THE EFFECTS OF CUTTING LENGHT AND IAA HORMONE ON THE ROOTING OF *EUONYMUS JAPONICUS* CUTTINGS

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Abstract. The aim of this study was to determine of the best size cutting length and concentration of IAA on rooting of *Euonymus* cuttings in the greenhouse. A factorial experiment was carried out in a compeletly randomized block design with 3 replications where each replication was comprised of 10 plantlets. Treatments were comprise of three stages of cutting length (7, 15 and 22 cm) and five stages of IAA concentrations (0, 500, 1000, 1500 and 2000 mgL^{-1}). Morphological and physiological traits such as number and leaf area, fresh and dry weight of plant, number and length of root, number and length of shoot and number of bud were recorded. The result indicated that the cutting length and concentration of IAA was significant on some of quantity charactristics of Euonymus. The best response was related to cutting length of 15 cm and 500 mgL^{-1} IAA.

Key Words*: Euonymus Japonicus*, IAA, cutting length, rooting, fresh weight, dry weight

Abbreviation

IAA- Indole-3-acetic acid

INTRODUCTION

Increasing in popularity of ornamental plants are important for marketing and great production (Cerveny and James, 2005). Many ornamental plants are propagated by asexual ways (Mohammad and hamid, 2014). *Euonymus* are primarily propagated by stem cuttings, but hardly produce adventitious roots by cuttings and is a problem for the vegetative propagation (Céline et al., 2006). Adventitious root formation is a key step in vegetative propagation of woody or horticulture species, and problems associated with rooting of cutting frequently result in significant economic losses (Mohammad and Hamid, 2014). Environmental factors such as temperature, rainfall and photoperiod can affect the amount of free auxin on mother plants (Negishi et al., 2014), determining unsatisfactory levels for rooting (Osterc and Stampar, 2011). In case of this, one way to facilitate the process of rooting is to the use of plant growth regulators such as auxins, which can induce root formation. Also, Auxins can form more uniform and vigorous roots and accelerate the process of formation of the seedlings (Pizzatto et al., 2011). In many plant species and among the auxins, IAA is typically the principal auxin used for rooting of cuttings. However, the usage of IAA concentrations different from species to species and are affected by genotype (Abu-Zahra et al., 2013). Generally, plants respond to an appropriate level of auxin and further increase in concentration becomes inhibitory or even phytotoxic (Pop et al., 2011). Few studies are conducted to reduce the time to use the seedlings and optimize *Euonymus* production. De et al. (2017) reported that the highest rooting number and length in Spondias pinnata Linn cuttings produced with application of cutting length of 15 cm supplemented with 1500 mgL^{-1} IAA. Laubscher and Ndakidemi (2008) reported that roots number and length increased with concentration of 500 mgL^{-1} IAA in Leucadendron laxum cuttings. Seran and Umadevi (2011) investigated the influence of IAA on the establishment of stem

cutting in Lemon (*Citrus Limon L.*) and found that the highest rooting percentage obtained in application of 2500 mgL^{-1} IAA.

As very little works has done in related to rooting in *Euonymus* cuttings, this experiment was carried out to standardize the IAA concentrations along with standard cutting length for better rooting.

MATERIALS AND METHODS

The experiment was conducted at the Horticultural Research Greenhouse, Velayat University, Iranshahr. The material consists of hardwood current-year shoots of *Euonymus* collected from 10 mother plants with six years old, three meters high and 14 cm diameter, planted on conditions of full sun. The stems were collected in early April 2017 located on the garden of Velayat University Campus.

The experiment was conducted in a factorial randomized design with 15 treatments and three replications. Each replication consisted of 10 cuttings. Factor A was contain of length of cutting and Factor B was contain of different concentrations of IAA. Stems were cut to obtain three cuttings lenght of each (7 cm = c1, 15 cm= c2 and 22 cm=c3). For all the cuttings, leaves were carefully removed to homogenize the samples. After preparation of the cuttings, these were immersed in Benomyl solution 5% for 10 minutes to phytosanitory and subsequently in the treatment solution with different concentrations of indole acetic acid (IAA) on base of cuttings for 10 seconds immersion (Stuepp et al., 2014). IAA powder was dissolved in 50% ethyl alcohol (Brown and Duncan, 2006). IAA solution used at concentrations of 0, 500, 1000, 1500 and 2000 mgL^{-1} (I1, I2, I3, I4 and I5 respectively). Control treated was distilled water. The cuttings were planted in Plastic pots (36 cm^2×6.0 cm) using washed sand as substrate in a plastic tunnel. The cuttings were watered twice a day. Adequate drainage was provided to prevent *Phytophtora* fungal infection of roots (Laubscher and Ndakidemi, 2008). The experiment was conducted in an acclimatized greenhouse, with an intermittent mist, average temperature of 24±2°C and 85% relative humidity.

