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The effects of cinnamic acid and IBA treatments on the rooting of wood cuttings of black mulberry (*Morus nigra* L.)†

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† This study is derived from a part of Nur Selin Karabulut's master thesis.

ABSTRACT

In this study, the effects of IBA and cinnamic acid applications on rooting of black mulberry cuttings taken from black mulberry trees grown in Tokat were treated. 6000 ppm Indole Butyric Acid (IBA), 6000 ppm IBA+100 ppm cinnamic acid (CA), 100 ppm cinnamic acid with control which no application was applied on the cuttings taken in July, September, November and January periods. The cuttings were kept during 60 days in rooting beds containing perlite and heated from the bottom. The highest rooting ratio (48.3%) and the highest average root number (3.1) were obtained from the cuttings taken November and January periods with 6000 ppm IBA + 100 ppm CA application. Among the applications and the periods, cuttings were taken, 6000 ppm IBA + 100 ppm CA application and July and January periods were superior to others.

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1. Introduction

Mulberry (*Morus* spp.) is the tree that creates the *Morus* genus of the *Moraceae* family of *Urticales* team, which can be grown in temperate, tropical and subtropical climates in the northern hemisphere by adapting to different climatic and soil conditions with high adaptability. The mulberry plant is common in many parts of the world (Datta, 2002). *Morus alba*'s homeland is China, Japan, Thailand, Malaysia and Burma; *Morus nigra* homeland is Turkey, Persia, Arabia, parts of Russia located in South Asia, and Syria; *Morus rubra*'s homeland is North America (Bellini et al., 2000). The species commonly made growing in Turkey; *Morus alba* (white mulberry), *Morus nigra* (black mulberry) and *Morus rubra* (red or purple mulberry). Turkey is one of the important producers with approximately 70000 tons of mulberry production (TUIK, 2020). In every region of Turkey which made the growing of fruit trees, mulberry cultivation can be done. Mulberry mostly likes warm, temperate and sunny regions. Optimum temperature demand of Mulberry is 24-28 °C (Özgen, 2010). It can be cultivated in places with annual rainfall amount is from 600 mm to 2500 mm and in places higher than sea level. The ideal humidity rate in mulberry cultivation is around 65-80% (Anonymous, 2013). Mulberry tree grows best in loamy, sandy-loam or clay-loam soils. The pH value of the soil should be 6.5-7. Groundwater should not be close to the soil surface, especially where the mulberry tree is planted. Mulberry is not selective in terms of soil and climate conditions except salty soils (Özgen, 2010). In mulberry production, vegetative methods such as grafting, layering tissue culture and production with cutting are used. In process of propagation by grafting, because of its high labor force and the lack of educated people to graft limits the success of grafting. In production with tissue culture, the desired level could not be reached in practical terms due to the need for special techniques and equipment. Due to the better results obtained compared to other vegetative reproduction methods, the most used method is the propagation by cutting (Anonymous, 2013). Propagation by cutting varies depending on the time of taking the cutting, the species and variety, the type of cutting, and the ecological conditions of the region where the main plant is grown. In the cutting reproduction method, generally, perlite is used in rooting environments and IBA hormone is used to increase rooting (Ryu, 1977). Many researchers working on the reproduction of mulberry with cuttings determined that rooting-stimulating hormones increase the root formation in mulberry cuttings (Şenel, 2002). In the cutting reproduction of mulberry plant, generally commercially available IBA's concentrations of 4000-8000 ppm are used. Soft cuttings are immersed in the prepared solution for 5-10 seconds and planted in the rooting medium. To achieve more successful root development, especially in rooting of wood cuttings, to heat the root regions at 21-27 °C during the day, and at 16-21 °C during the night will increase rooting by promoting cell division (Anonymous, 2013). Ünal et al. (1992) stated in their study that it did not reach maximum rooting rates with also IBA doses in black mulberry. In the studies to determine the rooting performance of black mulberry cuttings, rooting rates of up to 60% were reached by using different IBA concentrations (2000-6000 ppm) (Karadeniz and Şişman, 2003; Koyuncu and Vural, 2003; Erdoğan and Aygün, 2006). In many studies, it has been stated that the sugar and protein content, as well as the change in phenolic compounds within the plant, plays an important role in terms of adventive root formation (Kaur et al., 2002; Qaddoury and Amssa, 2004;

