

Propagation of *Rosa hybrida* L. cv. Dolce Vita by stenting and stem cutting methods in response to different concentrations of IBA

M. Pourghorban¹, S. Khaghani^{1(*)}, P. Azadi^{2,3(*)}, A. Mirzakhani⁴, M. Changizi¹

¹ Department of Agriculture, Arak Branch, Islamic Azad University, Arak, Iran.

² Department of Genetic Engineering, Agricultural Biotechnology Research Institute of Iran (ABRII), Agricultural Research, Education and Extension Organization (AREEO), P.O. Box 31535-1897 Karaj, Iran.

³ Department of Tissue Culture, Ornamental Plants Research Center, Horticultural Science Research Institute, Agricultural Research, Education and Extension Organization (AREEO), Mahallat, Iran.

⁴ Horticulture Crops Research Department, Markazi Agricultural and Natural Resources Research and Education Center, AREEO, Arak, Iran.



(*) Corresponding author:

azadip22@gmail.com
azadip@abrii.ac.ir
shahab.khaghani@gmail.com

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All relevant data are within the paper and its Supporting Information files.

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The authors declare no competing interests.

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Abstract: This study was conducted to investigate the effect of different concentrations of Indole-3-butyric acid (IBA) in propagation of *Rosa hybrida* L. cultivar Dolce Vita by stenting (cutting and grafting) and stem cutting methods in greenhouse conditions. Different concentrations of IBA (0, 1500, 3000 and 4500 mg/L) were considered on *Rosa hybrida* L. cv. Dolce Vita grafted onto *Rosa hybrida* L. 'Natal Briar' rootstock. Then, the stentings and stem cuttings were cultured in a cocopeat + perlite (in 1:1 ratio) medium under mist system. The research was conducted as a completely randomized design with three replications. The results suggested that all IBA treatments significantly increase rooting percentage compared with the control plants, and the highest specifications of roots and shoots were observed in 1500 mg/L IBA in both methods of propagation. The results approved the superiority of stem cutting in rooting. However, the higher content of chlorophyll a, b and total were obtained in stenting method.

1. Introduction

Rose flower (*Rosa hybrida* L.) is one of the world's most popular flowers among ornamental plants (Castilon *et al.*, 2006), and cut rose flower industry is the most important aspect of rose culture industry in the world (Bleeksma and Van Doorn, 2003) with a turnover of 735 million Euros in 2015 (Azadi *et al.*, 2016). Rose plants are propagated by seed and asexual methods such as stem cutting, grafting, budding, cutting-grafting (stenting), cutting-budding, root grafting and tissue culture (Salehi and Khosh-

Khui, 1997 a, b; Dole and Wilkins, 2005; Azadi *et al.*, 2007, 2013). Vegetative propagation is the sole method for maintaining desirable characters in a superior cultivar particularly when it is heterozygous and polyploid. The easiest and most common method of growing roses is the use of stem cuttings (Anderson and Woods, 1999). The success of rooting in stem cuttings depends on species and cultivar, growth season, the condition of cutting wood, type of cuttings like hardwood cuttings, semi hard wood cuttings, softwood and herbal cuttings, and many other factors (Hartmann *et al.*, 2002). However, stenting method is an efficient technique for quick propagation of plants. This method is an effective technique of rose propagation (Van de Pol and Breukelaar, 1982), in which cutting and grafting is performed simultaneously and the scion is grafted onto a non-rooted rootstock. Formation of the union and adventitious roots on the rootstock occurs simultaneously (Nazari *et al.*, 2009; Karimi, 2011; Babaie *et al.*, 2014). Cutting and grafting is also a valuable technique in propagation of several horticultural species including roses (Koepeke and Dhingra, 2013), conifers, rhododendrons, and also a number of citrus fruits, apples, plums and pears (Hartmann *et al.*, 2002), therefore the use of rootstocks in propagation is a common practice. Plant propagation by stenting has been practiced in several nurseries as a substitute for budding, since it has advantages such as better yield and quality, and resistance to crown gall disease (Park and Jeong, 2010). Efficiency and flower productivity in grafted plants is higher than those in plants growing on their own roots and this is due to the use of rootstock (Cabrera, 2002). Because of different climatic and soil conditions in different areas of the world, different rootstocks are recommended to be the main subject of attention of compatibility between rootstock and scion (Niu and Rodriguez, 2008). 'Natal Briar' is a rootstock of unknown lineage, probably coming from South Africa, and is becoming the desirable rootstock for hydroponic production systems in Netherlands, USA and Colombia (Cabrera, 2002). 'Natal Briar' is the rootstock for grafted roses, which represents 60-70% of the world's cultivation since it is easy to root and gives well stem length and head size (Otiende *et al.*, 2015). Exogenous auxin is shown to have a necessary role in root formation in cuttings and has an effect on the speed and the percentage of rooting of cuttings. Plants produce natural auxin in their fresh shoots and leaves, however, the synthetic auxin must be used for successful rooting to prevent cuttings death (Stefanic *et al.*, 2006; Kasim

