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Effects of Indole-3-Butyric Acid (IBA) and Cutting Type on Rooting of *Camellia sinensis* (L.) O. Kuntze

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Authors' contributions

This work was carried out in collaboration between all authors. Author HZ designed the study, performed the statistical analysis and wrote the protocol and the first draft of the manuscript. Authors HZ and AH managed the analyses of the study. Author OD managed the literature searches. All authors read and approved the final manuscript.

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ABSTRACT

The objectives of this study were to determine the effect of cutting type, and IBA (Indole-3-butyric acid) on rooting of Turkish tea (*Camellia sinensis* (L.) O. Kuntze) clone 'Fener-3'

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cuttings. The cuttings collected at 1 September in 2012. After pre-treating with 0, 2000, 4000 and 6000ppm IBA, the cuttings with full leaf and half leaf rooted in perlite medium in unheated trays under misting glasshouse for 60 days. And then, they all were removed from media to determine the survival rate, rooting rate, root number, root length, root diameter and root quality. In conclusion; survival percentages varied from 70.0% to 90.0%. The highest survival percentage (90%) was observed on full-leaf cuttings treated with 6000 ppm IBA. The rooting percentage varied from 43.3% to 78.3%. The best results were obtained for rooting (78.3%), which is 6000 ppm IBA doses, was gathered with full- leaf cutting. The root lengths varied from 9.83 to 14.77cm. The best result (14.77cm) was taken from 6000 ppm IBA dose based on the full leaf cutting. The root diameters varied from 0.99 mm to 1.30mm. The best result (1.30mm), prepared the full leaf cuttings, 6000 ppm IBA doses has been application. The number of root varied from 3.40 to 6.37 units. The best results (6.37 units) were taken 6000ppm IBA doses based on the full-leaf cuttings. The quality of the root varied from 1.93 to 3.50 points. The best result (3.50 points) was taken 6000 ppm IBA doses based on the full-leaf cuttings. Among all parameters the best type of cutting was full-leaf cutting, and 6000 ppm IBA was the most appropriate dose. The lowest rooting and rooting quality were taken from control (0 ppm IBA) treatment.

Keywords: Turkish tea; fener-3; IBA; cutting type; rooting.

1. INTRODUCTION

Camellia sinensis (L.) O. Kuntze (Family: *Theaceae*) commonly known as tea plant is one of the most important crops in the world [1]. The tea has received considerable interest in recent years as a medicinal agent. Young shoots of tea bushes are mainly processed into black tea, green tea, and oolong tea. Among these, green tea is most beneficial to human health. Recently reported pharmacological properties, e.g., antioxidant, anti-inflammatory, anti-mutagenic, and anti-carcinogenic effects also served to increase the popularity of green tea [2-5].

For tea crop production, Turkey is the fifty country in the world, but the yield crop is very low [6,7]. Possible causes of lower production are likely insufficient field applications, absence of high quality sapling and other reasons for sapling production and distribution.

Turkish tea clone ‘Fener-3’ was selected in Rize, Turkey, 20m in elevation in 1963. It is a hybrid exhibiting *Camellia sinensis* (L.) O. Kuntze kind of peculiarities. It has a strong structure and is durable to environmental conditions. Its leaves are dark green in color and ribbed appearance. Shoots are succulent, fragile and heavy. Shoot and first leaf are covered in plume let. Among Turkish tea clones, it is considered as second after Derepazarı-7 clone in terms of yield, and the values of bud shoot and polyphenol content [8].

In tea gardens of the Turkey, mostly the genetic purity of seeds has not existed because of seed replicated plant seedlings. Generally Chinese varieties have dominated in the gardens and, in addition, many of other types occurred with different forms of morphology, physiology, quality, and yield. Continuing tea production by seed would unavoidably result in new low yielding and quality types [9-12]. For these reasons today, tea has been farmed by vegetative propagation.

Conventionally, tea plants propagated by rooting hard-wood and semi-hardwood cuttings, budding, grafting and air layering. Mostly, semi-hardwood cuttings are used for large scale in propagation. Many propagators are not using the first 6-8 nodes of freshly growing shoots for propagation purpose because of difficulty to root. Commercial exploitation of the immature nodal cuttings is limited by heavy losses during rooting and hardening procedures. Normal cuttings without treatment of plant growth regulators are also difficult for rooting [8,13,14].