Assessment of cuttings

After 60 days of experiment installation, the following variables were evaluated (Fragoso et al., 2015). Number of callus per cutting, number of roots per cutting (counting the number of primary roots); length of the three longest roots per cutting (cm); the number and length of leaves of per cutting, the number of shoots and lenght per cutting, wet and dry weight of flowers, and the highest bud and flower number of each cutting. Total root length was measured (cm) using a vernier caliper. The flowers of all the plants were air-dried and then placed in an oven at 70 o^C for 24 hours. Dry shoots were weighted in grams by using an electronic balance CX-600.

Statistical analysis

All parameters were subjected to Analysis of Variance (ANOVA) to determine the level of significance of the treatments on the different cuttings of the *Euonymus* plant. All statistical analyses were conducted using R version 3.1.2 (R Development Core Team, 2014). The means were compared using Duncan's multiple range tests.

RESULT AND DISCUSSION

Effects of cutting length and IAA treatments on the rooting formation

The data on the number of roots was statistically significant (P ≤0.01) under all the treatments. The root number increased with application of IAA in all of the cuttings. The root number of *Euonymus* cutting ranged from 1.66 to 8.66. In T7 treatment, significantly (P ≤0.01) produced more roots (8.66) per cutting than all other treatments (Table 1a and 1b).

The callus number of *Euonymus* cuttings ranged from 3 to 24.33. Maximum callus number (24.33) was obtained in T7 treatment and minimum number (3) was obtained in T1 treatment (Table 1a and 1b). The root length of *Euonymus* was also significantly (P ≤0.05) increased in T7 treatment, with measurement of 83.37 cm (Table 1a and 1b). Analysis of variance showed that cutting length of Euonymus was effective on the callus number, root number and root length. The most callus number (13.46), root number (5.90) and root length (56.58 cm) induced in c2 followed by c3 and the lowest number and lenght produced in c1 (Table 1a). Reinhard (2003) reported that the highest number of root was observed in medium sized cutting and the lowest root number was seen in shorter and longer size of cutting. In view of this, inadequate supply of nutrients in shorter stem cuttings and consumption of most food material for lignification in large sized cuttings caused lower rooting and shooting percentage (Good and Tukey, 1956). The callus number, root number and length of Euonymus also were affected by auxin treatments (Table 1a). Stefancic et al (2005) and Chunn et al (2003) reported that some internal and external factors influenced the process of adventitious root formation. Among the internal factors, phytohormones especially the auxins had the most important role in initiation of lateral roots. Exogenous application of auxins results in development of lateral roots. Murthy et al. (2013) reported that better formation of roots in auxin treated cuttings might be due to accumulation of metabolites at the site of application, synthesis of new protein, callus formation, cell division and cell enlargement. The most callus number (17.44), root number (7.40) and length (74.90 cm) were observed in I2 treatment (Table 1a). Laubscher and Ndakidemi (2008) found that root length and number significantly increased in the 500 mgL-1 IAA treatments in comparison with other treatments and is agreemented with our results.

Table 1a

Effect of length of cutting and IAA concentration on root fomation of *Euonymus* cuttings

Treatments	Callus number	Root number	Root length (cm)	Shoot number	Shoot length (cm)	Leaf number	Leaf lenght (cm)
				Cuttings Length			
C1	9.46c	4.00c	47.36b	1.20c	4.88b	15.13c	11.08c
C2	13.46a	5.90a	56.85a	2.73a	6.22a	28.00a	16.56a
C3	11.06b	5.20b	52.92ab	2.20b	6.01a	24.26b	14.48b
F test	**	**	*	**	**	**	**
				Concentrations of IAA			
I1	4.33c	2.00d	5.52c	1.10c	5.62b	11.77c	4.17c
I2	17.44a	7.40a	74.90a	3.33a	6.80a	28.66a	21.66a
I3	17.22a	4.55c	43.96b	2.55b	6.06ab	28.66a	11.13b
I4	8.66b	5. 44b	66.55a	1.44c	3.56c	22.20b	12.00b
I5	9.00b	5.77b	70.96a	1.77c	6.44ab	21.00b	21.23a
F test	**	**	**	**	**	**	**

