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Propagation of Some Sea Buckthorn (*Hippophae rhamnoides L.*) Cultivars By Semi-Hardwood Cuttings

Mehmet Güneş, Onur Sefa Alkaç*, Osman Nuri Öcalan

Department of Horticulture, Agricultural Faculty, Tokat Gaziosmanpaşa University, Tokat, Turkey
**Corresponding author, onursefa.alkac5018@gop.edu.tr*

ABSTRACT: This research was carried out in the mist propagation unit of the Agricultural Research and Application Center of Tokat Gaziosmanpaşa University. Semi-hardwood cuttings of 'Hergo', 'Habego', 'Siberian Pearl', 'Leikora', and 'Pollmix' sea buckthorn cultivars were used as plant materials. Cuttings were taken in the middle of August and prepared in 15 cm length and treated with 0 (control), 500, 1000, and 2000 Indole-3 butyric acid (IBA). After ten weeks the cuttings were removed from the medium and callus rate (%), rooting rate (%), root length (cm), root number (piece), root wet weight (g), root dry weight (g) and sapling survival (%) were determined. The rooting rates were 76.67-97.00%, root lengths were 8.33-10.76 cm, root numbers were 3.67-5.91, fresh root weights were 0.52-0.70 g, dry root weights were 0.09-0.16 g, sapling survival varied between 71.23-87.45%, but almost no callusing occurred in the cuttings of the sea buckthorn cultivars. The highest rooting rate and root numbers were obtained after treatment with 1000 ppm IBA. 'Leikora' had the highest rooting rate among cultivars. 2000 IBA reduced the rooting rate of sea buckthorn semi-hardwood cuttings.

Keywords − *Callus, fogging, indole-3 butyric acid, rooting rates, root number*

1. Introduction

Sea buckthorn that closely relative with silverberry belongs to the *Hippophae* genus of *Elaeagnaceae* (Řezníček and Plek, 2008). Seven species of the genus have been identified so far. However, the most common among these species is *Hippophae rhamnoides*. There are eight subspecies of the mentioned species and spread over a wide area from the Atlantic coast of Europe to Northwest China (Bal et al., 2011). Plant dispersed in the North and East Anatolia regions of Turkey. *H. rhamnoides* naturally grow better at an altitude of about 2000 to 3000 m. They are resistant to extreme temperatures between -45 and + 43 °C. Sea buckthorn grows near the rivers, sunny steep slopes, and sandy regions (Dhyani et al., 2007). *H. rhamnoides* can also grow in barren soils, because it contains microorganisms that fix nitrogen in its roots. Sea buckthorn known in Turkey as sea buckthorn, white thorn, thorn bill, cıcılık, tea bush, ciskan, ılgaz, sincan etc. (Çakır, 2004; Murathan, 2017). Sea buckthorn is a shrub that can grow up to 3-4 m in average and fall leaves in winter (Michel et al., 2012). The plant generally blooms in April, and its fruit ripens in September-October in the north hemisphere. The branches of the plant are gray and thorny (Řezníček and Plšek, 2008). Its leaves are silvery-white in color. Small yellowish flowers bloom in spring, then leaves, and yellow-orange fruits follow (Li and Beveridge, 2003). Fruits can remain on the tree throughout the winter. It is dioecious in terms of fertilization biology. Females need a pollinizer to set fruit (Li and Beveridge, 2003). Traditionally, every part of the plant is being used, such as medicine, nutritional supplement, firewood, fencing, tree guard, windbreak, building construction, religious rites, agricultural implements, and soil fertility enhancement (Stobdan et al. 2013). The ripe fruit of the plant are orange/yellow and contain a single seed inside. The fruits are popular foods in the United States, Canada, Finland, Germany, and some other European countries. The fruits are evaluated for making jam, juice, marmalade, sugar, and cosmetic products (Murathan, 2017). *H. rhamnoides*., which pays attention, especially with its biochemical compounds, contains carotenoids, tocopherols, sterols, flavonoids, lipids, ascorbic acid, tannins, etc. Many of these compounds also have biological and therapeutic activity, including antioxidant, antitumoral, hepato-protective, and immunomodulatory properties (Guliyev et al., 2004). A remarkable aspect of the sea buckthorn is that its seeds are an exceptional source of omega seven fatty acids in the plant kingdom. The fact that *H. rhamnoides*, with its beneficial effects, is used as a raw material in the food, cosmetic and pharmaceutical industry, increases the production appeal of the plant. A profitable production is provided by the standardized plant material, which is a prerequisite for modern breeding. The way to obtain uniform plant material is to prefer vegetative propagation methods such as budding/grafting, layering, separating, propagation by cuttings, and tissue culture. Propagation by cutting is preferred for many fruit species since it is easy and practical for sapling mass production. However, the level success of cutting propagation in many fruit species is determined by the anatomical and physiological features of the plant as well as many external and internal reasons. Cuttings of some fruit species can be easily rooted, but adventitious root formation may be insufficient in others. Various studies have been carried out to increase the rooting performance (Yıldız et al., 2009).