Rooting success of cuttings have been affected by many factors such as type of cutting, mist, temperature control, collection times, use of shade, rooting media and materials structural factors, growth substance treatments, some chemical treatments, and environmental factors etc. [14-17]. Auxin has been also known to be intimately involved in the process of adventitious root formation in cuttings [14,18,19].

In 1928, the first experiments on cutting propagation of tea started in India, where types and varieties had important differences for rooting capabilities. Subsequent research revealed that cuttings of *Sinensis* varieties and its hybrids were rooted easily, but those of *Assamica* had some difficulties [13]. In Turkey, the first study on tea propagation by cutting was performed by Özbek et al. [9], reporting auxin applications stimulated the rooting significantly. Similar results were also reported for auxin in a subsequent study on 'Fener-3' tea clone [13]. In recent years several studies were carried out on the effects of plant growth regulators such as IAA, IBA and NAA [1,14,20,21] (Khan et al.; Rout; Ertürk et al.; Waheed et al.,), to promote rooting of tea (*Camellia sinensis* L.), IBA (Indole-3-butyric acid) were found to be most effective chemical. As the results of some experiments, 6000ppm [20], 50-100ppm (for 24 hours), 8000 ppm (for 5 sec.) [10], 75ppm (for 12 hours) [14], 2000ppm [1] and 2500-3000ppm [21], could be used to get better rooting.

To reach the desired level for tea cultivation in Turkey, it is compulsory that clonal cuttings should be produced from high yielding and quality tea types and tea plantations should be established by these cuttings. The aim of present study was to determine the type of cuttings, and the effect of plant growth regulators (IBA) on rooting percentage and quality of a hardly rooting of Turkish tea clone 'Fener-3'.

2. MATERIALS AND METHODS

2.1 Collection of Plant Material

The research was performed in Atatürk Tea and Horticultural Research Center in Rize. In this study, cutting taken from 'Fener-3' clones (*Camellia sinensis* var. *sinensis* (L.) O. Kuntze). The 'Fener-3' clone bushes garden established on a flat field (North: 41º01', East: 40º30', Altitude: 106). Soil structure was sandy – loamy. Soil analyses on soil taken in 20cm below of soil surface were the following:

pH: 4.65 - 5.35; organic matter : 0.14-3.96 %; total nitrogen content: 0.14–0.24%; available P_2O_5: 13 -30ppm; exchangeable K_2O: 80-370 ppm

2.2 Pretreatment of Cuttings

The 20-year-old 'Fener-3' clone bushes for cuttings were pruned in December and cuttings were taken from newly emerged shoots at 1 September in 2012. Well-developed disease free cuttings with full and half-leaf are 3.5 to 4cm in length were prepared and disinfected by

a fungicide (0.2% Benlate for 10 minutes) against fungus infections. After disinfection they were quickly washed three times in distillated water. These cuttings for all IBA applications and replications were bulked and then dipped approximately 1cm in IBA (0, 2000, 4000 and 6000 ppm) for 5 sec at the same time and allowed to air dry. IBA solution was freshly prepared by dissolving IBA powder (Merck Chemical Co.) in an ethanol/sterile water (50%/50%) solution. Cuttings in the control group were treated with 50% ethanol + 50% sterile water.

2.3 Rooting Media and Growth Conditions

After IBA applications, cuttings were placed in trays filled with perlite media to depth of 10cm under mist (15s/6min) in glasshouse maintained 21±2ºC. Rooting was performed in unheated glasshouse and the upper part rooting media was shaded by using of porous polyethylene with 70% light transmittance. For rooting, time-dependent automatic mist-propagation system was set at 70-90% level. The data on rooting and root growth were obtained after 60 days.

2.4 Scoring of Data in Rooting Experiment

At the end of rooting period, survival rate (%), rooting rate (%), root number (unit/cutting), root length (cm), root diameter (mm) and root quality (1-4 points) [22] were determined. The daily mean temperature and relative humidity measurements (1 hour's intervals) were used a mechanical data logger (HOBO U10 Temp/RH data logger).