*, ** Significant at 0.05 and 0.01 probability levels, respectively

Table 1b

Effect of length of cutting and IAA concentration on root formation of Euonymus cuttings

Treatments	Callus number	Root number	Root length (cm)	Shoot number	Shoot length (cm)	Leaf number	Leaf lenght (cm)
T1 (c1×I1)	3.00k	1.66i	5.50f	1.00e	4.00f	2.00h	4.00f
T2 (c1×I2)	16.33c	6.00cde	63.30abc	1.00e	4.80de	17.00g	9.00e
T3 (c1×I3)	12.00de	3.00h	34.00e	2.00cde	6.50bcd	17.00g	16.40c
T4 (c1×I4)	5.00ij	5.33ef	69.66abc	1.00e	5.00cde	20.60ef	18.00c
T5 (c1×I5)	11.00e	4.33g	66.77abc	1.00e	7.00b	19.00fg	8.00e
T6 (c2×I1)	6.00hi	2.66h	6.00f	1.33de	5.20bcde	17.00g	5.13f
T7(c2×I2)	24.33a	8.66a	83.37a	5.00a	8.70a	47.00a	30.00a
T8 (c2×I3)	17.00c	5.66def	58.23bcd	2.66c	6.33bcde	33.00c	8.40e
T9 (c2×I4)	13.00d	6.00cde	56.66cd	2.33cd	4.50e	23.00de	14.00d
T10 (c2×I5)	7.00gh	6.66c	80.00ab	2.33cd	5.33bcde	20.00ef	25.30b
T11 (c3×I1)	4.00gk	2.33hi	5.60f	1.00e	6.66bcd	16.33g	3.40f
T12 (c3×I2)	11.66de	7.66b	80.00ab	4.00ab	6.90bc	22.00de	26.00b
T13 (c3×I3)	22.66b	5.00fg	39.66de	3.00bc	5.37bcde	36.00b	8.60e
T14 (c3×I4)	8.00fg	5.00fg	73.33abc	1.00e	5.20bcde	23.00de	4.00f
T15 (c3×I5)	9.00f	6.33cd	66.11abc	2.00cde	7.00b	24.00d	30.00a
F test	**	**	*	**	**	**	**

*, ** Significant at 0.05 and 0.01 probability levels, respectively

Effects of cutting length and IAA treatments on the shoot formation

The data on the number of shoots was statistically significant ($P \leq 0.01$) under all the treatments. Highest number of shoots (5) was obtained at T7 treatment. Lowest number of shoots (1) was obtained in the T1 treatment. The data of shoot length were statistically significant ($P \leq 0.01$) under all the treatments. Shoot length of cutting was ranged from 4 cm to 8.70 cm. Highest shoot length (8.70 cm) was obtained in T7 treatment and lowest shoot length (4 cm) was recorded in T1 treatment(Table 1a and 1b). Shoot number and length of cutting were affected by cutting length and IAA concentrations. Highest shoot length (8.70 cm) was obtained in T7 treatment and lowest shoot length (4 cm) was recorded in T1 treatment (Table 1a and 1b). Alagesaboopathi (2012) found that highest shoot length was recorded due to increase in linear growth of stem by way of cell elongation.

Effects of cutting length and IAA treatments on the leaf formation

The leaf number was ranged from 2.00 to 47.00. Maximum leaf number (47.00) was obtained in T7 treatment. Minimum leaf number (2.00) was obtained in T1. Also, Leaf length was affected with cutting length and different concentrations of IAA. Maximum leaf length was observed in T7 treatment (Table 1a and 1b). The leaf number and length were affected by cutting length and IAA concentrations. Maximum leaf number (47.00) was obtained in T7 treatment. Minimum leaf number (2.00) was obtained in T1 (Table 1a and 1b). This result is agreemented with Dawa et al (2017) and Okunlola (2013) that reported development of new leaves in cuttings might be associated with increasing of root number and length in

treated cuttings, which helped them in better nutrient and moisture utilization from the growing medium and hence more growth in the form of new leaves and better survival.