and Rayya, 2009), and has been reported in many species, e.g. *Hibiscus rosa sinensis* (Kumar and Singh, 2012). For accelerating the formation of adventitious roots, auxin is widely used on the stem cuttings (Galavi *et al.*, 2013), and it increases the speed and percentage of rooting (Kasim and Rayya, 2009). Photosynthetic pigments in plants comprise chlorophylls a and b and these pigments play an important role in light absorption during photosynthesis (Lobato *et al.*, 2009). Carotenoids are a group of natural tetraterpenoid pigments distributed widely in plants, algae, fungi, and bacteria. Many flowers, fruits, and roots owe their vivid orange, yellow, and red colors to carotenoids. Carotenoids play necessary roles in photosynthesis and photoprotection (Domonkos *et al.*, 2013; Niyogi and Truong, 2013; Hashimoto *et al.*, 2016). This research was carried out to evaluate different concentrations of IBA in propagation of *Rosa hybrida L.* cultivar 'Dolce Vita' using stem cutting and stenting.

2. Materials and Methods

Greenhouse conditions

This research was carried out in February 2017 in a greenhouse with plastic cover located in Markazi Province, Arak, Iran. The greenhouse was equipped with mist system (Fig. 1 a) as well as hot water system for adjusting temperature and humidity. During the study cycle, the average temperature and relative humidity of day were maintained at $20\pm 5^{\circ}\text{C}$ and $85\pm 5\%$, respectively. At night, the mist system was automatically switched off and temperature was maintained at $10-15^{\circ}\text{C}$ and relative humidity of $65\pm 5\%$.

Plant materials and propagation (stenting and stem cutting)

Plant materials were prepared from a Rose commercial greenhouse (located in Markazi Province, Arak, Iran). The scions consisted of a single-node and one leaf (including 2 leaflets) were collected from *Rosa hybrida L.* cv. Dolce Vita as soon as their mother plants entered to faded flower stage. The scions were grafted onto a 4.0 cm length internode taken from the semi-hard wood cuttings of the rootstock *Rosa hybrida L.* 'Natal Briar'. In this method, scions and rootstocks with a suitable flat cut could be grafted together with the maximum overlap of the cambium layer (Fig. 1 b). The scions were selected based on thickness of the stem rootstock. Scions then were

grafted using splice grafting method. Scions and rootstocks with an appropriate smooth cut could be grafted together with the maximum overlap of the cambium layer. Plastic tape was used for wrapping the graft union. The end bud in the rootstock was removed for better rooting and avoiding sucker production (Fig. 1 c). Stem cuttings with at least 3 nodes and of 5-8 mm diameter and 15-20 cm length were selected from the middle portion of the vigorously growing shoots of *Rosa hybrida L. cv. Dolce Vita* as soon as their mother plants entered to faded flower stage. The end node of the stem cuttings were removed for better rooting. The distal end of rootstocks and stem cuttings were treated with different concentrations of IBA (1500, 3000 and 4500 mg/L) by quick dip method, and control plants were treated with distilled water. The stentings and stem cuttings were rooted in a mixture of cocopeat and perlite medium (in 1:1 ratio) under mist system. To prevent fungal infection, the beds were disinfected with fungicides 0.2% 'Captan' solution every two weeks. The grafted plants and stem cuttings were grown for 60 days under the mentioned greenhouse conditions. At the end of the study, the plants were removed from the culture medium and some of their morphological and biochemical traits including healing percentage, rooting, root number, longest root, fresh weight of roots, dry weight of roots, shoot percentage, leaf number, shoot number, longest shoot, content of chlorophyll (a, b and total) and carotenoid were measured.