2.5 Statistical Analysis

Experimental design used was a randomized complete block design with three replications. Each replication contained 20 cuttings spaced 5cm apart. Data expressed as percentage (rooting rate and survival rate) were transformed using the arc-sin$\sqrt{x}$ transformation. Data analyses were done using Univariate routine SPSS statistical package [23] via license of Ondokuz Mayis University. Design of the experiment was evaluated as 2X4 factorial. Before the ANOVA tests, homogeneity of variances were examined with Levene variance homogeneity test. Variances of all traits were found homogeneous ($P>0.05$). Duncan test was used for multiple comparisons.

3. RESULTS

3.1 Climatic Data

Experiment carried out in the glasshouse where daily mean relative humidity (%) and temperature (ºC) were recorded during 1 September to 1 November in 2012 years (Fig. 1). As shown in Fig. 1, mean daily temperatures varied from 12.7ºC to 24.6ºC. Mean daily relative humidity varied from 54.7% to 90.7%.

3.2 Survival Rate

The effects of IBA and type of cutting on the survival rate of tea cuttings are summarized in Tables 1, 2 and 3. Survival rate was insignificantly affected by the interaction of IBA treatment and cutting type ($P<0.05$) (Table 1). The survival percentages varied from 70.0% to 90.0%. The highest survival percentage (90%) was observed on full-leaf cuttings treated

with 6000 ppm IBA. The IBA application had insignificant effect (P<0.05) (Table 2). The best results were obtained (87.5%) from 6000 ppm IBA doses. The type of cutting had insignificant effect (P<0.05) (Table 3). The highest result (80.4%) was observed full-leaf cuttings.

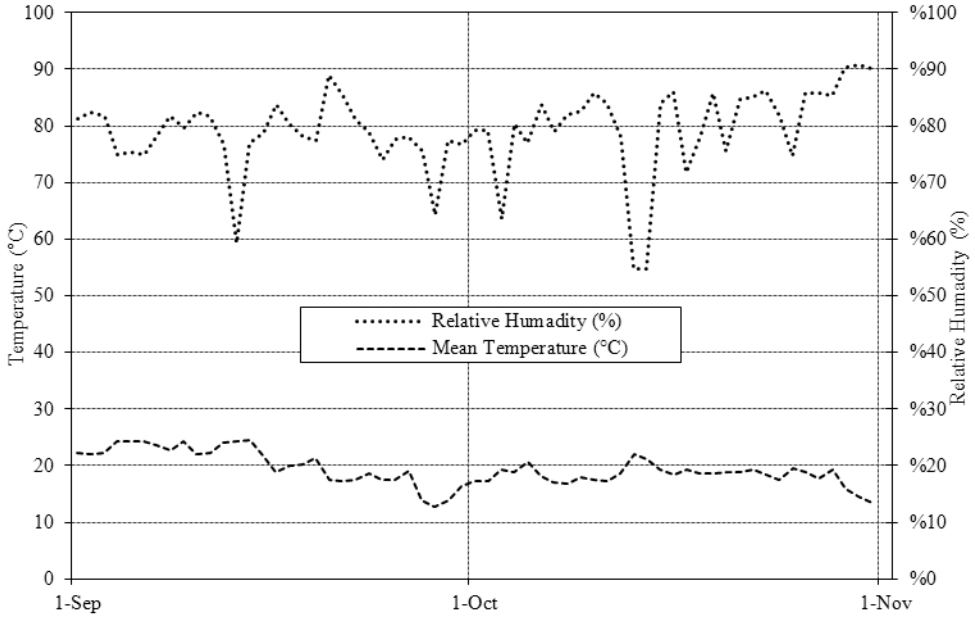

Fig. 1. Changing of daily mean temperature and relative humidity during the days after cutting

3.3 Rooting Rate

The rooting of tea cuttings are summarized in Table1, 2 and 3. Rooting was significantly affected by the interaction of IBA treatment and type of cutting (P<0.01) (Table 1). Rooting percentage varied from 43.3% to 78.3%. The best results were obtained for rooting (78.3%), which is 6000 ppm IBA doses, was gathered with full- leaf cutting. IBA doses had significantly effect (P<0.01) on rooting percentage (Table 2). The best result was obtained for rooting (76.7%), which is 6000 ppm IBA dose. In our study, the result showed that tea clone 'Fener-3' is able to root at low level 44.2% without any treatment. All the other treatments IBA increased rooting percentage. The type of cutting had insignificant effect (P<0.05) (Table 3). The highest result (65.0%) was observed full-leaf cuttings.

