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Improvement of Rooting in *Forsythia × intermedia* Cuttings by Plant Growth Regulators

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Abstract

Asexual propagation through cuttings is an appropriate method used for most ornamental plants. Success in increasing the percentage of rooting, reducing root emergence and increasing proliferation efficiency depends on several factors like plant growth regulators, especially auxins. Rooting of *Forsythia × intermedia* is difficult and requires specific hormonal treatments and proper cultivation beds. This work evaluated the rooting of *Farsythia × intermedia* cuttings under the action of different concentrations of plant growth regulators. The cuttings consisted of 15 cm long shoots with their basal leaves removed. The basal part of the cuttings was treated with 0, 500, 1000 and 1500 mg l^{-1} IBA and/or NAA. Stem cuttings were kept in hormones solution for 10 seconds. Then, they were planted in rice husk, sand and perlite as cultivation bed under greenhouse conditions. After two months, the percentage of rooted stem cutting, root number per stem cuttings, root length and root fresh and dry weights were determined on stem cuttings. The highest rooting percentage (51%) was obtained in cuttings treated with 1000 mg l^{-1} IBA + 1500 mg l^{-1} NAA. The maximum root number was obtained in cuttings treated with 1500 mg l^{-1} IBA + 1500 mg l^{-1} NAA. The maximum root length (4.66 cm) was obtained in stem cuttings treated with 500 mg l^{-1} IBA + 1000 mg l^{-1} NAA.

Keywords: Hormones, Oleaceae, Ornamental plants, Rooting bed, Rooting percentage.

INTRODUCTION

Forsythia (*Forsythia × intermedia*) belongs to the Oleaceae family. It is one of the known species of this family and plays an important role in the flower industry and landscape. Propagation of this ornamental shrub is performed by cutting. Cutting propagation is commonly used in the commercial production of ornamental crops (Blythe *et al.,* 2004). Desired individual characteristics are maintained through vegetative propagation resulting in a plant that is genetically identical to the mother plant, thus becoming the preferred method of propagation (Oliveira, 1989; Henrique *et al.*, 2006). Auxin is one of the most well-known plant growth regulators (PGR) that plays an important role in the early stages of adventitious root formation and elongation. It is an important way to improve rooting in cuttings of ornamental plants (Kroin, 1992). Cuttings of some plants root readily without a PGR treatment, while cuttings of other plants are promoted by PGRs treatment.

Enhanced promotion of rooting can be controlled by cutting collection time, cutting size, type of species and cultivar, conditions and age of the mother plant, plant nutritional conditions, type and health of the cutting, season when the cuttings are collected, rooting promoting treatments, conditions and type of substrates, irrigation, moisture and temperature, length, diameter, and degree of hardening of the cuttings, injury and heat treatments of the cuttings, the treatment concentrations of auxin-like compounds and other factors (Griffith, 1998; Hartmann *et al.,* 2011; Blythe *et al.,* 2004; Tsipouridis *et al.,* 2006).

Root-promoting PGRs for cutting propagation commonly contain indole-3-butyric acid (IBA), 1-naphthaleneacetic acid (NAA), or a combination of them (Blythe *et al.*, 2004). PGRs are normally applied to the basal part of cuttings using a liquid formulation (Hartmann *et al.*, 2011). Rooting on woody ornamental plants using auxins especially IBA and NAA, individually or in combination has been shown by some researchers (Hartmann *et al*., 2011; Blythe *et al.,* 2004; Henrique *et al*., 2006; Tsipouridis *et al.*, 2006; Tworkoski and Takeda, 2007). The aim of this study was to evaluate the effect of different concentrations of IBA and NAA on increasing the efficiency of asexual propagation, improving rooting of Forsythia × intermedia, determining the optimal concentration of IBA and NAA and introducing it to producers.

MATERIALS AND METHODS

Source of cuttings

Cuttings of leafy stem of forsythia (*Forsythia × intermedia*) with a height of 15 cm and diameter of 6.5 mm were prepared from mother plants in October, 2014. All cuttings were selected from the end of lateral shoots. To avoid the loss and reduce the minimum humidity, the cuttings were prepared in the early morning. Cuttings of forsythia were prepared with three apical leaves and a leaf removed from the basal node.

