T.C.

TOKAT GAZİOSMANPAŞA UNIVERSITY

INSTITUTE OF GRADUATE EDUCATION

Effect of Indole Butyric Acid on Rooting Characteristics of Sea Buckthorn (*Hippophae rhamnoides*) Hardwood Cuttings

Prepared by

Nashwan Naif ABDULLAH

Department of Horticulture

Master Thesis

TOKAT – 2022

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Supervisor

Prof. Dr. Mehmet GÜNEŞ

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SCIENTIFIC ETHICAL STATEMENT

According to the thesis writing guide of the Graduate Institute of Tokat Gaziosmanpaşa University, I declare that my master's thesis titled " **Effect of Indole Butyric Acid on Rooting Characteristics of Sea Buckthorn (*Hippophae rhamnoides*) Hardwood Cuttings**", which I have prepared under the supervision of Prof. Dr. Mehmet GÜNEŞ is an original work that complies with scientific ethical values and rules, and I will accept any legal sanctions if it is determinated otherwise.

14/01/2022

Nashwan Naif ABDULLAH

Effect of Indole Butyric Acid on Rooting Characteristics of Sea Buckthorn (*Hippophae rhamnoides*) Hardwood Cuttings

JURY MEMBERS

Head : Prof. Dr.:……………

Member: Assoc.Prof. .... :

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This thesis was accepted by the jury determined in the date of January 14th 2022 and 02 numbered session of the Board of Directors of Graduate Education Institute of Tokat Gaziosmapasa University.

Prof. Dr. Mehmet GÜNEŞ

Director of the Graduate Education Institute

DEDICATION

To the first teacher, to the light of all people, our

Prophet "Muhammad" (peace be upon him)

to my mother and father for their understanding

and for their overwhelming, support, morally and

financially

ACKNOWLEDGEMENTS

First of all, my profound thanks to Allah for giving me strength, patience and health to perform this study, and peace be upon our prophet "Muhammad" and these who follow him until the day of Judgment.

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Nashwan Naif ABDULLAH

Effect of Indole Butyric Acid on Rooting Characteristics of Sea Buckthorn (*Hippophae rhamnoides*) Hardwood Cuttings

Nashwan Naif Abdullah
Department of Horticulture
Superviser: Prof. Dr. Mehmet GÜNEŞ
January 2022, x + 30 pages

Summary

This study was conducted in the greenhouse of the Horticultural Department at the University of Tokat Gaziosmanpaşa, Agricultural Research and Application Center, from October 15th to December 15th, 2020. The study aimed to investigate the effect of four concentrations (0, 1000, 2000 and 4000 ppm) of Indole -3- butyric acid (IBA) on rooting of five cultivars of sea buckthorn ('Hergo', 'Habego', 'Leikora', 'Siberian Pearl' and 'Polmix') hardwood cuttings. The experiment was designed as a Completely Randomized Design with three replications. Twelve cuttings were kept in each replication. Obtained data were subjected to analysis of variance (One-way ANOVA) using IBM SPSS Statistics 26.0 and the Duncan test was used to compare the averages.

The treatment of cuttings with IBA especially 1000 and 4000 ppm significantly affected the callus rate which gained 10.55 % also the best-rooting rate was from the effect of 1000 ppm which obtained 54.44 %, while all other characteristics (root length, root numbers, fresh root weight, dry root weight and sapling survival) proved better results from the effect of control. Plant cultivars significantly affected the rooting of cuttings, best results of most characteristics were gained from 'Leikora' callus rate, rooting percentage, root length and roots number at 14.58 %, 54.86 %, 15.00 cm and 6.62 respectively, while best root fresh weight was obtained from 'Polmix' at 0.24 g, beside highest root dry weight was from roots of 'Habego' and 'Leikora' at 0.03 g and lastly best sapling survival 33.22 % was from 'Habego.'

Overall, the cuttings of 'Leikora' cultivar treated with 1000 ppm provided the best callus rate and root length (33.33 % and 31.18 cm), cuttings of 'Habego' treated with 2000 ppm gained the highest rooting rate (69.44%), cuttings of 'Leikora' treated with control provided best root number (17.79), cuttings of 'Polmix' treated with control obtained highest fresh root weight (0.59 g) and root dry weight (0.07 g) and lastly best sapling survival (58.73 %) was from 'Habego' treated with 1000 ppm.

Keywords: Sea buckthorn, IBA, propagation, hardwood cutting, rooting.

İndol Butirik Asit Dozlarının Yabani İğde (*Hippophae rhamnoides*) Odun Çeliklerinin Köklenme Özellikleri Üzerine Etkisi

Nashwan Naif Abdullah
Bahçe Bitkileri Anabilim Dalı
Danışman: Prof. Dr. Mehmet GÜNEŞ
Ocak 2022, x + 30 sayfa

Özet

Bu çalışma, Tokat Gaziosmanpaşa Üniversitesi Tarımsal Araştırma ve Uygulama Merkezi Bahçe Bitkileri Bölümü serasında 15 Ekim 2020-15 Aralık 2020 tarihleri arasında dört farklı konsantrasyondaki (0,1000, 2000 ve 4000 ppm) İndol -3- bütirik asit (IBA) ve beş yabani iğde çeşidinin ('Hergo', 'Habego', 'Leikora', 'Siberian Pearl' ve 'Polmix') odun çeliklerinin köklenmesi üzerine yürütülmüştür. Çalışma Tam Şansa Bağlı Deneme Desenine göre üç tekrrürlü olarak kurulmuş ve her tekerrürde 12 çelik bulundurulmuştur. Elde edilen veriler IBM SPSS Statistics 26.0 programı kullanılarak varyans analizine (One-way ANOVA) tabi tutulmuş ve ortalamalar Duncan testi çoklu karşılaştırma testine göre yapılmıştır.