Chlorophyll (a, b and total) and carotenoid contents measurement procedure

To measure the chlorophyll content, 0.2 g of fresh leaves were ground to a fine pulp by adding 10 ml of 80% acetone. The supernatant was transferred to a 25 ml volumetric flask. The washings were collected in the volumetric flask and the volume was made up to 25ml with 80% acetone. The solution was cen-

trifuged (5000 rpm) for five minutes. The absorbance of the solution was read at 663, 645 and 470 nm against the solvent (80% acetone) blank (Lichtenthaler, 1987).

$$\begin{aligned} \text{Chl a (mg/ml) tissue} &= [12.7(A_{663}) - 2.69 (A_{645})] V / 1000 W \\ \text{Chl b (mg/ml) tissue} &= [22.9(A_{645}) - 4.68 (A_{663})] V / 1000 W \\ \text{Total Chl (mg/ml) tissue} &= [20.2(A_{645}) + 8.02(A_{663})] V / 1000 W \\ \text{Car (mg/ml) tissue} &= [(1000 A_{470} - 1.8 \text{ Chl a} - 85.02 \text{ Chl b})/198] \\ &V / 1000 W \end{aligned}$$

where Chl= Chlorophyll, Chl a= Chlorophyll a, Chl b= Chlorophyll b, Car= Carotenoid, A= Absorbance at specific wavelengths, V= Final volume of chlorophyll extract in 80 % acetone (25 ml), and W= Fresh weight of tissue extracted (0.2 g).

Data collection and statistical analysis

Sixty days after planting, the stentings and stem cuttings were taken out from the media and some traits such as rooting percentage, shoot number, root number, root length (cm), leaf number, shoot length (cm), contents of leaf chlorophyll (a, b and total) and carotenoid (mg/g), root fresh and dry weight (g), and healing (graft-take) percentage were recorded for each stenting and stem cutting. After calculating fresh weight of roots, they were wrapped in paper envelopes and dried in oven at 60°C for 24 hours to calculate their dry weight. Content of leaf chlorophyll and carotenoid were determined using spectrophotometric method (Saini *et al.*, 2001). The experiment was conducted as a completely randomized design with three replications, and each replication consisted of 16 samples. The data were analyzed with SAS software and means were compared through Duncan test ($p < 0.05$).

3. Results

The influence of treatments on morphological parameters and graft healing of stentings are shown

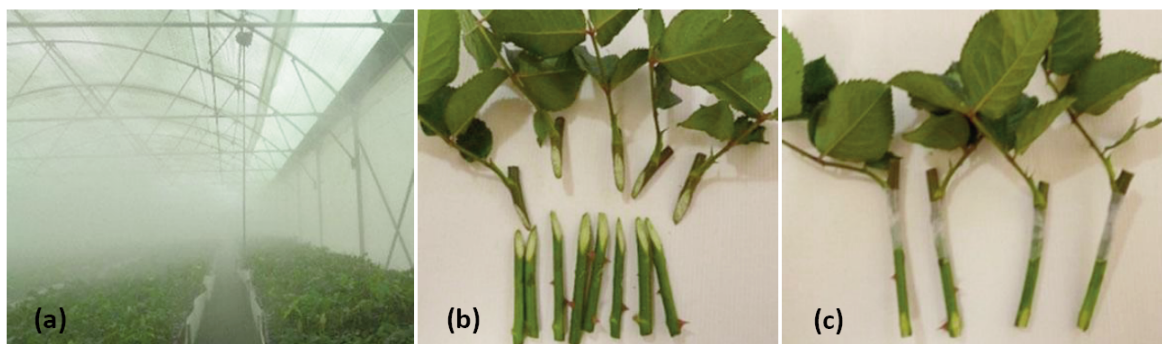


Fig. 1 - Greenhouse space and the stages of preparation of stentings. Mist system in the greenhouse (a), Flat cut in Scions and rootstocks (b), Connection scions and rootstocks by plastic tape, and remove end bud (c).

