 6.51
	IBA 5,000 ppm	96.67 ± 5.77	3.33 ± 5.77	1.83 ± 0.91	7.45 ± 2.80
	IAA 3,000 ppm	100.00 ± 0.00	0.00 ± 0.00	1.57 ± 1.76	6.70 ± 4.65
	IAA 5,000 ppm	93.33 ± 11.55	0.00 ± 0.00	1.25 ± 0.70	5.43 ± 2.46
	NAA 3,000 ppm	93.33 ± 11.55	3.33 ± 5.77	1.44 ± 0.71	6.54 ± 2.85
	NAA 5,000 ppm	96.67 ± 5.77	3.33 ± 5.77	1.49 ± 0.63	5.83 ± 2.25
	Control	90.00 ± 0.00	3.33 ± 5.77	1.29 ± 0.58	4.04 ± 2.30
	Average	95.71 ± 6.76	1.90 ± 4.02	1.56 ± 1.19	6.29 ± 3.88

Table 2. The results of variance analysis

	RP		CP		RL		RN	
	F	*p*	*F*	*p*	*F*	*p*	*F*	*p*
GM	2.400	0.133	4.000	0.055	7.526	0.006*	1.239	0.266
PH	1.511	0.211	0.500	0.803	2.005	0.064	5.199	0.000 *
GM × PH	0.222	0.966	0.500	0.803	0.817	0.557	0.535	0.782

Note: * $p < 0.01$: there is a statistically significant difference at the 99% confidence level.

ment in GM-1, it occurred with very low percentages in the GM-2. The highest values in terms of root length and the number of roots were found as 2.01 cm and 7.83 roots in IBA 3,000 ppm treatment in GM-2, respectively. Approximately twice as high results were obtained compared to control cuttings (1.29 cm and 4.04 roots) in the same medium. The results of the variance analysis performed to determine whether there are statistically significant differences in terms of greenhouse media, phytohormones and greenhouse media × phytohormones interaction regarding RP, CP, RL and RN data are given in Table 2.

According to the results of variance analysis, there was no statistically significant difference in RP and CP in terms of greenhouse media, phytohormones and greenhouse media × phytohormones interaction, while it was found that there were statistically significant differences between the greenhouse media in terms of RL and between the phytohormones in terms of RN at 99% confidence level. The graphical representation of the changes in the greenhouse media in terms of RL and RN is given in Figure 3.

When the graphs regarding the RL and RN data were examined, higher results were obtained in the GM-2 in terms of root length, while there is a general balance be-