1000 ve 4000 ppm IBA ile muamele edilen çeliklerden %10.55 ile en yüksek ortalama kallus ve %54.44 oranı ile en yüksek ortalama köklenme oranı elde edilmiştir. Çalışılan diğer tüm özellikler (kök uzunluğu, kök sayısı, kök yaş ağırlığı, kök kuru ağırlığı ve fidan sağ kalım oranı) kontrolden daha iyi sonuçlar vermiştir. Çeşitlerin köklenme özellikleri üzerine etkisi önemli olmuştur. Kallus oranı, köklenme yüzdesi, kök uzunluğu ve kök sayısı sırasıyla %14.58, %54.86, 15.00 cm ve 6.62 ile 'Leikora' çeşidinden elde edilirken, en iyi kök taze ağırlığı 0.24 g ile 'Polmix' çeşidinden elde edilmiştir. En yüksek kök kuru ağırlığı 0.03 g ile 'Habego' ve 'Leikora' çeşitlerinden ve son olarak en iyi fidan sağ kalım oranı %33.22 Habego çeşidinden elde edilmiştir. Genel olarak, 1000 ppm ile muamele edilen Leikora çeşidinin çelikleri ve ile en iyi kallus oranı (%33.33) ve kök uzunluğunu (%31.18), kontrol ile muamele edilen Leikora (%80.55) ve 2000 ppm ile muamele edilen 'Habego' çeşidinin çeliklerinden (%69.44) en yüksek köklenme oranınları elde edilmiştir. Kontrol ile muamele edilen 'Leikora' çeliklerinden en yüksek kök sayısı (17.79), kontrol ile muamele edilen 'Polmix' çeşidinin çeliklerinden en yüksek kök taze (0.59 g) ve kök kuru ağırlığı (0.07 g) ve son olarak en yüksek fidan hayatta kalma oranı (%58.73) 1000 ppm ile muamele edilmiş 'Habego' çeşidinden elde edilmiştir.

Anahtar Kelimeler: Yabani iğde, IBA, odun çeliği, çoğaltma, köklenme,

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ABBREVIATIONS

cm	:	Centimeter
et al.,	:	Co-worker
g	:	Gram
^{o}C	:	Degree centigrade
cv	:	Cultivated variety

1. INTRODUCTION

Sea buckthorn is native to Turkey, Central Europe, Western China and Central Asia (Yücel, 2012). The plant is widely distributed from the Atlantic coast of Europe to Northwest China and grows naturally in the North and East Anatolian Regions of Turkey (Guliyev et al., 2004). Sea buckthorn is a close relative of silverberry and belongs to the *Elaeagnaceae* family (Reznicek and Plek, 2008). The Greek words *hippo*, which means horse and *phaos*, which means to shine, are the origins of the common name sea buckthorn. This was because its leaves and young branches were used as fodder for horses, causing them to gain weight quickly and their coat to become shinier (Rongsen, 1992).

The sea buckthorn species can vary from tree (*H. salicifolia*) to a dwarf shrub (*H. tibetana*) and bushy (*H. rhamnoides*) form, depending on their growth habits. The botanical classification of sea buckthorn species has changed over the years. Li and Beveridge (2003) reported that sea buckthorn was initially identified by Servrttaz (1908) as only *H. rhamnoides* and its subspecies were *rhamnoides*, *salicifolia* and *tibetana*. Rousi (1971) reclassified *Hippophae* into three different species as *H. rhamnoides, H. salicifolia* and *H. tibetana*, considering the morphological differences. The researcher divided *Hippophae rhamnoides* into 9 subspecies: *carpatica, caucasian, fluviatilis, gyantsensis, mongolica, rhamnoides, sinensis, turkestanica* and *yunnanensis*. These subspecies differ in size, shape, number of main lateral veins on leaves, and amount and color of stellate hairs. Even within each subspecies, there are racial differences. Taxonomists have debated over the exact classification of the *Genus* due to variation found in the Himalayas and nearby areas of Central Asia, where the primary differentiation of the *Genus* took place. Liu and He (1978) added a fourth species (*H. neurocarpa*) that naturally grows in China's Qinghai–Xizang plateau. Chinese taxonomists have changed the classification several times over the years. Lian and Chen (1997), Lian (1988) and Lian et al. (2000) reported the genus *Hippophae* as 6 species and 12 subspecies. These; *H. salicifolia, H. rhamnoides (carpatica, caucasica, fluviatilis, mongolica, rhamnoides, sinensis, turkestanica* and *yunnanensis) H. goniocarpa (litangensis and goniocarpa), H. gyantsensis, H. neurocarpa (stellatopilosa)* and *H. neurocarpa (stellatopilosa)* and *H. neurocarpa*.

Hippophae rhamnoides is the most common species in this *genus*. It is a shrub that can reach a height of 3-4 m and sheds its leaves in the winter (Michel et al., 2012). Sea buckthorn is a hardy deciduous bush that bears clusters of juicy fruits, about the size of a small pea, greenish at first but turns orange, red, or yellow as they mature (Ahmad and Kamal 2002). The plants have extensive roots, with taproots up to 1.1 m deep and horizontal roots up to 2.5 m high on five-year-old plants. The leaves are small (usually 3 to 8 cm long and 0.4 to 1 cm wide), alternate, linear, lanceolate, and have silvery stellate scales on the backside that reflect sunlight and minimize moisture loss (Lu, 1992). Since sea buckthorn leaves contain numerous nutrients and bioactive substances, they are used to make various products. It is commonly known as 'Seabuckthorn' or 'Tsestalulu,' and can grow well in harsh environmental conditions such as high altitude, extreme temperature ranging from -43 °C to +40 °C, and water scarcity (Janifer et al., 2013). Sea buckthorn is also known as white thorn, thorn bill, tea bush, ciskan, cıcalık, ılgaz, sincan, and other names in Turkey (Çakır, 2004; Murathan, 2017).

Any part of the plant has traditionally been used for medicine, dietary supplements, firewood, fencing, tree guards, windbreaks, building construction, religious rituals, agricultural implements, and soil fertility enhancement, among other things (Stobdan et al. 2013). Hardwood cuttings are one of the most cost-effective and simple methods of vegetative propagation for deciduous woody plants (Hartmann et al., 2002). Softwood and hardwood effectively propagate sea buckthorn (Li and Beveridge 2003).