in Table 1. The presented data clearly revealed that different concentrations of IBA significantly affected various traits of roots and shoots. Maximum rooting percentage, root number, average root length, and fresh and dry weight of roots were recorded in 1500 mg/L of IBA. The highest grafting success, number of leaves, number of shoots per stenting, and average shoot length were observed in 1500 mg/L of IBA concentration (Table 1). An example of rooted stentings is shown in figure 2 (a-d). The influence of treatments on morphological parameters in stem cuttings is also shown in Table 2. Maximum rooting percentage, root number, and fresh and dry weight of roots in stem cuttings were recorded in 3000 mg/L of IBA. An

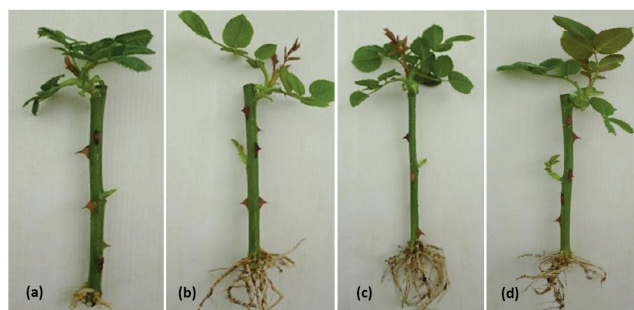


Fig. 3 - Rooting level at different concentrations IBA in *Rosa hybrida* L. cv. Dolce Vita stem cuttings. Control plant (a), Concentration of 1500 mg/L IBA (b), Concentration of 3000 mg/L IBA (c) and Concentration of 4500 mg/L IBA (d).

Table 1 - The effect of IBA treatments on growth parameters and graft healing of *Rosa hybrida* L. cv. Dolce Vita stentings

IBA (mg/L)	Healing (%)	Rooting (%)	Root number	Longest root (cm)	Roots fresh weight (g)	Roots dry weight (g)	Shoot (%)	Leaf number	Shoot number	Longest shoot (cm)
0	37.50 b	35.41 b	1.96 b	0.63 c	0.25 c	0.02 c	37.5 b	1.25 b	0.44 b	2.22 b
1500 mg/L	66.66 a	81.25 a	9.60 a	3.00 a	0.95 a	0.12 a	66.66 a	2.50 a	0.70 a	5.32 a
3000 mg/L	43.75 b	50.00 b	4.54 b	1.93 b	0.60 b	0.07 b	43.75 b	1.96 ab	0.46 ab	3.51 b
4500 mg/L	37.50 b	41.66 b	3.73 b	1.71 b	0.51 b	0.06 b	37.50 b	1.59 b	0.37 b	2.80 b

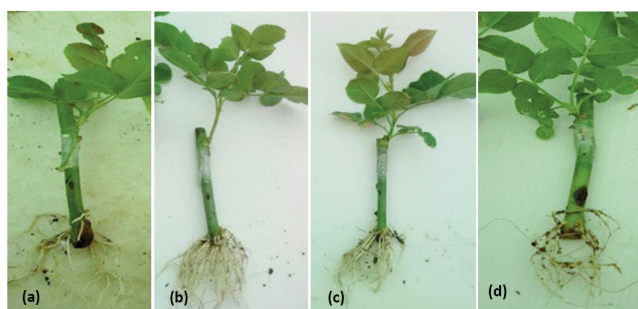


Fig. 2 - Rooting level at different concentrations IBA in *Rosa hybrida* L. cv. Dolce Vita stentings. Control plant (a), Concentration of 1500 mg/L IBA (b), Concentration of 3000 mg/L IBA (c) and Concentration of 4500 mg/L IBA (d).

mg/L of IBA. However, there was no significant difference between treatments (1500 and 3000 mg/L) in most of the traits of stem cuttings (Table 2). Results showed that contents of leaf Chlorophyll (a, b and total) and carotenoid in stentings and stem cuttings were affected by different concentrations of IBA ($p < 0.01$). An example of rooted stentings is shown in figure 4 (a-d).