Effects of cutting length and IAA treatments on the Fresh weight and dry weight

The data on fresh weight were statistically significant (P ≤0.01) under all the treatments. Fresh weight was ranged from 96.66 to 44.66 g. Maximum fresh weight (96.66 g) was obtained in T7 treatment. Minimum fresh root weight (44.66 g) was obtained in T1 treatment (Table 2a and 2b). The data on dry weight were statistically significant (P ≤0.01) under all the treatments. Dry weight was ranged from 7 to 1.40 g. Maximum dry weight (7 g) was obtained in T7 treatment and least dry root weight (1.40 g) in T1 treatment (Table 2a and 2b). Fresh and dry weight were affected by cutting length and IAA concentrations. IAA increased the fresh weight of the plant by production of more number and length of roots. Maximum fresh weight (96.66 g) was obtained in T7 treatment (Table 2a, 2b). Seran and Umadevi (2011) found that water and nutrients was absorbed by growing roots from soil and more growth induced more weights of shoot. Dry weight of cutting of Euonymus was ranged from 1.40 to 7.00 g. Similar result was reported by Pinto et al. (2011).

Table 2a

Effect of length of cutting and IAA concentration on fresh weight, dry weight and bud number of *Euonymus* cuttings

Treatments	Fresh weight (g)	Dry weight (g)	Bud number
Cutting length			
C1	56.51c	3.73b	2.40b
C2	78.85a	5.49a	7.20a
C3	67.93b	5.13a	3.00b
F test	**	**	**
Concentrations of IAA			
I1	53.34c	3.62c	2.66c
I2	80.20a	5.85a	9.22a
I3	63.46b	5.67a	2.22c
I4	64.10b	3.83bc	1.88c
I5	77.68a	4.94ab	5.00b
F test	**	**	**

*, ** Significant at 0.05 and 0.01 probability levels, respectively

Effects of cutting length and IAA treatments on the bud number

The data on the bud number were statistically significant (P ≤0.01) under all the treatments. Number of buds was ranged from 1 to 18. Highest number of buds (18) was obtained in T7 treatment and minimum number of buds (1) was obtained in T1 treatment (Table 2a and 2b). Bud formation increased with application of cutting length and IAA concentrations. Highest number of buds (18) was obtained in T7 treatment and minimum number of buds (1) was obtained in T1 treatment (Table 2a and 2b). This result is agreemented with Dey et al. (2017) that found the reason might be that IAA treatment increased the accumulation of food reserve, resulting in higher bud formation.

Table 2b

Effect of length of cutting and IAA concentration on fresh weight, dry weight and bud number of *Euonymus* cuttings

Treatments	Fresh weight (g)	Dry weight (g)	Bud number
T1 (c1×I1)	49.10 ef	1.40f	1.00f
T2 (c1×I2)	57.33 ef	4.10cde	7.00bc
T3 (c1×I3)	60.40def	5.00abcde	2.00ef
T4 (c1×I4)	49.33ef	3.10ef	1.66ef
T5 (c1×I5)	66.40cde	5.06abcde	3.00e
T6 (c2×I1)	61.26def	4.46bcde	1.00f
T7(c2×I2)	96.66a	6.46ab	18.00a
T8 (c2×I3)	83.33abc	6.36abc	2.66e
T9 (c2×I4)	66.33cde	4.40bcde	1.66ef
T10 (c2×I5)	86.66ab	5.76abcd	5.00d
T11 (c3×I1)	49.66ef	5.00abcde	6.00cd
T12 (c3×I2)	86.66ab	7.00a	17.00a
T13 (c3×I3)	46.66f	5.66abcd	2.00ef
T14 (c3×I4)	76.66bcd	4.00de	3.00e
T15 (c3×I5)	80.00abc	4.00de	7.00bc
F test	**	**	**

*, ** Significant at 0.05 and 0.01 probability levels, respectively

CONCLUSIONS

Cutting length and IAA treatments had significant effects on the rooting of Euonymus cuttings. Cutting length of 15 cm and IAA in 500 mgL^{-1} produced the most number and length of root in Euonymus cuttings.

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