Plant growth regulators are natural or synthetic compounds that change plants' growth and development by influencing their physiological due to these processes (Kakimoto, 2003). The use of synthetic growth regulators to promote rooting is successful (Srivastava, 2002). They significantly improve the chances of rooting success and play an essential role in regenerating roots in cuttings and their survival (Chaudhari, 2016). Indole-3-butyric acid (IBA) has long been thought to be a synthetic auxin and was a natural auxin. Recently, it has been discovered that it occurs naturally in several plant species, including *Arabidopsis* (Schneider et al., 1985; Ludwig - Muller and Epstein, 1991; Ludwig-Muller et al., 1993). It is the most popular auxin for inducing adventitious roots in stem or leaf cuttings (Srivastava, 2002). It is the best root promoter because of its high auxin activity and a relatively slow enzymatic mechanism (Hartmann et al.,2002). Auxins are thought to promote adventitious root production in stem cuttings by promoting the initiation of lateral roots primordia and enhancing carbohydrate transport to the cutting base, both of which are essential for root growth (Hartmann et al., 1990 and Leakey, 1983). Auxins as plant growth regulators, physiological effects, and practical applications make for an enticing horticultural research tale (Tincker, 1936). All growth regulators are not created equal when it comes to rooting efficiency. Indole Butyric Acid is the most popular and widely used growth regulator for ornamental species to achieve a high percentage of rooting success (Kundu et al,1987). The rooting percentage and number of roots per cutting are affected by different IBA concentrations (Mehraj, 2013). Propagation by cutting is suitable for many fruit or ornamental species because it is easy and practical for the mass production of saplings. However, in species, the ratio of success of cutting propagation is related to anatomical and physiological characteristics of the plant and a variety of external and internal factors. Some fruit cuttings are easily rooted, but adventitious root formation may be inadequate in others. Various studies have improved rooting efficiency (Yıldız et al., 2009).

More research is required to have more information about the benefits of sea buckthorn and expand and develop its cultivation. To increase the success of the rooting characteristics of sea buckthorn, studies have been carried out by considering factors such as plant growth-promoting substances, rooting beds, mulches, exposure to cold and cutting thickness. It is essential to decide the plant's best propagation methods to access these purposes. Within the scope of a project carried out in our department, it was aimed to determine the most appropriate propagation time by targeting the propagation of sea buckthorn with green, semi-wood and hard-wood cuttings. So, this study aimed to see how different doses of indole-3 butyric acid (IBA) affected the rooting success of hardwood cuttings from five sea buckthorn cultivars under the mist propagation system.

2. LITERATURE REVIEW

Sea buckthorn (*Hippophae rhamnoides* L.) is a multipurpose, hardy, deciduous shrub, an ideal plant for soil erosion control, land reclamation, wildlife habitat enhancement, and farmstead protection. It has high nutritional and medicinal values for humans. It is a promising new crop, and recently it has attracted considerable attention from researchers, producers, and industry (Li and Schroeder, 1996). For this reason, it has great importance to investigate and find the most suitable propagation method and develop the cultivation of sea buckthorn. The season in which hard-wood cuttings are taken and the time of year they are taken may affect the rooting of stem cuttings (Qrunfleh and Ayoub, 2006). Between October and March, hardwood cuttings can be successfully rooted (Singh and Gupta 2003).

Although sea buckthorn is easy to propagate vegetatively, currently, there is little information on reliable techniques to propagate the plant repetitively within a year vegetatively. To address this, three experiments were conducted to study whether season and chilling affected the successful rooting of cuttings. Four cultivars, 'Chuskaya,' 'Golden Rain', 'Lord', and 'Sunny', were used in the season- and chilling-effect experiments. Hardwood and softwood cuttings from field-grown plants did not root from October to December. The percent of rooted cuttings in January was cultivar-dependent. The number and percent of rooted greenhouse-produced cuttings were significantly affected by the length of chilling. Most cuttings were produced and the highest percentage was rooted when the plants chilled for at least 6 weeks. 'Lord' had the most and 'Golden Rain' the few rooted cuttings. The study indicated that greenhouse-grown stock plants were a viable source of sea buckthorn cuttings for vegetative propagation. Researchers reported thatwhen combined with field-grown sources, it is possible to produce sea buckthorn planting material year-round (Dale and Galic 2018).

Researchers have been working to standardize the fast method of propagating sea buckthorn from softwood cuttings, which needs to be reviewed for their adaptation in the cultivation program, in light of the increasing demand for sea buckthorn in commercial cultivation and afforestation for ecological rehabilitation of degraded mountainous lands. Global research has indicated that propagating seabuckthorn from softwood cuttings from one-year-old shoots with lengths ranging from 15 to 40 cm, taken between late June and August, is advantageous. Cuttings with only a few leaves on top proved the most successful rooting. Various researchers have employed growth hormones such as IBA, NAA, and IAA to speed up root production in seabuckthorn. For root development, IBA and NAA were the most valuable hormones. Cuttings are first immersed in water, then in a growth hormone solution at a lower concentration at room temperature for 14-16 hours or at a higher concentration for a few seconds before being planted in a mist system soil medium with 90% humidity. The soil medium in the mist bed is usually made of sand on top of the soil layer, providing ideal root growth conditions. Saplings can be planted in a nursery or the field the same season or the following year (Virendra et al. 2014).