Kevresan et al., 2007; Satish et al., 2008). Researchers have stated that some phenolic compounds promote rooting while others prevent (Schneider and Wightman, 1974; Faust, 1989; Bandurski et al., 1995). It has been determined that especially when cinnamic acid is used together with IBA, it has a positive effect in terms of rooting performance (Padney and Pathak, 1981).

This study aimed to investigate the effects of cinnamic acid, IBA, and period differences on rooting in black mulberry cuttings that are difficult to root.

2. Materials and methods

The research was carried out in the rooting greenhouse of Tokat Gaziosmanpaşa University Agricultural Application and Research Center in 2014-2015. The research was planned in four different periods with four different applications and three repetitions. In the study, cuttings taken from old branches were prepared in 15 cm length and 15 cuttings were planted in each repetition. Cuttings were put in a heated mist propagation unit which was filled with perlite. Keeping the rooting temperature and humidity values at the desired level were achieved by a controlled automatic system. Before planting, the cuttings were immersed in a fungicide solution to protect against fungal infections. While preparing the solutions, the active ingredients were first dissolved in methanol and then water and methanol were added to the mixture according to the dilution ratio to be used. 1 cm of the cuttings from the bottom was immersed in 6000 ppm IBA, 100 ppm CA and 100 ppm CA + 6000 ppm IBA solutions for 5 s. Then, the cuttings were kept for 1-2 min and the alcohol on them was allowed to evaporate. Since the period differences are considered, planting was done in 4 periods. Cuttings planted on tables were kept in a rooting medium for 2-3 months. At the end of this period, measurements were made on the cuttings removed from the rooting medium.

Callus rate (%): Callus cuttings were counted and expressed in % (Edizer et al., 2016).

Rooting rate (%): Rooted cuttings were counted and expressed in % (Edizer et al., 2016).

Average number of roots per plant (number/plant): All roots of cuttings rooted in each plot were counted and recorded and the average of the results was calculated (Edizer et al., 2016).

Average root length (mm): The average of the results was calculated in mm by measuring with a digital caliper the roots of the cuttings rooted in each plot (Saracoglu et al., 2016).

Root diameter (mm): The diameters of the cuttings rooted in each parcel were measured with a digital caliper and the results were calculated in mm (Saracoglu et al., 2016).

The study was arranged according to the random parcel trial pattern in three repetitions. Fifteen cuttings were used in each repetition. Obtained data were subjected to analysis of variance (ANOVA) by using SAS software, and LSD test was preferred for comparing the averages.

3. Results and discussion

As it can be understood from the Table 1 it was determined that the effect of plant growth regulator doses on black mulberry wood cuttings callus rate was statistically significant at 5% level. The highest callus rate was obtained from 6000 ppm IBA dose in January (97.8 %). The lowest callus rate was obtained from 100 ppm CA dose in November (2.2%). In terms of average callus rate treatments 6000 ppm IBA had the highest callus rate. It was found that the callus rate was highest in July, while the lowest callus rate in November. In the study of Özkan et al. (1995), it was stated that the best callus formation occurred in green cuttings with 6000 ppm IBA application.

Table 1. The effect of plant growth regulator concentrations applied on callus rate (%)

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	88.9 A-a	82.2 A-a	2.2 B-b	68.9 A-a	60.6 a
100 ppm CA + 6000 ppm IBA	93.3 AB-a	64.4 B-a	31.1 C-ab	95.6 A-a	71.1 a
6000 ppm IBA	86.7 A-a	68.9 A-a	35.6 B-a	97.8 A-a	72.2 a
Control	89.0 A-a	75.6 A-a	24.4 B-ab	93.3 A-a	70.6 a
Average	89.47 A	72.8 B	23.3 C	88.9 A	68.6

The differences between the period averages shown in the same capital letter in the same row and the application averages shown in the same column in the same lower case are not important ($P<0.05$).