Treatments

Five cuttings were placed in a container containing 0, 500, 1000 and 1500 mg l^{-1} from both of IBA and NAA for 10 seconds. Then, they were held out for 10 seconds to remove residual PGRs and were cultured in cultivation bed. Cultivation bed used for this experiment was a mixture of sand, perlite and rice husk (1:1:1 v/v). According to the plan, seedling's plastics were filled with 13 cm bed combinations. It should be noted that rice husk was first poured at the bottom of pots and the pots were filled with the sand and perlite. Before putting the cuttings, beds were watered and beaten to achieve a uniform surface. To prevent any physical damage to the cuttings, a hole was first created in the bed before placing the cuttings and then the cuttings were treated with different concentrations of PGRs.

Measurement of traits

Measured traits included the percentage of rooting, root number, root length, and root fresh and dry weight. These traits were measured after two months of cultivation in bed. To determine the fresh weight, the roots were separated from cuttings by a blade and were weighed. To determine the dry weight, roots were separately put in a container and were placed in an oven at 105°C for 24 h. Cuttings of each treatment were removed from the bed and the number of rooted cuttings was counted and accordingly rooted cuttings percentage was used as the main criteria to evaluate treatments. Length of root is an appropriate criterion to evaluate its growth and development because of contacting with the soil.

Statistical analysis

Results were evaluated with analysis of variance and LSD test at 5% probability level to compare treatments with the control. Statistical analysis was performed using the SAS system, release 9.2. The charts were drawn using MS-Excel software.

RESULTS

Rooting percentage

Analysis of variance (ANOVA) showed that the simple effect of IBA and NAA as well as the interaction effect of these two factors on rooting percentage is statistically significant at the 1% probability level (Table 1). Cuttings in all treatments rooted and all cuttings produced new roots. Mean comparison of the data showed that the maximum percentage of rooting (51%) was obtained in cuttings treated with 1500 mg l^{-1} IBA + 500 mg l^{-1} NAA and the minimum one (31%) was related to the cuttings treated with 500 mg l^{-1} NAA + no IBA (Fig. 1). There was no trend in rooting percentage with increasing PGRs concentration.

Root number

Table 1 shows that the simple effects and interaction of IBA and NAA is statistically significant on the root number ($P<0.01$). Rooting was seen in all cuttings for both samples; samples to which PGRs were applied and those to which PGRs were not applied (Fig. 2). Comparison of

Table 1. Analysis of variance of the effects of IBA and NAA on measured traits on *Forsythia × intermedia*

S.O.V	df	Rooting percentage	Root number	Root length	Root fresh weight	Root dry weight
IBA	3	0.0155**	185.5**	4.21**	0.178**	0.0011*
NAA	3	0.0171**	105.4**	1.99**	0.077**	0.0005ns
IBA × NAA	9	0.0225**	63.89**	1.459**	0.047**	0.0012*
Error	32	0.00046	13.14	0.245	0.010	0.00026
CV (%)		5.29	24.54	21.58	41.42	49.64

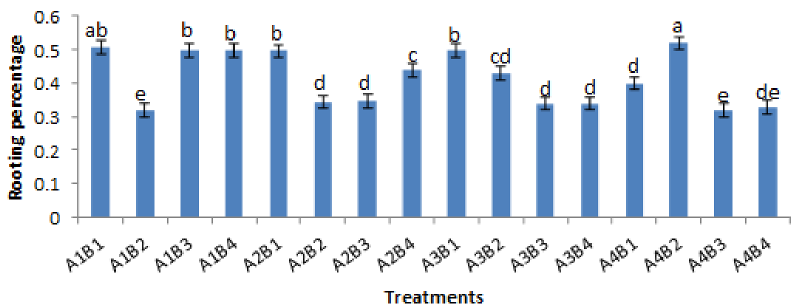

Fig. 1. Effect of IBA and NAA on rooting percentage of *Forsythia × intermedia*

B1: 0 mg l^{-1} NAA
B2: 500 mg l^{-1} NAA
B3: 1000 mg l^{-1} NAA
B4: 1500 mg l^{-1} NAA

