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Rooting of black elderberry (*Sambucus nigra* L.) by treating of indole-3-acetic acid

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ABSTRACT

Lack elderberry (*Sambucus nigra* L.) is a perennial plant in the form of a shrub or semi-shrub in the Adoxaceae family. It spreads naturally in the Black Sea, Central Anatolia, Southern and Eastern Anatolia regions of Turkey. Black elderberry fruits are traditionally used as food and medicine in Europe. Hippocrates considered it the greatest medicinal plant of nature. In traditional treatment, its flowers are used as a diaphoretic analgesic. Its fruits are used to treat prostate and hemorrhoids; pharmaceutical products are used to treatment cold and flu symptoms. Propagation from seed is complex due to the plant's high seed dormancy. Therefore, the plant is propagated by cuttings. This study aimed to root the elderberry annual and biennial wood cuttings by IAA (Indol-3-Acetic Acid) application. Cuttings were taken on 09.11.2020 from annual and biennial wood branches, 10-15 cm long, with two nodes. On the same day, the cuttings were treated with 0-5-15-30 ppm doses of IAA for 15 seconds, then planted in 25x30 cm pots filled with a mixture of worm manure+peat+soil+perlite at a ratio of 1:1:1:1.5. According to the experimental design of split plots randomized blocks, the experiment was established in the greenhouse condition with 3 replications and 5 plants in each replicate (pot). In this research, it was observed that the first rooting was 17 days after planting. The root lengths were measured by removing them from the pots 120 days after planting, waiting for the strengthening of root development. As a result of the research, the best root length and development were determined by control (20.47 cm) and 30 ppm (22.53 cm) treatments. Annual cuttings (96%) are rooted better than biennial cuttings (76%). While the highest rooting rate in stem cuttings was obtained with 30 ppm (100%) IAA application, weak shoot development was observed in the identical wood cuttings. Control (93%) cuttings followed the rooting rate, but shoot development was better than other applications. As a result, considering the root length and shoot development, it was concluded that two-node annual wood cuttings taken in November could be used for rooting and reproduction without the need for IAA growth hormone.

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1. Introduction

Black elderberry (*Sambucus nigra* L.) is a perennial herb in the Adoxaceae family, found in a shrub or semi-shrub form (Donoghue et al., 2003; Dundar, 2009). While it spreads in different places in the world such as subtropical regions of Asia, North Africa, and North America (Finn et al., 2008; Młynarczyk et al., 2018), it grows in the Black Sea, Central Anatolia, and Southern and Eastern Anatolia regions in our country (Ozdemir et al., 2019).

All plant parts of black elderberry, primarily fruits and flowers, are rich in phytochemicals such as carbohydrates, lipids, flavonoids, phenolic acids, terpenoids, and alkaloids (Finn et al., 2008). It is a medicinal plant, defined by Hippocrates as "the medicine chest" (Agalar, 2019).

In addition to the traditional use of fruit and flower extracts to treat colds and flu, it has been clinically tested. Today, there are industrially produced elderberry-based pharmaceutical products (Ulbricht et al., 2014; Porter and Bode, 2017). However, the whole part of the plant is used for bronchitis, cold, cough, upper respiratory tract diseases, pain reliever, diuretic, and anti-inflammatory (Zakay et al., 2004; Odabaş, 2020).

Moreover, its fruits are also used in the food industry in Europe to produce pies, jellies, jams, ice cream, yogurt, and alcoholic beverages (Senica et al., 2016).

Thanks to the benefits provided by these characteristics, the interest in black elderberry, which has a wide usage area, has increased, and cultivation studies have begun (Lorenzo et al., 2018; Milena et al., 2019). Black elderberry, which spreads especially in Northern Anatolia in Turkey, can be propagated vegetatively or generatively (Ozdemir et al., 2019). In elderberry seeds, the germination rate of seeds is low due to dormancy (Leif et al., 2011). Therefore, it is propagated vegetatively by cuttings.

Various applications are performed in propagating with cuttings. Plant growth regulators are at the forefront of these applications. The purpose of applying these regulators is to

accelerate the root formation of cuttings, to increase the number and quality of roots per cutting (Zenginbal et al., 2006; Seker et al., 2010).

The most commonly used hormone in plant growth regulators is auxin. Auxins promote plant growth by increasing cell division and rooting (Saini et al., 2012). Indole-3-acetic acid (IAA) is the only naturally synthesized hormone in plants. While IAA stops the branching in the shoots during the development of the plants, it promotes root development by increasing the branching in the root (Sezgin and Kahya, 2018).

This study investigated the effects of optimum wood cutting age and IAA (Indole-3-Acetic Acid) doses on rooting in *Sambucus nigra* L.