In Turkey, there has not been a commercial production of sea buckthorn yet. Studies are needed to know the uses of sea buckthorn and to extend and improve its cultivation. So, there is a need to determine the optimum propagation methods of the plant to serve these purposes. In this study, it was aimed to determine the effects of indole-3 butyric acid (IBA) doses on the rooting success of the semi-hardwood cuttings taken from the five sea buckthorn cultivars in the vegetation period.

2. Material and Methods

The research was carried out in the mist propagation unit in the greenhouse of Tokat Gaziosmanpaşa University, Agricultural Research and Application Center. Semi-hardwood cuttings of ‘Hergo’, ‘Habego’, ‘Siberian Pearl’, ‘Leikora’, and ‘Pollmix’ sea buckthorn cultivars were used as the material in the study. Cuttings were taken in the middle of August 2019, averaged as 15 cm long, then removed enough of the lower leaves so none will be in contact with the rooting medium or buried after planting and treated with 0 (control), 500, 1000 and 2000 IBA concentrations. The basal of 4-5 cm of each cutting was dipped in IBA solution for 5 s and then were placed into the rooting beds in a depth of 10 cm in rows. Cuttings for the control treatment were dipped into a solution consisting solely of 50% ethanol+water. Perlite was used as the rooting medium. The rooting medium was heated to about 24±1 ^{o}C with electric soil cables when needed. Cuttings were regularly misted in the unit for 5 s with an interval of 5 min during rooting. The cuttings were in intermittent mist during the daylight h and allowed to dry at night. Callus rate (%), rooting rate (%), root length (cm), number of roots (number), fresh root weight (g), dry root weight (g), and sapling survival (%) determined after ten weeks.

The cuttings kept in the rooting medium for ten weeks under the mist propagation conditions were removed, and callus rate, rooting rate, root length, root number, and root dry matter ratio were determined. Callus rate was determined the rate of rooting by proportioning the cuttings that formed callus to the total number of cuttings; root length was determined by proportioning rooted cuttings to the total number of cuttings; root length of rooting cuttings are measured and divided by the number of roots, the number of roots; the number of roots per cuttings and the rate of root dry matter were determined by proportioning the roots with wet weight to dry weight in the oven (Yıldız et al., 2009).

The experiment was designed as a factorial design with three replications. Twelve 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

In this study, the effects of four IBA concentrations on callus and rooting rates, root length, root number, root wet and dry weights, and seedling retention rate of five sea buckthorn cultivars cuttings were found statistically significant. Also, interaction of factors (cultivar x IBA concentration) on root wet and dry weights and seedling retention rates IBA concentration were detected as significant ($p<0.05$).