A1: 0 mg l^{-1} IBA
A2: 500 mg l^{-1} IBA
A3: 1000 mg l^{-1} IBA
A4: 1500 mg l^{-1} IBA

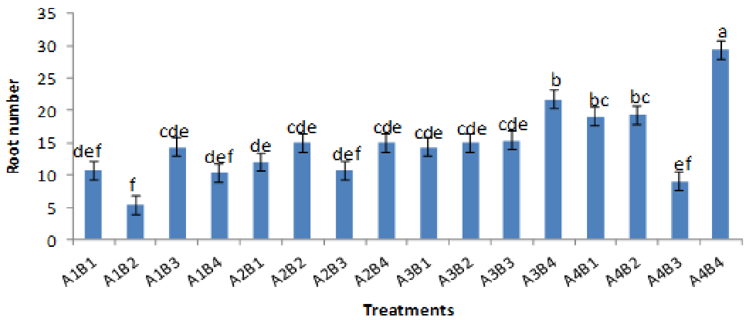

Fig. 2. Effect of IBA and NAA on root number of *Forsythia* × *intermedia*

B1: 0 mg l-1 IBA	A1: 0 mg l-1 NAA
B2: 500 mg l-1 IBA	A2: 500 mg l-1 NAA
B3: 1000 mg l-1 IBA	A3: 1000 mg l-1 NAA
B4: 1500 mg l-1 IBA	A4: 1500 mg l-1 NAA

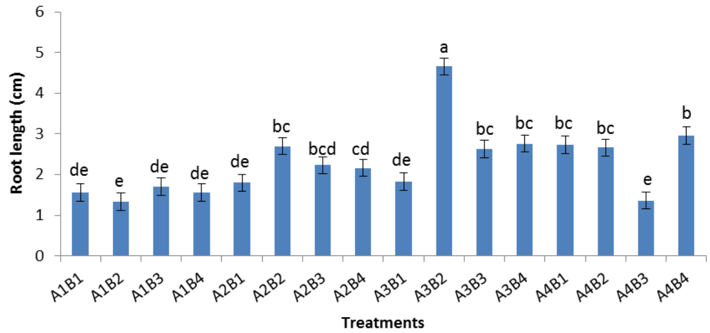

Fig. 3. Effect of IBA and NAA on root length of *Forsythia* × *intermedia*

B1: 0 mg l-1 IBA	A1: 0 mg l-1 NAA
B2: 500 mg l-1 IBA	A2: 500 mg l-1 NAA
B3: 1000 mg l-1 IBA	A3: 1000 mg l-1 NAA
B4: 1500 mg l-1 IBA	A4: 1500 mg l-1 NAA

the average interaction of these two factors showed that the highest number of roots (29.33) was obtained in cuttings treated with 1500 mg l^{-1} IBA + 1500 mg l^{-1} NAA. The least number of roots (5.33 per cutting) was related to the treatment of 500 mg l^{-1} NAA + no IBA (Fig. 2).

Root length

According to the results of the analysis of variance, the effects of the PGRs was found to be significant on root length at P< 0.01 (Table 1). Mean comparison of the data showed that the maximum root length (4.66 cm) was obtained in treatment of 500 mg l^{-1} IBA + 1000 mg l^{-1} NAA and the lowest one (1.33 and 1.36 cm) was obtained in treatment of 500 mg l^{-1} NAA + no IBA and 1500 ppm IBA + 1000 mg l^{-1} NAA, respectively (Fig. 3). The role of IBA is clear in enhancing the root length.

Root fresh weight

In this study where IBA and NAA, individually or in combination, were applied, significant differences were observed at the 1% level in root fresh weight (Table 1). According to the results obtained, PGRs application increased root fresh weight considerably, so that the maximum fresh weight (0.620 g) was obtained in cuttings treated with 1000 mg l^{-1} IBA + 1500 mg l^{-1} NAA. The minimum fresh weight of root (0.046 g) was obtained in cuttings treated with 500 mg l^{-1} NAA + no IBA (Fig. 4).