2. Materials and methods

2.1. Plant materials

The research was carried out in the greenhouse conditions of Samsun Ondokuz Mayıs University Faculty of Agriculture Department of Field Crops. As plant material, *Sambucus nigra* L. wood branch cuttings (single and biennial) were used during the dormant period of the plant (Figure 1; A-B). The cuttings taken were prepared to be 10-15 cm long. The upper part of the prepared cuttings was covered with grafting paste to prevent water loss (Figure 1; C).

a b c

Figure 1. One-year wood branch cuttings (a), two-year wood branch cuttings (b), cuttings with grafting paste (c).

Rooting medium was prepared from soil, peat, vermicompost, and perlite at a ratio of 1:1:1:1.5. This mixture was filled in 25x30 cm pots and watered, then planting was done, and the pots were kept in greenhouse conditions throughout the experiment. As a hormone, 5 ppm, 15 ppm, 30 ppm doses of commercial IAA were prepared and compared with the untreated control (zero-dose).

2.2. Methods

The study investigated the effects of wood cutting age and IAA doses on rooting in *Sambucus nigra* L. In the present experiment, cutting ages were placed in the main plots, and IAA doses were placed in the sub-plots according to the split plots trial design in random blocks with 3 replications. Each pot is planned with one replication per dose. In each pot (repeat) 5 wood cuttings were placed. A total of 120 wood branch cuttings were used.

The 2-3 cm lower part of the branch cuttings were planted in pots after being kept in the solution prepared for each application on 13 October 2020 at 15 s. After planting, irrigation was done with daily checks. No disease or damage was recorded during the experiment.

In the observations made after planting, the first rooting was seen in applying of 30 ppm (one-year wood cutting) between IAA applications on the 17th day. However, the research was continued for 120 days to observe root length and shoot development. Rooting rate (%) and root length (cm) were determined by removing the cuttings from the pots on the 120th day of rooting.

The variance analysis of the obtained data was performed with the MSTAT-C statistical package program. The DUNCAN multiple comparison tests determined the difference between the means according to the importance levels.

3. Results and discussion

As it can be understood from Table 1, the data obtained on root length and rooting rates with IAA (Indole-3-Acetic Acid) application in one-year and two-year wood cuttings of black elderberry (*Sambucus nigra* L.) are given in Table 1-2.

According to the analysis of variance results, the root length was statistically significant ($p<0.01$) among the applied IAA doses, and it was not significant for the cutting age and the cutting age x dose interactions.

Although statistically, the difference between means of applications is insignificant, root length was longer in one-year cuttings (20.18 cm) than two-year cuttings (18.41 cm). Among the dose applications, the longest root was obtained from 30 ppm (22.53 cm) applications, followed by control (20.47 cm) applications. The shortest average root length was obtained from the application of 15 ppm (15.97 cm).

The longest root was measured for two-year cuttings at 30 ppm (23.33 cm) and the shortest at 15 ppm (12.87 cm). In cuttings other than 30 ppm between applications, increasing the cutting age decreased the root length (Table 1).

When the IAA application × cutting age interaction was examined, the mean root length was measured as 20.93 cm in one-year cuttings without IAA (control) and 20.00 cm in two-year cuttings. In general, root and shoot development of one-year cuttings was better than that of two-year cuttings (Table 1; Figure 2. A-E). Mean root lengths of one-year and two-year cuttings were measured as 19.00 cm and 17.47 cm, respectively, at a dose of 5 ppm of IAA application, and shoot growth was better developed in one-year wood-branch cuttings than in two-year cuttings. (Table 1; Figure 2. B-F). Root length was measured as 19.07 cm and 12.87 cm, respectively, in one- and two-year cuttings at the applied 15 ppm dose (Table 1; Figure 2. C-G), while in 30 ppm IAA application, it was recorded as 21.73 cm and 23.33 cm, respectively (Table 1; Figure 2. D-H).

Table 1. Root length of IAA application in one-year and two-year wood cuttings of black elderberry (*Sambucus nigra* L.)

Doses (ppm)	Average Root Lengths (cm)		
	Annual wood cuttings	Perennial wood cuttings	Mean *
Control	20.93	20.00	20.47 [ab]
5	19.00	17.47	18.23 [bc]
15	19.07	12.87	15.97 [c]
30	21.73	23.33	22.53 [a]
Mean	20.18	18.41	

*Means in columns with the same letter do not differ according to Duncan test at P<0.01

Table 2. Rooting rates of IAA application in one-year and two-year wood cuttings of black elderberry (*Sambucus nigra* L.)

Doses (ppm)	Rooting rates (%)		
	Annual wood cuttings	Perennial wood cuttings	Mean (%)
Control	100	86	93
5 ppm	93	66	80
15 ppm	93	60	73
30 ppm	100	100	100
Mean	96	76	

Figure 2 . Control one-year wood cuttings (A), 5 ppm one-year wood cutting (B), 15 ppm one-year wood branch steel (C), 30 ppm one-year wood branch steel (D), control two-year wood branch steel (E), 5 ppm two-year wood branch steel (F), 15 ppm two-year wood branch steel (G), 30 ppm two-year wood branch steel (H)

While the highest rooting rate was obtained with 30 ppm (100%) IAA application in one- and two-year cuttings, weak shoot development was observed. In terms of rooting rate, it was followed by one-year (100%) and two-year (86%) wood branch cuttings of the control application. It was followed by one-year (100%) and two-year (86%) wood branch cuttings of the control application in terms of rooting rate. Shoots of control's cuttings developed better than other applications. The lowest rooting rate was measured in two-year cuttings at 15 ppm application.