The emergence of callus was just seen in the control application of ‘Siberian Pearl’ and ‘Pollmix’ cultivars with a rate of 5.55% and 2.77%, respectively (Table 1).

The differences between the rooting rates of cultivars were significant ($p<0.05$). The cultivar with the highest rooting rate was ‘Leikora’ (97.00%), while the lowest rooting was found in ‘Hergo’ with 76.67%. There are no significant differences, ‘Habego’, ‘Leikora’, ‘Pollmix’ and between, ‘Hergo’ and ‘Siberian Pearl’. The differences between IBA concentrations were also found significant ($p<0.05$). The highest rooting rate is 96% and attained from 1000 ppm IBA treatment, whereas the lowest rooting rate was obtained from 2000 ppm IBA (79.07%). Considering the IBA concentrations, 0, 500, and 1000 were in the same group, and 2000 were in the other group. Cultivar x IBA interaction was also found significant ($p<0.05$). When the averages are analyzed, the highest rooting rate (100%) was measured at 500 in ‘Habego’ and 1000 in ‘Leikora’ and ‘Pollmix’ (Table 1). Eskimez et al. (2020), stated that the application of IBA increases the rooting capacity of apple.

The difference between the root lengths of cultivars was found as significant ($p<0.05$). The longest roots were measured in ‘Leikora’ (10.76 cm), and the shortest roots were measured from ‘Siberian Pearl’ (8.33 cm). The difference between IBA applications was also found as significant ($p<0.05$). The maximum root length was determined from 1000 IBA treatment (10.25 cm), and the lowest root length was determined in control (8.72 cm). Cultivar x IBA interaction was found significant ($p<0.05$). When the IBA x cultivar interaction was examined, the maximum root length was measured in ‘Leikora’ and 1000 treatment (12.50 cm). The lowest root length was determined in the ‘Siberian Pearl’ variety and the control application (7.27 cm) (Table 1).

The difference between the number of roots of cultivars was significant ($p<0.05$). The highest number of roots was determined in ‘Leikora’ (5.91), and the least number of roots was recorded in ‘Siberian Pearl’ (3.67). The difference between IBA concentrations was significant ($p<0.05$). The highest number of roots was determined after treatment with 2000 IBA (5.27), while the minimum number of roots was found after the treatment of 1000 IBA (4.33). Cultivar x IBA interaction was found significant ($p<0.05$), and the highest number of roots was obtained after the treatment of 2000 (8.12) in ‘Leikora’. The minimum number of roots was measured in ‘Hergo’ after treatment of 1000 (3.82) (Table 1).

The effect of IBA concentrations on the fresh root weight of cultivars was found as significant ($p <0.05$), but there was no significant difference ($p> 0.05$) between the cultivars. The highest root wet weight was measured in 2000 ppm IBA concentration (0.9 g), while the lowest root fresh weight was found in control (0.44 g). ‘Hergo’ and ‘Siberian Pearl’ were found as significant at the cultivar x IBA interaction (p<0.05). While the maximum fresh root weight was measured in ‘Siberian Pearl’ after treatment of 2000 IBA (1.16 g), and the lowest root fresh weight was found in ‘Siberian Pearl’ after treatment with 500 IBA (0.23 g). However, when the researched concentrations of ‘Habego’, ‘Leikora’, and ‘Pollmix’ were applied, the difference between root weights was not significant (p>0.05) (Table 1).

The effect of IBA concentrations on root dry weight was not significant (p> 0.05). The highest root dry weight was measured in ‘Pollmix’ (0.16 g), and the lowest root dry weight was measured in ‘Habego’ (0.09 g). The maximum root dry weight was measured after the application of 2000 IBA (0.18 g), and the lowest root dry weight was measured in control (0.1 g). When the cultivar x IBA interaction was examined, no significant difference was found (p> 0.05). In cultivar x IBA concentration interaction, the highest dry weight was measured in ‘Siberian Pearl’ in 2000 ppm IBA application (0.26 g), while the lowest root dry weight was determined in ‘Habego’ (0.06 g) (Table 1).