Root dry weight

LSD test showed that the highest root dry weight (0.091 g) was obtained in treatment of

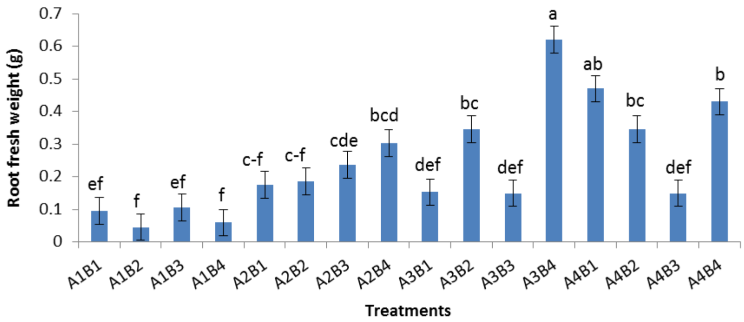

Fig. 4. Effect of IBA and NAA on root fresh weight of *Forsythia × intermedia*

B1: 0 mg l^{-1} IBA	A1: 0 mg l^{-1} NAA
B2: 500 mg l^{-1} IBA	A2: 500 mg l^{-1} NAA
B3: 1000 mg l^{-1} IBA	A3: 1000 mg l^{-1} NAA
B4: 1500 mg l^{-1} IBA	A4: 1500 mg l^{-1} NAA

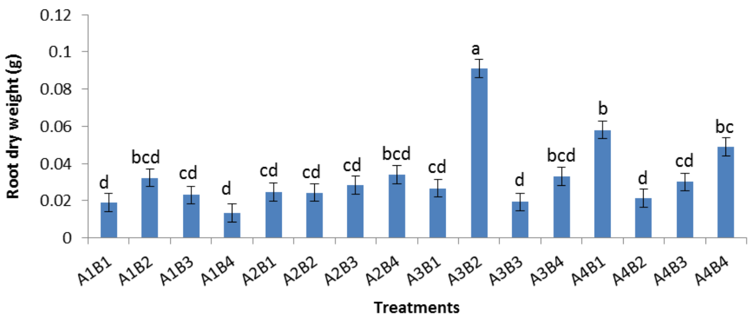

Fig. 5. Effect of IBA and NAA on root dry weight of *Forsythia × intermedia*

B1: 0 mg l^{-1} IBA	A1: 0 mg l^{-1} NAA
B2: 500 mg l^{-1} IBA	A2: 500 mg l^{-1} NAA
B3: 1000 mg l^{-1} IBA	A3: 1000 mg l^{-1} NAA
B4: 1500 mg l^{-1} IBA	A4: 1500 mg l^{-1} NAA

1000 mg l^{-1} IBA + 500 mg l^{-1} NAA and the lowest root dry weight (0.013 g) was obtained in treatment of 1000 mg l^{-1} NAA + no IBA (Fig. 5). Our findings showed that there was no a direct relation between increasing IBA and NAA concentrations and enhancing the root dry weight. Table of analysis of variance (Table 1) revealed that simple effect of IBA and interaction of IBA and NAA were statistically significant at the 5% level, but the simple effect of NAA was not significant.