The effect of plastic mulching, colored shade netting, spacing and cutting thickness on rooting success and growth of sea buckthorn (*Hippophae rhamnoides* L.) through hardwood cuttings are investigated. The silver black plastic mulching film resulted in 10 percent higher rooting success and significant plant growth. Suppression of weed emergence by the plastic mulch resulted in 75.8 percent time-saving in manual weeding by farmworkers. No significant gender difference in rooting success was observed. Reduction in light intensity by 66 percent using green shade net resulted in a significant reduction in rooting and growth of nursery plants. Three different spacing between cuttings did not show a significant difference in rooting and growth-related parameters suggesting that cuttings can be planted denser (3"×3") under mulching to get a higher number of nursery stock per unit area. Cutting thickness showed a significant effect on rooting success. The highest rooting percentage was observed in pencil thickness cuttings (7.5 ± 1.6 mm dia) followed by cuttings with 2.9 ± 0.8 mm and 11.3 ± 1.7 mm basal diameter. The result of the present study could facilitate the establishment of a vegetative propagation method wherein faster growth and the more significant number of cuttings can be propagated with a higher rooting success rate (Dolkar et al. 2017)

One-year-old growth stem cutting (2.9 ± 0.8 mm thickness), which is less than half of the conventional pencil thickness (7–8 mm) hardwood cutting, was studied for vegetative propagation of seabuckthorn (SBT). The rooting trial tested the effects of 14 media and 6 concentrations of indole-3-butyric acid treatment. Over 94 % rooting was observed in all the media tested. The mean rooting percentage was 97.6 ± 2.2. Exogenous auxin treatments lack any benefit and are over 97 % without hormonal treatment. Sevenfold more cuttings were obtained per plant compared to pencil thickness hardwood cuttings, which is the conventional method of SBT propagation. The result of the study could facilitate the establishment of a vegetative propagation method wherein a more significant number of cuttings per plant can be propagated with a higher rooting success rate without the need for exogenous application of rooting hormone (Dolkar et al., 2017)

Under open field conditions, the effect of different concentrated auxin dosages on rooting percentage, number of roots per rooted cuttings, and root length of male and female stem cuttings of *Hippophae salicifolia* was investigated. The cuttings pretreated with 50 mg/L IBA had the best rooting properties (rooting percentages of 50.00 and 76.67 percent, root number of 2.3 and 3.0, and root length per rooted cuttings of 2.5 and 3.5 cm, respectively). As a result, cuttings pretreated with 50 mg/L IBA are recommended for vegetative propagation of *H. salicifolia* via cuttings. Furthermore, this research contributes significantly to developing a simple, low-cost propagation technique for large-scale cultivation and future domestication of the elite genotype for improved nutritional security, socio-economic upliftment, and sustainable rural development in the Indian Himalayan Region (Dhyani et al. 2012).

Güneş et al., (2020) used semi-hardwood cuttings as plant materials of sea buckthorn cultivars taken in the middle of August and prepared in 15 cm length and treated with 0, 500, 1000, and 2000 ppm IBA. The cuttings were removed from the medium after ten weeks, the best rooting rate (96.00 %), length of root (10.25 cm) were recorded from 1000 ppm IBA, while roots number (5.27), fresh weight of root (0.90 g), dry weight of roots (0.18 g) were from 2000 ppm, on the other side best survival of sapling was (88.33 %) obtained from 500 ppm.

Hammo et al. (2015) indicated when treated hardwood stem cuttings of *Photinia fraseri* with IBA at different concentrations (0, 2000, 4000, 6000 and 8000) ppm that the maximum rooting percentage 68.33 %, roots number/cutting 3.50 and length of longest root 4.91cm was obtained from cuttings treated with 8000 ppm, while 6000 ppm revealed the best dry weight of shoots 2.81 g. In contrast, control revealed the lowest results of all parameters. Siddiqui and Hussain (2007) used cuttings from *Ficus Hawaii* as plant materials and tested them with different concentrations of Indole Butyric Acid (IBA) of 1000, 2000, 3000, 4000, and 5000 ppm. The findings revealed that IBA treatment caused substantial variance. Maximum root length (11.5 cm) and roots per cutting (13 pieces) were recorded in cuttings treated with 4000 ppm IBA, as compared to the minimum (0.6 cm) and (2.1) in the control treatment.

According to Zenginbal et al., (2014), when semi-hardwood cuttings of *Camellia sinensis* were treated with IBA at concentrations of 4000 and 6000, rooting percentage, root length, root number, and root quality all improved. In comparison to 0 and 2000 ppm, respectively.

Shiri et al. (2019) studied the effect of indole 3 -butyric acid (IBA) hormone concentration on *D. erecta* propagated from tip cuttings under greenhouse conditions was investigated. The IBA hormone was used in three concentrations (2500, 5000, and 7500 ppm), with regulation of 0 ppm. Cutting survival, root length, and number were all taken into account. The results showed that the optimum IBA concentration is 5000 ppm compared to the other treatment.

Mehraj et al., (2013) revealed that when mature stem cuttings of *Bougainvillea spectabilis* were treated with IBA, they showed significant differences in comparison with control, especially 1000 ppm, which was superior over other treatments in all parameters (rooting percentage, roots number, length of longest root) although both of 500 and 2000 ppm resulted in the same rooting percentage, the best rooting survival was obtained from 1000 ppm.

Saffari and Saffari (2012) researched cuttings of *Dodoneae viscosa* L. to investigate the effects of different concentrations of Indole 3-butyric acid (0, 2000, 4000, and 6000 ppm) on their rooting response. The effects of various hormone concentrations on the number of roots, percentage stem cutting survival, root fresh weight, and dry weight were significant. With 4000 ppm IBA, the best results were obtained compared to all other treatments. The effect of Indole butyric acid (IBA) on rooting of 'Night Jessamine' (*Cestrum nocturnum*) stem cuttings were studied by Rahbin et al. (2012). Then 15 cm long cuttings from each section of the shoot were prepared and handled with 0 (distilled water), 1000, 1500, 2000, and 4000 mg/L IBA for 5 seconds before being cultured in the sand and peat-moss pots. Rooting percent, grown cutting percent, root number in each cutting, root length, and root fresh and dry weight were all measured after 75 days. IBA 2000 and 4000 mg/L were substantially better than other concentrations.

Janifer, et al. (2013) treated hardwood cuttings of seabuckthorn **(***Hippophae rhamnoides*) with auxins IBA at different concentrations to know its effect on roots and shoots characteristics. The recorded results indicated that the dip application of cuttings at 300 ppm of IBA for 48 hours was best for treating the cuttings, which provided a significant rooting percentage and roots number.