Regression analysis was performed because the doses of IAA applied on the rooting of elderberry had a very

significant effect. As a result of orthogonal fragmentation, it was determined that the effect of different IAA doses on root lengths in *Sambucus nigra* L. was quadratic. The equation that emerged in the regression curve shown in Figure 3 was determined as y = 0.0241 x 2 - 0.6609 x + 20.644, and the R^2 value was determined as 0.9954. It was concluded from the formula -b/2xa in the equation that the minimum mean root length would be obtained at a dose of 13.71 ppm IAA.

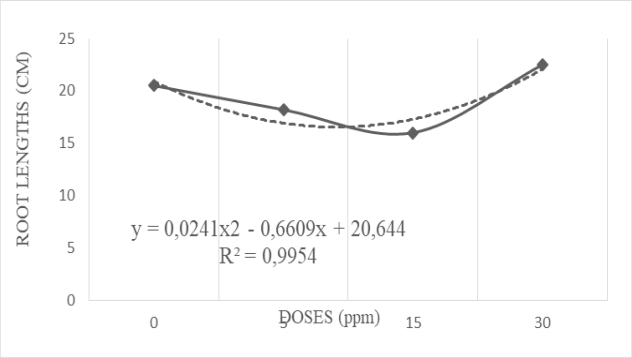

Figure 3. Regression graph of the effect of applied different doses of IAA on rooting

Studies have shown that successful rooting of plant cuttings does not depend solely on the use of auxins. External factors such as the environment in which the cuttings are located, the time and duration of irrigation, and the ambient temperature influence rooting (Tiberia et al., 2011). The studies determined that IAA gave successful rooting percentage, root length, and several roots, and it could be used for rooting hard cuttings of berberis species (Nautiyal and Purohit, 1986).

Although Gudeva et al. (2017) reported that 5 ppm IAA concentration had a positive effect on rooting in elderberry, this dose was found to be behind the control and 30 ppm application in our study. Our study determined that cutting age (one and two-year) affected on rooting rate and root length (Table 1-2). The rooting rate (96%) was higher in one-year cuttings than in two-year cuttings (76%). As Çetin (2016) stated, as the age of the rootstock from which the cuttings were taken increased, the rooting rate of the cuttings decreased. While Gerçekcioglu et al. (2008) reported that 250 ppm IBA application increased rooting in young cuttings in *Sambucus nigra* L. Villa et al. (2018) determined that cuttings taken from the apical meristem and middle regions of black raspberry were rooted before. Moreover, Kucuk et al. (2010), Villa et al. (2018) and Adekola et al. (2012) reported in their studies on rooting of stem cuttings of different plants with different growth hormones that there is no need for hormone application for rooting, and that rooting is superior to other applications in

While Murtic et al. (2015) recommended a 0.8% IBA dose for rooting *Sambucus nigra* L. cuttings, Seker et al. (2010) recommended 6000 ppm IBA application in *Arbutus unedo* L., Ozelbaykal and Gezerel (2005) stated that 4000-6000 ppm IBA dose in the rooting of 'Gemlik' olive cultivar, and Seran et al. (2011) stated that 2500 ppm IAA dose in lemon stem cuttings increased rooting.

The literature shows that applied rooting hormones and cutting ages showed different effects according to plant species. Our research discovered that one-year wood cuttings with a length of 10-15 cm and at least two nodes could be utilized successfully for rooting *Sambucus nigra* L. without the need for growth hormone.

4. Conclusion

Our study determined root length and root growth best in control dose and 30 ppm dose applications. One-year wood branch cuttings root better than two-year cuttings. While 30 ppm IAA administration resulted in the greatest rooting rate in one-year wood branch cuttings, weak shoot development was seen in the identical cuttings. One-year wood cuttings followed the control application in terms of rooting rate; however, shoots of the control cuttings developed better than in other applications.

Therefore, considering the root length and shoot development, it was determined that one-year wood cuttings with at least two nodes collected in November could be utilized successfully for rooting and propagation without the need for IAA growth hormone.

Compliance with Ethical Standards

Conflict of Interest

The authors declare that they have no conflict of interest.

Authors' Contributions

Nazlıcan Sönmezışık: Investigation, Writing, Formal analysis, Data curation. **Şahane Funda Arslanoğlu**: Methodology, Investigation, Conceptualization, Validation, Writing - original draft, Visualization. **Rümeysa Öztürk**: Investigation, Data collection, Formal analysis. **Mehmet Han Baştürk**: Investigation, Data collection, Formal analysis.

Ethical approval

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Data availability

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Consent for publication

Not applicable.

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