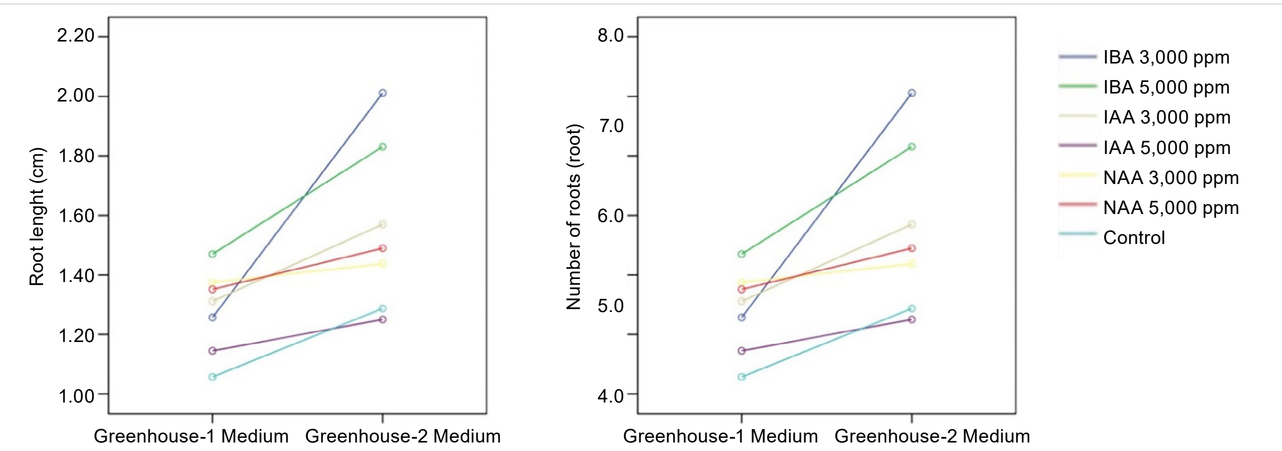

Figure 3. The state of root morphology in greenhouse media related to RL and RN

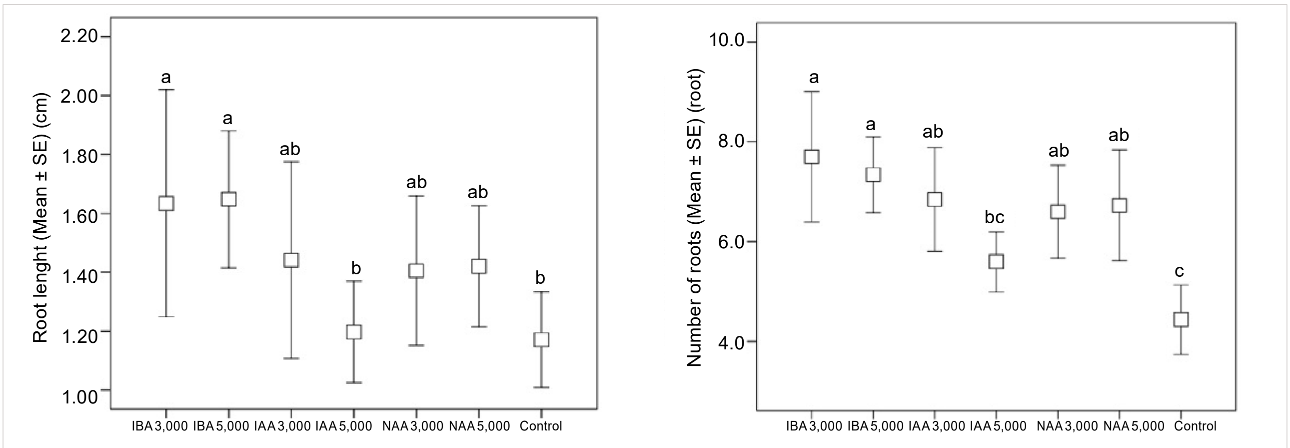

Figure 4. Duncan's test results for RL and RN

tween greenhouse media in terms of the number of roots. However, only NAA 5,000 ppm treatment and control cuttings were found to have lower results in GM-2 concerning the number of roots. Additionally, Duncan's test results regarding the measured characteristics are schematized in Figure 4.

Since there were no significant differences between the phytohormones applied in terms of rooting percentage and callus percentage, groupings did not occur. Three different groups emerged in terms of root length and four different groups in terms of the number of roots. Accordingly, in terms of both root morphological characteristics, it was determined that IBA 3,000 and IBA 5,000 ppm treatments formed the first groups together, while control cuttings formed the last groups.

Discussion and conclusion

This study examined the effects of different rooting table temperatures and phytohormones on rooting in the propagation by softwood cutting of *Buxus sempervirens* L. subsp. *sempervirens*, 29 days after planting, the first root formations were determined in IBA 3,000 and NAA 5,000 ppm treatments in GM-2. In addition, after the rooting period of 57 days, 100% rooting percentage was determined and the cuttings were removed from the rooting media. Vieira et al. (2018) reported that a period of about 116 days is needed for the rooting of semi-hardwood cuttings of *B. sempervirens*. In our study using softwood cuttings for *B. sempervirens* subsp. *sempervirens*, full rooting was achieved in almost half the time (57 days) reported by Vieira et al. (2018).