Singh, et al. (2015) used rooting hormone IBA at concentrations of 1000, 1500, 2000 ppm and control for treating hardwood cuttings of *Grewia asetica* L. and discovered that the best concentration was 2000 ppm, which resulted in the best percentage of rooting and survival, root length.

Ligustrum ovalifolium was studied by Hammo et al. (2015). Between March 1st and August 1st, 2010, researchers at the Agricultural College/ Salahalden University investigated the impact of IBA concentrations (0, 1000, 2000, 3000) mg.l-1 on the rooting capacity of *Ligustrum ovalifolium* stem cuttings. Compared to the other concentrations, the findings show that the 2000 and 3000 mg.l-1 IBA causes a substantial increase in rooting percentage 68.92, 68.42 percent, longest root 3.41, 3.36 cm, roots dry weight 0.31, 0.31 g. The 3000 mg.l-1 is substantially superior in root number 5.91 root/plant, with the highest values for cuttings handled with 2000 and 3000 mg.l-1.

Dolkar, et.al (2016) evaluated the different concentrations of IBA (0, 200, 500, 1000, 2000 and 5000) ppm on hardwood cuttings of seabuckthorn (*Hippophae rhamnoides* L.) and found 5000 ppm as the best concentration for providing the highest roots number and 500 for obtaining the most extended length of the root.

Indole butyric acid (IBA) on the rooting of *Myrtus communis* cuttings was investigated. The research was carried out in the Forestry and Timber Trees Department of the Horticulture Research Institute of the Agriculture Research Center in Giza, Egypt, base and terminal cuttings of Common myrtle shrubs formed from tissue culture two consecutive seasons of 2014 and 2015. The results showed that IBA concentration significantly impacted survival percentage, root number, and root length. Dipping cuttings in 4000 ppm IBA significantly reported the highest studied characteristics in both seasons compared to untreated cuttings (control) (Abd El Hameed, 2018).

Akhtar et al. (2002) During the years 2001 to 2002, this research was conducted at the Department of Horticulture, University of Agriculture, Faisalabad, on the Rose project. This experiment used rose cuttings from two rose varieties, *Rosa damascene* and *Rosa centifolia*. In addition to control, the basal portion of their stem cuttings was treated with 500 ppm and 1000 ppm concentrations of IBA using a quick dip method. The results show a significant advantage of 1000 ppm over the other treatments.

Güneş, et al., (2020) used semi-hardwood cuttings as plant materials of five cultivars of sea buckthorn, namely 'Hergo', 'Habego', 'Leikora', 'Siberian Pearl', and 'Polmix' to now difference between them, after recording data found highest results of callus rate (1.39%) was from Siberian Pearl' cultivar. Whereas best rooting rate (97.00%), root length (10.76 cm), roots number (5.91), root fresh weight (0.7 g) from Leikora. And better root dry weight (0.16g) was from Polmix. And lastly highest survival of sapling (87.45%) was from *Siberian Pearl.*

Shaymaa et al. (2017) investigated the rooting response of three olives *(Olea europae)* cultivars, namely (Arbokonia, Hojblanka and Arbokonia 18) dwarf Spanish origin, using hardwood cuttings, from the obtained data at the end of the specified period found Hojblanka cultivar provided best rooting percentage, while highest root length was from Arbokonia 18 and root dry weight from Arbokonia.

Also from another experiment carried out by (Bisi et al 2016) on hardwood cuttings of fifteen cultivars of the fig tree (Brunswick', 'Calabacita', 'Negro de Bursa', 'Mini Figo', 'Lampa Preta', 'Lemon', 'Troiano', Nazaré', 'Três num Prato', 'Princesa', 'Colo de Dama', 'Montes', 'Bêbera Branca', 'Pingo de Mel', and 'Roxo de Valinhos') showed the highest result of sprouting percentage from Calabacita cultivar, rooting percentage and root number from Brunswick and Negro de Bursa cultivars respectively.

Muhammad et al (2019) investigated the difference of three olive cultivars cuttings ("Arbequina, Arbosana and Koroneiki") on rooting success and found that the best callus percentage was from Koroneiki cultivar while the highest rooting success was from the Arbosana cultivar.

Elisabeta et al (2012) showed from research on cuttings of two rose cultivars precisely (Christopher Columbus and Vay Vicend) that better results of rooting percentage, roots number and root length were obtained from Christopher Columbus in contrast, Vay Vicend showed lowest results.

Fardin et al. (2015) found from research carried out on twelve cultivars of *Rosa damascene* (from Kurdistan 1 to Kurdistan 12) that maximum results of rooting and callusing percentage, root fresh weight were from Kurdistan 5 while highest root number and root length was from Kurdistan 2 in contrast best root dry weight were showed from Kurdistan 7 (Ayoub & Qrunfleh, 2006) noticed when planted semi hardwood cutting of two cultivars of olive (*Nabali* and *Raseei*) in different planting dates January, February, March and September to December found a better percentage of rooting and the number of roots per cutting from *Raseei* cultivar comparison with 'Nabali' cultivar.

Planted hardwood cuttings of seven cultivars of two species of Actinidia, *Actinidia deliciosa* cultivars (AD20, Hayward, Katiuscia, Kramer, Tomuri) and *Actinidia argute* cultivars (Francesca, Jumbo) after recording data at the end of specified time found best data of rooting percentage, the number of formed roots per cutting and root length from Hayward cultivar of *Actinidia deliciosa* comparison with all other used cultivars (Petilica et al., 2016).