3.4 Root Length

Results in Table 1 show that there were insignificant affected by the interactions between IBA concentration and cutting type on the root length of tea cuttings at 60 days after establishment. The root lengths varied from 9.83 to 14.77cm. The best result (14.77cm) was taken 6000 ppm IBA dose based on the full leaf cutting. The root length was insignificant effect (P<0.01) of IBA application (Table 2). The best results were obtained (13.98cm) 6000ppm IBA doses. The type of cutting had significant effect (P<0.05) (Table 3). The highest result (13.95cm) was observed full-leaf cuttings.

Table 1. Interactive effect of IBA concentration and cutting type on rooting and root growth of semi-hardwood cuttings of tea clone (Fener-3) at 60 days after establishment

Type of cutting	IBA doses (ppm)	Survival rate (%)	Rooting rate (%)	Root length (cm)	Root diameter (mm)	Root number (unit/ cutting)	Root quality (0-4 points)
Half – Leaf	0 (control)	70.0[1](56.84±1.81)[2]	45.0(42.37±5.08)cd	9.93±1.12	1.20±0.04ab	4.17±0.38cd	2.47±0.47
	2000	76.7(40.43±16.53)	55.0(47.88±1.67)bcd	9.83±1.30	1.03±0.05c	3.40±0.23d	1.93±0.35
	4000	76.7(61.14±1.14)	71.7(57.86±1.07)ab	12.00±1.63	0.99±0.06c	4.77±0.64bc	2.83±0.52
	6000	85.0(67.21±0.00)	75.0(60.00±0.00)a	13.20±0.00	1.18±0.00ab	5.90±0.00ab	3.20±0.00
Full – Leaf	0 (control)	73.3(59.33±4.76)	43.3(41.15±1.92)d	12.90±2.89	1.27±0.09ab	5.00±0.17bc	3.00±0.38
	2000	78.3(62.90±5.16)	63.3(53.39±8.08)abc	14.13±1.15	1.21±0.02ab	5.37±0.49abc	3.00±0.15
	4000	80.0(63.43 ±0.00)	75.0(60.00±0.00)a	14.00±0.00	1.12±0.00bc	5.60±0.00ab	3.00±0.00
	6000	90.0(71.56±0.00)	78.3(62.48±3.05)a	14.77±1.05	1.30±0.00a	6.37±0.52a	3.50±0.15
	Significant	0.105	0.003	0.175	0.002	0.001	0.082

*[1] Original data; [2] Transformed data; * Mean within a column followed by the same letter are not significantly different*

Table 2. Combined mean of rooting and root growth at different IBA concentration of tea clone (Fener-3) at 60 days after establishment

IBA Doses (ppm)	Survival rate (%)	Rooting rate (%)	Root length (cm)	Root diameter (mm)	Root number (unit/cutting)	Root quality (0-4 points)
0 (control)	71.7[1](58.08±2.34)[2]	44.2(41.76±2.45)c	11.42±1.54	1.23±0.05a	4.58±0.27b	2.73±0.29
2000	77.5(51.67±9.23)	59.2(50.63±3.89)b	11.98±1.23	1.12±0.05ab	4.38±0.50b	2.47±0.29
4000	78.4(62.29±0.72)	74.4 (58.93±0.68)a	13.00±0.85	1.05±0.04b	5.18±0.34ab	2.92±0.24
6000	87.5(69.39±0.97)	76.7(61.24±1.47)a	13.98±0.59	1.24±0.03a	6.13±0.26a	3.35±0.10
Significant	0.098	<0.001	0.398	0.011	0.010	0.106

*[1] Original data; [2] Transformed data; * Mean within a column followed by the same letter are not significantly different*

Table 3. Combined mean of rooting and root growth at different cutting type of tea clone (Fener-3) at 60 days after establishment

Type of cutting	Survival rate (%)	Rooting rate (%)	Root length (cm)	Root diameter (mm)	Root number (unit/cutting)	Root quality (0-4 points)
Half – Leaf	77.1[1](56.4±4.65)[2]	61.7 (52.03±2.47)	11.24±0.66b	1.10±0.03b	4.56±0.32b	2.61±0.22b
Full – Leaf	80.4 (64.3±2.01)	65.0 (54.25±3.12)	13.95±0.73a	1.23±0.03a	5.58±0.22a	3.13±0.11a
Significant	0.133	0.582	0.012	0.011	0.015	0.048