When Table 2 is examined, the highest rooting rate in black mulberry wood cuttings dose of 100 ppm CA+6000 ppm IBA in July (%88.9), while the lowest rate of rooting at the dose of 100 ppm CA in November (%0.0). In terms of average rooting rate, treatment of 100 ppm CA+6000 ppm IBA has highest rooting rate. In the black mulberry wood cutting the highest rooting rate in January, while in September the lowest rooting rate. In the study conducted by Kako (2012) the lowest rooting rate was determined in the control groups. Similarly, Mohammed and Kako (2021) stated that high IBA doses were effective in increasing the rooting rate.

As can be seen from Table 3 dose of 100 ppm CA+6000 ppm has the highest average number of root in July (5.7), the lowest average number of root 100 ppm CA dose in November (0.0). In terms of doses applied in black mulberry cuttings average the number of root was highest in 100 ppm+6000 ppm IBA dose, followed by 6000 ppm IBA and 100 ppm CA doses. It was found that the average number of root was highest in July, while the lowest average number of root in November.

Table 2. The effect of plant growth regulator concentrations applied on the rooting rate (%)

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	31.1 B-b	11.4 BC-a	0.0 C-b	60.0 A-a	25.6 b
100 ppm CA + 6000 ppm IBA	88.9 A-a	8.9 D-a	31.1 C-a	64.4 B-a	48.3 a
6000 ppm IBA	83.0 A-a	6.6 C-a	22.2 C-a	55.5 B-a	41.8 a
Control	22.3 B-b	6.7 B-a	24.4 B-a	46.7 A-a	25.0 b
Average	56.3 A	8.4 C	19.4 B	56.7 A	35.2

The differences between the period averages shown in the same capital letter in the same row and the application averages shown in the same column in the same lower case are not important (P<0.05).

Yıldız et al. (2009) found that the highest number of roots with 6000 ppm IBA application in the wood cuttings taken in winter and green cuttings taken in the summer period. Similarly, Edizer et al. (2016) stated that high IBA doses were effective in increasing the number of roots. As it can be understood from the Table 4 it was determined that the effect of plant growth regulator doses on black mulberry wood cuttings root length was statistically significant at 5% level. The highest root length was obtained from 100 ppm CA + 6000 ppm IBA dose in July (109.1 mm). The lowest root length was obtained from 100 ppm CA dose in November (0.0 mm). In terms of root length treatments 100 ppm CA+ 6000 ppm IBA had the highest root length. It was found that the root length was highest in July, while the lowest root length in September. Many researchers found that the highest average root length with 6000 ppm IBA application in the wood cuttings taken in winter and green cuttings taken in the summer period (Yıldız et al., 2009; Cekic et al., 2013).

Table 3. The effect of plant growth regulator concentrations applied on the average root number

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	1.6 A-b	2.5 A-a	0.0 A-a	3.2 A-a	1.8 a
100 ppm CA + 6000 ppm IBA	5.7 A-a	2.7 AB-a	1.7 B-a	2.3 B-a	3.1 a
6000 ppm IBA	4.6 A-ab	3.0 A-a	1.6 A-a	2.8 A-a	3.0 a
Control	2.3 A-b	2.2 A-a	1.5 A-a	2.1 A-a	2.0 a
Average	3.6 A	2.6 AB	1.20 B	2.6 AB	2.6

The differences between the period averages shown in the same capital letter in the same row and the application averages shown in the same column in the same lower case are not important. (P<0.05).