Kishan and Raju, (2008) experimented on hardwood cuttings of *bougainvillea* cultivars (Sonnet, cherry blossom, Mahara, Dr. R.R. Pal, Refulgence, Glabra, Golden Glow, and Zakiriana). The maximum average rooting percentage of two years (90.80%) was registered in cultivar Sonnet followed by Refulgence (89.50%), whereas the minimum rooting percentage (63.33%) was observed in cultivar Cherry Blossom. The maximum number of primary roots (9.25) was found in cultivar Mahara, the maximum average length of primary roots (17.33 cm), plant height (112.33 cm) and length of branches (74.12 cm) were recorded in cultivar Dr. R.R. Pal. Variety Shubhra recorded a maximum (7.96) number of branches over the others. It may be concluded from the two-year study that in terms of rooting percentage, the cultivar Sonnet, Refulgence, Glabra, Golden Glow and Zakiriana were found to be promising and recorded higher rooting percentage (73.11-90.80%) while remaining cultivars ranged from 63.33 to 68.33 percent.

3. MATERIAL AND METHODS

3.1 Study location

The research was conducted in mist propagation in the greenhouse of Tokat Gaziosmanpaşa University Agricultural Research and Application Center. The experiment was conducted from October 15th to December 15th (2020).

Figure 1. Location of the study

3.2. Source of used cuttings in the study

Hardwood cuttings of Hergo, Habego, Siberian Pearl, Leikora and Polmix sea buckthorn cultivars were cut and collected from stock plants parcels in the garden of the Horticulture Department and used as material in the study. Cuttings were taken in the middle of October 2020. Cuttings prepared as average 15 cm length were treated with 0 (control) 1000, 2000 and 4000 ppm IBA concentrations. Basal (4-5 cm) of each cutting set was dipped in IBA solution for 10 seconds and then allowed to evaporate the alcohol for 1 min. and was placed into the rooting beds in a depth of 10 cm in clonal rows of 12 cuttings. 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 at about 21±1 °C with electric soil cables (if needed). Cuttings were regularly misted in the rooting area for 10 seconds with an interval of 5 min. The cuttings were misted during the daylight hours and allowed to mist at night.

Figure 2. Source of cuttings

Callus rate (%), rooting rate (%), root length (cm), root number (number), root fresh weight (g), root dry weight (g) and sapling survival rate (%) of cultivars hardwood cuttings were determined by removing the cuttings kept for ten weeks in rooting medium.

3.3 Preparation of Indole -3- butyric acid (IBA) solution

The IBA concentrations that were used for this study was prepared by dissolving 0.5 g of IBA powder (from Merck company 99 % purity) in 250 ml of ethanol (100 %) on a stirrer instrument for obtaining 1000 ppm after the powder was dissolved completely, the volume was completed to 500 ml with distilled water, and 2000 ppm was prepared by dissolving 1g of IBA and 4000 ppm by dissolving 2g and the other steps was same as those used for 1000 ppm.

Figure 3. Preparation of Indole -3- butyric acid (IBA) solution.

3.4. Experimental measurements

The cuttings kept in the rooting medium for ten weeks 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 Güneş et al. (2020) 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).

3.5 Statistical Analysis of Data

The experiment was designed as a Completely Randomized Design with three replications. Twelve cuttings were kept in each replication. Obtained data were subjected to analysis of variance (One-way ANOVA) using IBM SPSS Statistics 26.0 and Duncan test was used to compare the averages.

Figure 4. Experiment design.

4. RESULTS

One of the essential characteristics determined in cutting propagation studies is the rate of callus formation. In our study, the effect of cultivars on the rate of callus formation (%) was significant. However, the effect of IBA treatment and cultivar *x IBA* treatment interaction was insignificant (Table 4.1). While the highest average callus formation rate was obtained from Leikora is 14.58%, the lowest mean callus rate was obtained from Habego is 1.39%. The average callus formation rates of the treatments varied between 4.44-10.55%; the callus formation rates of the IBA treatment x cultivars interaction varied between 0.00-19.44%.

Table 4.1. The effect of IBA concentrations (ppm) on callus rate (%) of hard-wood cuttings of sea buckthorn cultivars

IBA	Cultivar					Mean
	Habego	Hergo	Leikora	S. Pearl	Polmix	
Control	2.78	11.11	8.33ab**	0.00b	0.00	**4.44**
1000	0.00^{B}***	0.00^{B}	$33.33a^{A}$	$16.66ab^{AB}$	2.78^{B}	**10.55**
2000	0.00	19.44	2.78b	0.00b	8.33	**6.11**
4000	2.78	11.11	13.89ab	19.44a	5.55	**10.55**
Mean	**1.39b***	**10.41ab**	**14.58a**	**9.03ab**	**4.17b**	

*: The difference between the general means indicated in different lower-case letters on the same line is significant at 0.05.

**: The difference between the averages indicated in different lower case letters on the same column is significant at 0.05

***: The difference between the averages indicated in different upper-case letters on the same line is significant at 0.05.

From the data in (Table 4.2) it can be observed that there was a significant difference among cultivars on the rate of rooting percentage and the highest percent was recorded for Leikora 54.86 % in contrast lowest rates were obtained from Siberian Pearl was 34.02 %. Also, from effect of IBA treatment it can be shown that the cuttings treated with 1000 ppm provided highest rate of rooting 54.44 % while this rate decreased to the lowest rate 24.44 when cuttings were treated with 4000 ppm. Moreover, for the effect of interaction between cultivar *x IBA* treatment, it is clear that Leikora cuttings treated with 0 ppm proved the highest percent of rooting was 80.55 % conversely the cuttings of Siberian Pearl cultivar treated with 4000 ppm gives lowest percentage 11.11%.

Table 4.2. The effect of IBA concentrations (ppm) on rooting rate (%) of hard-wood cuttings of sea buckthorn cultivars

IBA	Cultivar					
	Habego	**Hergo**	**Leikora**	**S. Pearl**	**Polmix**	**Mean**
Control	58.33	58.33a**	80.55a	33.33a	33.33	**52.77a**
1000	63.89	52.77ab	63.89ab	47.22a	44.44	**54.44a**
2000	69.44	36.11ab	49.99ab	44.44a	50.00	**49.99a**
4000	25.00	16.66b	25.00b	11.11b	44.44	**24.44b**
Mean	**54.16a***	**40.97ab**	**54.86a**	**34.02b**	**43.05ab**	

*: The difference between the averages indicated in different lower-case letters on the same line is significant at 0.05.