Healing percentage in stentings

The presented data clearly revealed that maximum grafting success (66.66%) was in 1500 mg/L of IBA, and the minimum healing percentage was observed in control and 4500 mg/L of IBA (Table 1).

Table 2 - The effect of IBA treatments on growth parameters of *Rosa hybrida* L. cv. Dolce Vita stem cuttings

IBA (mg/l)	Rooting (%)	Root number	Longest root (cm)	Roots fresh weight (g)	Roots dry weight (g)	Shoot (%)	Leaf number	Shoot number	Longest shoot (cm)
0	83.33 b	13.95 d	5.06 b	1.23 d	0.12 c	93.75 a	4.8 a	1.69 a	7.87 b
1500 mg/L	95.83 a	22.94 b	8.21 a	1.76 b	0.16 b	95.83 a	4.83 a	1.61 ab	11.20 a
3000 mg/L	97.91 a	26.49 a	7.73 a	2.01 a	0.22 a	97.91 a	4.29 b	1.41 ab	10.46 a
4500 mg/L	85.41 b	20.72 c	5.80 b	1.55 b	0.15 bc	85.41 a	3.39 c	1.35 b	8.73 b

example of rooted stem cuttings is shown in figure 3 (a-d). Different concentrations of IBA had significant effect on rooting percentage, root number, and fresh and dry weight of the roots. The highest rooting percentage, root number, fresh and dry weight of roots was obtained in stem cuttings treated with 3000

Root indices and fresh and dry weight of roots

The highest percentage of rooting were observed in stentings (81.25%) and stem cuttings (97.91%) treated with 1500 and 3000 mg/L of IBA, respectively, and the minimum rooting percentages (35.41% and 83.33%) in both propagation methods were

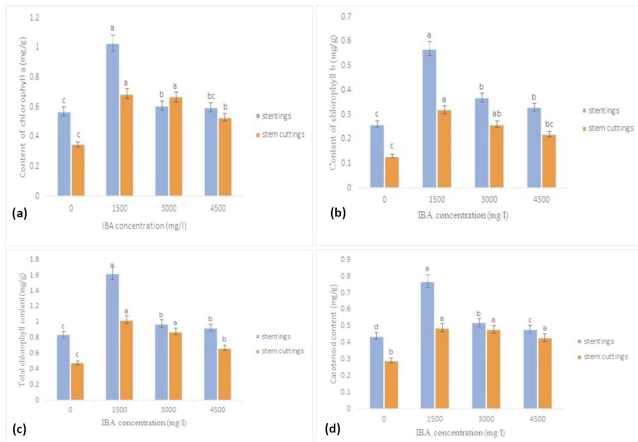


Fig. 4 - Comparison content of chlorophyll and carotenoid (mg/ml) in *Rosa hybrida* L. cv. Dolce Vita stentings and stem cuttings treated with different concentrations of IBA (mg/L). Content of Chlorophyll a (a), content of Chlorophyll b (b), total chlorophyll content (c) and carotenoid content (d).

recorded in control plants (Table 1, 2). Based on this study, different concentrations of IBA affected root number in stentings and stem cuttings. The maximum root number in stentings (9.60) and stem cuttings (26.49) were observed in 1500 mg/L and 3000 mg/L of IBA, respectively (Table 1 and 2). Results showed the greatest average root length in stentings (3 cm) and stem cuttings (8.21 cm) on 1500 mg/L of IBA, and the smallest rooting length in stentings (0.63 cm) and stem cuttings (5.06 cm) were observed in control plants (Table 1 and 2). The greatest root fresh weights were found in 1500 and 3000 mg/L of IBA respectively in stentings (0.95 g) and stem cuttings (2.01 g), and the lightest root fresh weights in both propagation methods were observed in control plants. The greatest dry weight of the root in stentings (0.12 g) and stem cuttings (0.22 g) were also observed at 1500 and 3000 mg/L of IBA, respectively, and the lightest dry weight of the root in both propagation methods were recorded in the control plants (Table 1 and 2).