Table 1. The effect of IBA concentrations on rooting of semi-wood cuttings of sea buckthorn cultivars

IBA (ppm)	**Cultivar**					
	Habego	**Hergo**	**Leikora**	**Siberian Pearl**	**Pollmix**	**Average**
			Callus rate (%)			
0	0	0	0	5.55 a A+	2.77 ab*	1.67 A
500	0	0	0	0 B	0	0 B
1000	0	0	0	0 B	0	0 B
2000	0	0	0	0 B	0	0 B
Average	0	0	0	1.39 a	0.69 ab	
			Rooting rate (%)			
0	97.00a A	82.67ab A	94.00ab	77.33b B	97.00a AB	89.60 A
500	100a A	83.00b A	100a	91.33ab AB	94.00a AB	93.67 A
1000	94.33 A	88.67 A	100	97.00 A	100 A	96.00 A
2000	71.67ab B	52.33b B	94.00a	91.33a AB	86.00a B	79.07 B
Average	90.75a	76.67b	97.00a	79.07b	94.25a	
			Root length (cm)			
0	8.79	8.21	9.92 B	7.27 B	9.38	8.72 B
500	9.83ab	9.27bc	10.54a AB	6.72c B	9.14ab	8.90 B
1000	9.94b	9.41b	12.50a A	8.61b AB	10.79ab	10.25 A
2000	8.72	9.88	10.07 B	10.71 A	9.22	9.72 AB
Average	9.32bc	8.94 bc	10.76 a	8.33c	9.64ab	
			Number of roots			
0	4.86	4.82	4.10 C	4.05	4.83	4.53 AB
500	4.5ab	4.55ab	5.57a BC	3.37b	4.02b	4.40 AB
1000	4.86ab	3.82bc	5.87a B	2.85c	4.25bc	4.33 B
2000	4.23b	5.19b	8.12a A	4.43b	4.39b	5.27 A
Average	4.61b	4.6b	5.91a	3.67b	4.37b	
			Fresh root weight (g)			
0	0.30b	0.45a AB	0.69a	0.39ab B	0.36ab	0.44 B
500	0.65ab	0.26b B	0.81a	0.23b B	0.46ab	0.48 B
1000	0.53	0.61 AB	0.71	0.43 B	0.48	0.55 B
2000	0.99	0.78 A	0.58	1.16 A	1.00	0.90 A
Average	0.62	0.52	0.7	0.55	0.57	
			Dry root weight (g)			
0	0.06 a	0.1 ab	0.14 a	0.1 ab	0.09 ab	0.10
500	0.09	0.07	0.13	0.09	0.17	0.11
1000	0.08	0.12	0.17	0.15	0.15	0.13
2000	0.15	0.18	0.11	0.26	0.22	0.18
Average	0.09	0.12	0.14	0.15	0.16	
			Sapling survival (%)			
0	-	-	-	85.71 AB	74.54	80.13 AB
500	-	-	-	100 A	76.67	88.33 A
1000	-	-	-	89.68 AB	75.55	82.62 AB
2000	-	-	-	74.40 B	58.15	66.28 B
Average	-	-	-	87.45 a	71.23 b	

*: The difference between lowercase letters and average means in the same line (p <0.05) is significant.

+: The difference between the averages associated with different capital letters in the same column (p <0.05) is significant.

The effect of different IBA concentrations between ‘Pollmix’ and ‘Siberian Pearl’ on sapling survival was found to be significant ($p<0.05$). The ‘Siberian Pearl’ has been identified as having the highest sapling survival rate, with 87.45%. The lowest sapling survival rate was measured at 71.23% in ‘Pollmix’. When the averages between different concentrations were examined, the highest sapling survival rate was found after treatment with 500 IBA (88.33%), and the lowest sapling survival rate was found at 2000 IBA (66.28%). Cultivar x IBA concentration interaction was statistically significant ($p <0.05$). This interaction resulted in the highest sapling survival rate in ‘Siberian Pearl’ after treatment with 500 IBA (100%), while the lowest sapling survival rate was found in ‘Pollmix’ after application of 2000 IBA (58.15%) (Table 1).