**: The difference between the averages indicated in different lower case letters on the same column is significant at 0.05

The data are shown in Table (4.3) clarifies that the longest root length obtained from the Leikora cultivar was 15.00 cm compared with the shortest root length shown from Hergo cultivar 2.91. from the effect of IBA treatment on root length, it is noticeable that iba concentration is not significantly different from treatment with control. From the interaction between cultivar *x* treatment, there was a significant difference with 31.18 being the most extended root length from cutting of Leikora cultivar treated with 1000 ppm in contrast cutting of the same cultivar when treated with 4000 ppm the shortest root length were taken at 1.69.

Table 4.3. The effect of IBA concentrations (ppm) on root length (cm) of hard-wood cuttings of sea buckthorn cultivars

IBA	Cultivar					
	Habego	**Hergo**	**Leikora**	**S. Pearl**	**Polmix**	**Mean**
Control	4.11	3.50	17.04	13.78	18.10	**11.30**
1000	2.23	2.40	31.18	16.29	3.25	**11.07**
2000	2.96	3.65	10.12	10.96	2.63	**6.06**
4000	2.97	2.10	1.69	2.67	2.79	**2.44**
Mean	**3.07b***	**2.91b**	**15.00a**	**10.92ab**	**6.69ab**	

*: The difference between the averages indicated in different lower-case letters on the same line is significant at 0.05.

The data written in (Table 4.4) shown that the best root number was obtained from cuttings of leikora cultivar were 6.62 which was significantly different from all other cultivars, especially with habego cultivar were 2.00 root number found.also from the IBA treatment on cuttings was shown that control had highest root number as compared with all other IBA treatment which provided reverse effect from the interaction between cultivar *x* treatment best root number were found from cutting of leikora cultivar treated with control which was significantly different with all other interaction treatment especially habego cutting treated with 4000 ppm were 1.39.

According to data of (Table 4.5) noticeable, the highest fresh root weight was taken from cutting of Pollmix cultivar was 0.24g, significally different from all other cultivars, especially with Leikora cultivar were 0.05g. Also, the IBA treatment on cuttings showed that control had the highest fresh root weight than all other IBA treatments, which provided reverse effect, especially 4000 ppm treatment, which was 0.03g. from the interaction between cultivar *x* treatment highest fresh root weight was found from cutting of Polmix cultivar treated with control which was significantly different with all other interaction treatment, especially Leikora and Siberian Pearl cutting treated with 4000 ppm were 0 g .

Table 4.4. The effect of IBA concentrations (ppm) on root number (piece/cutting) of hard-wood cuttings of sea buckthorn cultivars

IBA	Cultivar					
	Habego	**Hergo**	**Leikora**	**S. Pearl**	**Polmix**	**Mean**
Control	2.30	3.22	17.79	2.33ab	2.33	**5.60a****
1000	2.02	3.19	3.02	3.02a	3.42	**2.93b**
2000	2.31	2.48	1.85	1.67b	1.89	**2.04b**
4000	1.39	2.67	3.83	1.67b	2.50	**2.41b**
Mean	**2.00b***	**2.89b**	**6.62a**	**2.17b**	**2.53b**	

*: The difference between the averages indicated in different lower-case letters on the same line is significant at 0.05.

**: The difference between the averages indicated in different lower case letters on the same column is significant at 0.05

Table 4.5. The effect of IBA concentrations (ppm) on fresh root weight (g) of hard-wood cuttings of sea buckthorn cultivars

IBA	Cultivar					
	Habego	**Hergo**	**Leikora**	**S. Pearl**	**Polmix**	**Mean**
Control	0.17	0.08	0.09	0.19	0.59	**0.22**
1000	0.10	0.07	0.07	0.08	0.21	**0.11**
2000	0.07	0.05	0.05	0.03	0.06	**0.05**
4000	0.03	0.04	0.00	0.00	0.11	**0.03**
Mean	**0.09**	**0.06**	**0.05**	**0.08**	**0.24**	

From data available in (Table 4.6), it is clear that the highest dry root weight taken from roots of cuttings of Habego and Leikora cultivars were 0.03g, significally different from all other cultivars, particularly with Siberian pearl cultivar were 0.009g. Also, the IBA treatment on cuttings showed that control and 1000ppm had the same highest dry root weight 0.03 g compared with all other IBA treatments, which gave the reverse effect, especially 4000 ppm treatment were 0.001g. from the interaction between cultivar x treatment highest dry root weight was found from treated cutting of Leikora cultivar with 1000 ppm which was significantly different with all other interaction treatment, precisely Hergo, Leikora, Siberian Pearl and Polmix cutting treated with 4000 ppm were 0 g .

Table 4.6. The effect of IBA concentrations (ppm) on dry root weight (g) of hard-wood cuttings of sea buckthorn cultivars

IBA	Cultivar					
	Habego	**Hergo**	**Leikora**	**S. Pearl**	**Polmix**	**Mean**
Control	0.05	0.05	0.04a*	0.02	0.07	**0.04a**
1000	0.05	0.01	0.06a	0.02	0.02	**0.03a**
2000	0.01	0.02	0.02ab	0.03	0.02	**0.02ab**
4000	0.00	0.00	0.00b	0.00	0.00	**0.00b**
Mean	**0.03**	**0.02**	**0.03**	**0.01**	**0.02**	

*: The difference between the averages indicated in different lower-case letters on the same column is significant at 0.05.

From data of (Table 4.7) showed that cuttings from the Habego cultivar had the best sapling survival percent 33.22% which was different significantly from the cuttings of other cultivars more notably with 2.38%.also from IBA treatment, the control had the best sapling survival 24.59% in contrast the other IBA treatment has least percent of sapling survival with 1.33% shown to be the minor percent from 4000 ppm. The sapling survival from the interaction between cultivar x treatment the best percent were taken from the interaction of Habego with 2000 ppm were 54.17% compared with the lowest percent 0.00 were taken from the interaction of 4000 ppm with all cultivars except Siberian Pearl.