Number of leaves, number and length of shoots

In stentings, highest leaf number (2.50), shoot number (0.70) and shoot length (5.32 cm) were observed in 1500 mg/L of IBA. The lowest leaf number (1.25) and shoot length (2.22 cm) were recorded in control plants, while the lowest shoot number (0.37) was observed in 4500 mg/L of IBA (Table 1). In stem cuttings, highest leaf number (4.83) and shoot length (11.20 cm) were observed in 1500 mg/L of IBA, while highest shoot number (1.69) was observed in control plants (Table 2).

Contents of leaf Chlorophyll (a, b and total) and carotenoid

In this study, contents of chlorophyll a, b and total and contents of carotenoid were measured in stentings and stem cuttings. In 0.2 g of fresh leaves, the highest content of chlorophyll a (1.03 and 0.69 mg/g), chlorophyll b (0.57 and 0.32 mg/g), total chlorophyll (1.62 and 1.03 mg/g) and carotenoid (0.77 and 0.49 mg/g) were observed in the 1500 mg/L of IBA in stentings and stem cuttings, respectively (Fig. 4 a-d).

4. Discussion and Conclusions

Physiologically, stenting is more complicated than cutting propagation since formation of the graft union must occur simultaneously with rooting and there are interactions between photosynthesis, root formation, and bud development (van de Pol *et al.*, 1986). Karimi (2011) also reported that in stenting, the graft union must be formed before root initiation. Therefore, after leaf formation on the scion, carbohydrates and natural hormones are produced and transmitted from the leaves to the rootstock for growing. Another study reported that rootstock plays an important role in the entire process of root growth, and propagation through bench grafting will bring success. In propagation of *Chinese hibiscus* through stenting showed that IBA treatments significantly increased rooting percentage (Izadi and Zarei, 2014). The effect of different concentrations of auxin on rooting of *Stewartia pseudocamellia*, reported that rooting percentages in cuttings treated with rooting hormones (71.9% to 93.6%) are higher than rooting percentages (53%) in the control plants (Nair *et al.*, 2008). For root formation on stem cuttings, natural or synthetic auxins are essential. Auxin increases the formation of adventitious roots in many species through facilitating carbohydrates and nitrogen materials transfer to the cutting base and motivating primordial root. The high level of auxin may have a negative effect on root length. Auxin leads to transfer of leaf carbohydrate and nitrogen to the roots and therefore causes an increase in the root dry weight (Hartmann *et al.*, 2002). Al-Salem and Karam (2001) reported that auxin concentration had a significant effect on rooting regardless of its chemical structure or type. The maximum rooting percentage, number of root, length, and fresh and dry weight were obtained by basal cuttings treatment with 24 mM of IBA. The maximum dry weight of roots may be

ascribed to increased roots length and number of roots (Ingle and Venugopal, 2009). Auxins promote adventitious root formation and formed roots enhance the uptake of water and mineral nutrient and production of hormones (cytokinins) which are required for shoot growth and development (Otiende *et al.*, 2015). Growth amount of shoots and roots are interdependent (Tonutti and Giulivo, 1990). The probable cause for increase in shoot length may be the better utilization of carbohydrates, nitrogen and other nutrients which has been assisted by growth regulators (Chandramouli, 2001). Izadi and Zarei (2014) reported that the highest leaf number was observed in stentings with higher root number. Buds and leaves are considered significant factors to improve root induction (Hartmann *et al.*, 2002). Leaf chlorophyll content was affected by the interaction between cultivars and propagation methods and was significant in all grafted cultivars; as a result, leaf chlorophyll content and quality index were higher in grafted plants compared to those propagated by cuttings. This might be the effect of rootstock (Nazari *et al.*, 2009). In conclusion, the study demonstrated that the application of IBA plays an important role in success of *Rosa hybrida L. cv. Dolce Vita* propagation through stenting and stem cutting methods. IBA had significant effect on rooting compared to control plants, and the highest rate of rooting percentage was observed in 1500 mg/L IBA in both methods of propagation. Superiority of stem cutting in the case of percentage of rooting was approved. However, the higher content of chlorophyll a, b, total and carotenoids were obtained in stenting which probably will affect the quality and flower size at reproductive stage. It is needed further study on the growth stages of the plant until to the flower harvesting in both cuttings and stenting method.

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