In the study conducted by Banko and Stefani (1986) on the cutting propagation of *Buxus sempervirens*, nodal (cuttings were cut just below a node) and internodal (cuttings were cut above a node) cuttings were used. They stated that the highest rooting percentage was determined as 78.5% in nodal cuttings that wounding and IBA combined application were applied. In another study, control, IBA 1,500 mgL^{-1}, IBA 3,000 mgL^{-1} and IBA 6,000 mgL^{-1} for *B. sempervirens* semi-hardwood cuttings had the highest rooting percentage of 97.5% (Vieira et al. 2018). In our study, the highest rooting percentage (100%) was determined in IBA 3,000, IBA 5,000, IAA 3,000, NAA 3,000 and NAA 5,000 ppm treatments in GM-1 and IBA 3,000 and IAA 3,000 ppm treatments in GM-2. In general, callus formation did not occur in many treatments, while the highest values in terms of root length (2.01 cm) and the number of roots (7.83 roots) occurred in IBA 3,000 ppm treatment in GM-2. However, the higher average number of roots (6.69 roots) was obtained in GM-1 having 5°C higher rooting table temperature compared to GM-2. As a result of the study, higher results were obtained in phytohormone-treated cuttings compared to control cuttings in terms of RP, RL and RN. Banko and Stefani (1986) reported that the longest root lengths obtained in *B. sempervirens* were 2.40 cm (nodal cuttings) and 2.60 cm (internodal cuttings) in the combination of wounding and IBA treatment. For *B. sempervirens* semi-hardwood cuttings, Vieira et al. (2018) obtained the highest number of roots and the longest root length with 3.73 roots and 2.55 cm, respectively, in the control cuttings, while IBA 6,000 mgL^{-1} took the second place with 3.66 roots and 2.45 cm. Therefore, while similar results were obtained with the longest root length (2.01 cm) detected in *B. sempervirens* subsp. *sempervirens* softwood cuttings used in our study, higher results were obtained in terms of the highest number of roots (7.83 roots). Based on this, it can be stated that IBA phytohormone (IBA 3,000 ppm) applied in lower concentrations results in better root morphology with the higher number of roots.

There are many studies investigating the effects of auxins such as IBA, IAA and NAA in cutting propagation. Some researchers reported that auxins had positive effects on rooting in their studies conducted on *Vaccinium corymbosum* L. (Turna et al. 2013), *Ficus benjamina* L. (Topaçoğlu et al. 2016), *Taxus baccata* L. (Bayraktar et al. 2018a), *Elaeagnus umbellata* Thunb. (Bayraktar et al. 2018b) and *Salix anatolica* Ziel. et D. Tomasz. (Yildirim et al. 2020). In parallel with these studies, in our study it was determined that the cuttings treated with auxins (IBA, IAA and NAA) provide better rooting than the control cuttings, especially in terms of root morphology. Also, the fact that the rooting table temperature was 5°C higher than the ambient air temperature (GM-1) had positive effects on rooting. On the other hand, it has been determined that when propagating with softwood cuttings of *B. sempervirens* subsp. *sempervirens*, half the time required for rooting is saved compared to the study conducted by Vieira et al. (2018), using *B. sempervirens* semi-hardwood cuttings. In addition, it has been determined that high rooting success can be achieved with different auxins (IAA and NAA) and low concentrations, except for IBA used in conducted studies (Banko and Stefani 1986, Vieira et al. 2018), and thus different alternatives have emerged for phytohormone use. As a matter of fact, in a study, it was reported that the re-establishment of natural boxwood forests can be contributed by planting the seedlings that will be obtained by propagating with the cuttings taken from the surviving and resistant boxwood individuals in areas where there are no factors that cause boxwood blight (Özkaya 2020). Based on the results obtained in the present study, although boxwood natural distribution areas suffered damage in Türkiye as in the world, it will be possible to make these areas productive by using the quality seedlings to be produced with softwood cuttings of boxwood and making appropriate silvicultural interventions. In addition, as a result of the production to be made with the method used in this study, the large number of seedlings required for boxwood, which is used extensively in landscaping works, will be easily produced in a short time.

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