*[1] Original data; [2] Transformed data; * Mean within a column followed by the same letter are not significantly different*

3.5 Root Diameter

The root diameter of tea cuttings are summarized in Table 1, 2 and 3. The root diameter was significantly affected by the interaction of IBA treatment and type of cutting ($P<0.01$) (Table 1). The root diameters varied from 0.99mm to 1.30mm. The best result (1.30mm), prepared the full leaf cuttings, 6000 ppm IBA doses has been application. The IBA application had significant effect ($P<0.05$) (Table 2). The best result was obtained (1.24mm) 6000ppm IBA doses. The type of cutting had significant effect ($P<0.05$) (Table 3). The highest result (1.23mm) was observed full-leaf cuttings.

3.6 Root Number

When looked at the impact on the root number (Table1, 2 and 3), IBA dose x type of cutting had a significant effect ($P<0.01$) (Table 1). The number of root varied from 3.40 to 6.37 units. The best results (6.37 units) were taken 6000 ppm IBA doses based on the full-leaf cuttings. Root numbers were significantly affected ($P<0.01$) by application of IBA. The highest result (6.13 units) was obtained 6000 ppm IBA application. The type of cutting had significant effect ($P<0.05$) on the root number of tea cutting. Full-leaf cuttings had greater root number than half-leaf cuttings.

3.7 Root Quality

Analyzed data on the root quality the IBA treatment x type of cutting interaction and IBA treatment were insignificant (Table 1 and 2). The quality of the root varied from 1.93 to 3.50 points. The best result (3.50 points) was taken 6000 ppm IBA doses based on the full-leaf cuttings. The type of cutting had significant effect ($P<0.05$) on the root quality of tea cutting (Table 3). Full-leaf cuttings had greater root number than half-leaf cuttings.

4. DISCUSSION

These temperature and relative humidity values were compatible with those reported previously [24].This experiment was designed to investigate the factors (IBA and type of cutting), which might affect the rooting performance of semi-hardwood cuttings of tea clones ‘Fener-3’. As a result of all these findings, among all parameters the best type of cutting was full-leaf cutting, 6000 ppm IBA was the most appropriate dose. IBA treated cuttings generally rooted significantly better than water-treated (control) cuttings. In present study, control cuttings rooted poorly, but responded to high concentration of IBA (6000 ppm). Applications of elevated concentration of IBA increased rooting. Indeed, Weaver [25] stated that growth regulators (auxins) changed the number and the type of root was a manufacturer of the strong fringe root. Also, Kinez [13], Ayfer et al. [10], Khan et al. [20], and Rout [14], Waheed et al. [21] also reported that treating cuttings with auxin (IBA) increased the percentage of rooting, root initiation, root number and as well as uniformity of roots in *Camellia sinensis*. It is evidence that the cuttings of different fruit species require different optimum concentrations of IBA to promote rooting. Dipping the bases of hardwood peach, plum, mulberry and kiwifruit cuttings in solution containing IBA in the range of 2000 to 6000ppm was effective in stimulating root production [19,26-28]. In terms of type of cutting in cutting to the full-leaf cutting had better results than a half-leaf cuttings. In cutting the leaves and buds, prepared had a positive effect on the number of root and root formation, Hartmann et al. [17], the presence of leaf cuttings physiological and morphological report of activities carried out

more quickly. In addition, Ayfer et al. [11], Zenginbal and Özcan [19], the presence of a leaf cuttings rooting and root state that has a positive impact in terms of number.

5. CONCLUSION

It is necessary to use high quality sapling in establishing the garden to catch the desired yield and quality levels in tea cultivation in Turkey. For this purpose, saplings, produced vegetative (via in vitro culture or cutting) should be used instead of ones, produced via seeds. As a result of this study, rooting of 'Fener-3' Turkish tea clones cuttings for rooting in order to ensure a successful, good selection of cuttings and appropriate conditions must necessary. Therefore misting and shading systems and perlite media should be used for successfully rotting of cuttings. In addition, among all parameters the best type of cutting was full-leaf cutting, and 6000 ppm IBA was the most appropriate dose. The lowest rooting and rooting quality were taken from control (0 ppm IBA) treatment.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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