4. Discussion

No callus formation was observed in any cultivar except in the control treatments of ‘Siberian Pearl’ and ‘Pollmix’. This study showed that callus formation is not a prerequisite for rooting of cuttings. Callus formation is seen as an advantage for slow-rooted plant species (Hartmann et al. 1997), but it is not a direct relationship with root formation (Skolidis et al. 1990).

Rooting rates increased in the researched cultivars depending on the rising of the IBA concentration up to 1000 (96.00%). Also, there was a decrease in the mean rooting rate of cultivars of ‘Hergo’ and ‘Siberian Pearl’ when compared with others. 100% rooting rate was obtained from ‘Habego’ after 500 IBA application. The highest rooting rates were measured in 1000 ppm IBA application in ‘Leikora’, ‘Siberian Pearl’, and ‘Pollmix’. Considering the concentrations and cultivar of sea buckthorn, it was determined that the best application was 1000 ppm IBA. It has been reported that rooting success is 96% (Avdeev 1976) in sea buckthorn cultivars and over 86% (Avdeev 1984). Dolkar et al. (2016) applied different IBA concentrations to one-year wood cuttings of sea buckthorn cultivars and achieved 97.6% rooting. Li and Schroeder (1996) reported that 90% of the wood cuttings taken in mid-March were rooted. However, Avdeev (1976) stated that the cuttings taken during intense growth are less rooted, and this varies between cultivars. In our study, the rate of rooting was found to be relatively high compared to Singh and Gupta (2003), which are compatible with similar studies previously performed on sea buckthorn.

The lowest root length was obtained in ‘Siberian Pearl’ with 8.33 cm. Considering the IBA concentrations, the longest roots were 10.25 cm, again after treatment with 1000. Kara et al. (2011) reported that the root length in rosemary was 7.1 cm after the application of 4000 IBA, and the root length increased as the concentration of IBA increased. In our study, the difference between root length in some varieties was insignificant, while in some varieties, IBA in concentration 1000 and 2000 caused longer roots compared to control and 500. Dolkar et al. (2016) measured that as the concentrations of IBA increased, the number of roots increased and that there were 7.3 roots at 5000 IBA. In our study, the maximum number of roots (5.27) was formed after the application of 2000 IBA. It is thought that as the level of IBA concentration increases, the number of roots may increase. In rosemary (Kara et al. 2011) and *Dodoneae viscosa* (Saffari and Saffari 2012) the highest number of roots was 28.8 and indicated the number for *Dodoneae viscosa* resp. after the application of 4000 IBA.

While the highest fresh root weight was measured in ‘Habego’, ‘Hergo’, ‘Siberian Pearl’, and ‘Pollmix’ at 2000 ppm IBA, it was determined at 500 IBA in ‘Leikora’. When comparing the root dry weight of cultivars and IBA concentrations, there were no statistically significant differences. Dale and Galic (2018) identified root dry weights as similar.

In our study, the best results of the sapling survival rate were in the 'Siberian Pearl' and 'Pollmix' at 500 ppm and 1000 ppm IBA concentrations (100% - 76.67%).

As a result; when the parameters such as rooting rate, root number, and root length of sea buckthorn semi-hardwood cuttings are taken into consideration, the most suitable IBA concentration was 1000 ppm; it was possible to propagate semi-hardwood cuttings of sea buckthorn successfully without applying any exogenous plant growth regulator. It has been concluded that concentrations higher than 1000 ppm IBA may negatively affect the rooting success rate of sea buckthorn semi-hardwood cuttings.

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