DISCUSSION

Current study showed that PGRs were effective on rooting of *Forsythia × intermedia*. Similar findings have been reported by many researchers (e.g. Hartmann *et al.*, 2011; Blythe *et al.*, 2004; Henrique *et al.*, 2006; Tsipouridis *et al.*, 2006; Tworkoski and Takeda, 2007). NAA at 500 mg l^{-1} is effective when combined with IBA especially at 1500 mg l^{-1}. Our results showed that IBA is better than NAA for rooting of cuttings. This finding is consistent with many other results obtained by other researchers (Henrique *et al.*, 2006). Henrique *et al.* (2006) revealed for *Pinus* cuttings that the percentage of rooted cuttings treated with IBA presented a higher root formation response in comparison with NAA. These workers showed that rooting was highest in cuttings treated with 4000 mg l^{-1} + 100 mg l^{-1} paclobutrazol. Based on Hartmann *et al.* (2011), IBA is the best auxin for general application because it is nontoxic to plants over a wide concentration range than NAA, and is proper in promoting rooting of a large number of plant species. Nelson et al. (1992) used IAA, IBA, NAA, and indole-3-propil in *Pinus* and showed that IAA and IBA presented good results, while NAA and indole-3-propil showed poor results in the rooting of cuttings. IBA may enhance rooting via increased internal-free IAA. In some woody plants species, NAA is a better PGR for rooting of cuttings than other PGRs (Ibrahim *et al.*, 2015). Some researchers induced rooting in peach cuttings by applying IBA in concentrations from 500 to 2500 mg l^{-1} (de Oliveira *et al.*, 2003; Tsipouridis *et al*., 2005). Tworkoski and Takeda (2007) showed that rooting declined at 1250–2500 mg l^{-1} in peach. One of the most important reasons for different results of rooting on cuttings in various species is the content of endogenous PGRs. Higher endogenous PGRs concentrations improve rooting in some species. Rooting success in many species also is affected by the collection date of the cuttings (Tworkoski and Takeda, 2007). Our results showed that rooting was induced in all cuttings (treated with and without PGRs). Similar results were observed in some studies (Tworkoski and Takeda, 2007; Sağlam *et al*., 2014). Taghavi *et al*. (2012) found that the number of root is significantly affected by the type of cutting and PGRs. They also stated that the increase in the concentration of PGRs (IBA and NAA) up to 7500 ppm improved the number of roots but further increase in concentration reduced the number of roots. Pirkhazri *et al.* (2010) showed that the effect of different concentrations of IBA on the number of roots in cuttings of apple and reported that the maximum number of roots in cuttings was obtained in cutting treated with 2500 ppm and the lowest one is related to the control.

Sağlam *et al*. (2014) showed for *Salvia fruticosa* Mill. that the high concentrations of IBA, NAA and IAA caused the notable increase in root length while lowering the application of these PGRs did not affect root length. They demonstrated that the longest roots were obtained from NAA and IAA applications while the shortest roots were obtained from IBA application. But, the present study showed that the application of low concentration of IBA + NAA induced better root length on cuttings. Similar to our finding, Ibrahim *et al*. (2015) showed that the highest values of root cutting and root length were recorded with the treatments of IBA especially at 250 ppm of IBA.

PGRs cause initial meristematic activity and stimulate the growth and development of the formed roots (Hartman *et al.*, 2011). Transferring rizocalines and combined compound improve rooting indices such as fresh weight (Hartman *et al.*, 2011). In the present study, the use of auxin improved root fresh weight. The results indicated that auxin improves root dry weight and that higher IBA concentration results in higher root dry weight. At the same time with stimulation of rooting by auxin, the mobilization of carbohydrates from the leaves to the root helps rooting greatly increasing dry weight (Fathi and Ismaelpour, 2000). Different beds and PGR levels had a significant effect on root dry weight of *Dodoneae viscosa* L. cuttings (Saffari and Saffari, 2012). A study on *Citrus* showed that the highest root dry weight was obtained in 2000 ppm NAA. Sağlam *et al.* (2014) showed that the higher concentrations of IBA, NAA and IAA induced higher increase in root weight of *Salvia* while lower doses of PGRs did not induce root weight. The lowest root weights were obtained from those for which PGR was not applied. They showed that the highest root weight was obtained from NAA and IAA which was higher than those to which IBA was applied. Our finding was inc contradiction with this result so that the highest weight of roots was obtained in cutting treated with IBA plus NAA.

CONCLUSION

The current experiment showed a clear difference in rooting between cuttings treated with different concentrations of IBA and NAA. In IBA and NAA application, especially in 1500 and 500 mg l^{-1} application, notable increases in most traits were observed. The use of IBA and NAA for rooting of cuttings can help increase the percentage of rooted cuttings and prevent death of cuttings. Totally, IBA is better than NAA for rooting of *Forsythia* × *intermedia* cuttings.

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