Table 4.7. The effect of IBA concentrations on sapling survival (%) of hard-wood cuttings of sea buckthorn cultivars

IBA (ppm)	**Cultivar**					
	Habego	**Hergo**	**Leikora**	**Siberian Pearl**	**Polmix**	**Mean**
0	20.00	9.52	24.54	38.89	30.0	**24.59a****
1000	58.73A	0.00B	3.03B	15.0B	16.67B	**18.68a**
2000	54.17	0.00	20.83	6.67	27.78	**21.89a**
4000	0.00	0.00	0.00	6.67	0.00	**1.33b**
Mean	**33.22a***	**2.38b**	**12.10b**	**16.81ab**	**18.61ab**	

*: The difference between the averages indicated in different lower-case letters on the same line is significant at 0.05.

**: The difference between the averages indicated in different lower case letters on the same column is significant at 0.05

5. DISCUSSION

From the data in Table (4.1 and 4.2) which show the effect of IBA on cuttings characteristics evident that the best callus rate and the rooting rate was from the effect of 1000 and 4000 ppm these results resemble the data of Güneş, et al., (2020) on semi-hardwood cuttings of sea buckthorn cultivars which showed 1000 ppm as best concentration for getting the highest result of rooting rate and Mehraj et al., (2013) on mature stem cuttings of *Bougainvillea* spectabilis. These findings show that IBA is the best root promoter due to its rapid auxin activity and a completely slow demolition enzymatic system, as confirmed by Hartmann et al. (1990) as the rooting hormone encourages adventitious forming of roots in several types of cuttings by increasing carbohydrate hydrolysis and easily translocating it to the base of cuttings, causing primordial root growth and promoting initiation. It also affects the accumulation of metabolites at the treated site, the synthesis of new proteins, the creation of calluses, cell division, and cell enlargement (Villar and Gadget –Faurobert, 1997). Alternatively, to enhance the total number of cuttings forming Greenwood and Berlyn by accumulating auxin at the site of root formation and concentrating sucrose or its derivative at the site of root formation (1973). Auxins can also regulate meristematic tissue activity and increase the supply of plastic substances at root formation sites. According to Kumari et al. (2010), auxins can aid in the production of other plant hormones and control bud formation, cell enlargement, and root initiation. This, in turn, increases the ability of cuttings to absorb water and nutrients (Bonaminio and Blazich, 1983 and Blazich, 1988).

From the data in tables (4.3,4.4,4.5,4.6, and 4.7) on root number, root length, fresh root weight, dry root weight, sapling survival which indicated the result of control was better than IBA concentrations These results could be attributed to the addition of a high concentration of IBA above the tolerance level, which can hinder plant growth (Blazich, 1988). When auxin is administered exogenously at high levels, it can have a harmful effect because it encourages the synthesis of ethylene, which can impede growth (Trigiano and Gray, 2004). Furthermore, according to research, high auxin levels have been linked to the death of cell components in cuttings (Mansori et al., 2018**.** Furthermore, these results may be due to cuttings with adequate concentrations of endogenous IAA, which caused the addition of exogenous IBA to have a reverse effect on the mentioned characters. Furthermore, it is not recommended to immerse cuttings in hormone solution as it is used in research; instead, it is preferable to immerse cuttings based on hormone solution (Al-Atrakchii, 2021).

From the data presented in all tables (4.1-4.7) there was a difference in best results from cultivar to cultivar; these results resemble data of Güneş et al. (2020) on semi-hardwood cuttings of five cultivars of sea buckthorn namely ('Hergo', 'Habego', 'Leikora', 'Siberian Pearl', and 'Polmix'). Muhammad et al. (2019) on three olive cultivars cuttings ("Arbequina, Arbosana and Koroneiki") and (Bisi et al. 2016) on hardwood cuttings of fifteen cultivars of the fig tree, these differences in obtained data may be caused by variation in the physiological status of the cutting tissues on stock plants resulting in the occurrence of gradients in the cellular activity or in the level of assimilates or growth regulators or in the level of significations or may be caused from the differences in endogenous auxin levels in cuttings and inherent genetic characteristics of cultivars, (Hartmann et al., 1990, 1997), or maybe attributed to their anatomy have an exclusive potential for rooting and adventitious rooting formation in stem cuttings which is influenced by the diverse endogenous factor (Gyana, 2006).

6. CONCLUSIONS AND RECOMMENDATIONS

Depending on the attained data from the carried-out study, the following conclusions can be drawn:

The treatment of cuttings during late summer and early autumn with IBA concentrations is unnecessary. It may contain sufficient endogenous IAA and other rooting co-factors and promoters to obtain the best rooting cuttings results. Overall, the easiest cultivar of sea buckthorn for obtaining the best-rooted cuttings from used cultivar is Leikora**,** followed by Habego then Hergo. However, other cultivars provided the best results of a specific parameter. Leikora cultivar is the best for obtaining the best-rooted cuttings, then Habego followed by Hergo.

According to carried out study, it is recommended not to use IBA for cuttings during this time of year as it is unnecessary.

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Appendix 1

The research was conducted in mist propagation in the greenhouse of Tokat Gaziosmanpaşa University Agricultural Research and Application Center (40° 19' 57'' N extent. 36° 28' 36'' E), at altitude 590 m; the experiment was conducted during the period from 15th october to December 15th (2020)

Appendix 2

Table 4.6. The effect of IBA concentrations (ppm) on dry root weight (g) of hard-wood cuttings of sea buckthorn cultivars

IBA	Cultivar					
	Habego	**Hergo**	**Leikora**	**S. Pearl**	**Pollmix**	**Average**
Control	0.05	0.05	0.04a*	0.02	0.07	**0.04a**
1000	0.05	0.01	0.06a	0.02	0.02	**0.03a**
2000	0.01	0.02	0.02ab	0.03	0.02	**0.02ab**
4000	0.00	0.00	0.00b	0.00	0.00	**0.00b**
Average	**0.03**	**0.02**	**0.03**	**0.01**	**0.02**	