As stated in Table 5 doses of 100 ppm CA and 6000 ppm IBA have widest root diameter in July (1.9 mm), while dose of 100 ppm CA has narrowest root diameter in November (0.0 mm). In terms of root diameter treatments 100 ppm CA have widest root. It was found that root diameter was widest in July, while the narrowest root diameter in September. Yıldız et al. (2009) found that the highest root diameter ratio with 6000 ppm IBA application in wood cuttings taken in the winter period, green cuttings are taken in summer period and semi-wood cuttings taken in October. In addition, many researchers stated that the lowest root diameter values were obtained from the low IBA doses samples (Cekic et al., 2013; Singh, 2018).

Table 4. Effect of applied plant growth regulator concentrations on mean root length (mm)

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	68.3 A-a	50.4 A-a	-	84.9 A-a	67.9 a
100 ppm CA + 6000 ppm IBA	109.1 A-a	37.8 B-a	50.9 B-a	89.5 AB-a	71.8 a
6000 ppm IBA	86.9 A-a	24.1 B-a	44.7 AB-a	50.9 AB-a	51.6 a
Control	88.9 A-a	24.3 B-a	46.0 AB-a	49.5 AB-a	52.2 a
Average	88.3 A	34.1 B	47.2 B	68.7 A	60.9

The differences between the period averages shown in the same capital letter in the same row and the application averages shown in the same column in the same lower case are not important. (P<0.05).

4. Conclusion

In our study, it was investigated the changes in the rooting performance of the cuttings as a result of using indole butyric acid and individually and together cinnamic acid in different cuttings taking periods in black mulberry plant. According to the findings, the best rooting rate was found as 88.9% in the cuttings treated with 6000 ppm IBA + 100 ppm CA in July. The best mean root number was 5.7 in the cuttings treated with 6000 ppm IBA + 100 ppm CA in July and the highest root length was 109.1 mm in the cuttings treated with 6000 ppm IBA + 100 ppm CA. 100 ppm CA + 6000 ppm IBA treatment was generally more successful and it was determined that the periods in January and July were the most successful. The most successful treatment terms of rooting rate, which is of great importance for rooting performance, was found as 100 ppm CA + 6000 ppm IBA treatment in July. According to the results of this study, we

can say that 6000 ppm IBA + 100 ppm CA treatment on the cuttings taken in July and January is the most suitable growth regulator dose for rooting, root number, root length and root diameter in rooting wood cuttings of black mulberry variety in Tokat region.

Table 5. The effect of applied plant growth regulator on average root diameter (mm)

Application	Callus rate %				
	July	September	November	January	Average
100 ppm CA	1.9 A-a	1.0 AB-a	-	1.6 A-a	1.5 a
100 ppm CA + 6000 ppm IBA	1.8 A-a	0.4 B-a	0.7 AB-a	1.0 AB-a	0.9 a
6000 ppm IBA	1.9 A-a	0.6 B-a	1.0 AB-a	0.8 AB-a	1.1 a
Control	1.4 A-a	0.9 A-a	0.9 A-a	1.3 A-a	1.1 a
Average	1.8 A	0.7 B	0.9 B	1.2 B	1.2

The differences between the period averages shown in the same capital letter in the same row and the application averages sho wn in the same column in the same lower case are not important. (P<0.05).

This study, in which we investigated the effect of cinnamic acid and indole butyric acid treatments on rooting in black mulberry cuttings, is important in terms of providing a source for hormone applications in future studies of reproduction with cutting. Studies to be done on this subject in more detail, to development of mulberry cultivation and sapling production in Turkey will be beneficial. When the findings we obtained were examined, the highest values in callus formation, rooting rate, average root number, average root length, and root diameter were reached in 6000 ppm IBA and 100 ppm CA + 6000 ppm IBA applications. Considering the period, the best results were obtained in July.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Nur Selin Karabulut: participated in data collection, analysis, description, and draft the manuscript. **Onur Saraçoğlu**: participated in supervision of the work starting from the proposal up to final draft, edited and revised the manuscript.

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