International Journal of Horticultural Science and Technology
Vol. 7, No. 1; March 2020, pp 27-36
Print ISSN: 2322-1461 Online ISSN: 2588-3143
DOI: 10.22059/ijhst.2020.280528.293
Web Page: https:// ijhst.ut.ac.ir, Email: ijhst@ut.ac.ir

Propagation of Three Cultivars of *Rosa hybrida* L. through Stenting Method

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(Received: 3 May 2019, Accepted: 24 November 2019)

Abstract

In the commercial production of roses, introducing a method to reduce the time of propagation with maximum success is crucial. This study was conducted to investigate the effect of different concentrations (0, 1500, 3000, 4500 mg/L) of Indole-3-butyric acid (IBA) on propagation of three cultivars of *Rosa hybrid* L. ('Dolce Vita', 'Samurai' and 'Utopia') by stenting (simultaneous cutting and grafting) method under greenhouse conditions. The cultivars were grafted onto *Rosa hybrida* L. 'Natal Briar' rootstock. The factorial experiment was performed in a completely randomized design with three replications. The stentings were cultured in coco peat and perlite medium at 1:2 ratio in a greenhouse under mist system. After 60 days, the percentage of grafting, rooting percentage, root number, longest root length and fresh and dry weights of roots and shoot traits (including leaf number, shoot number, and longest shoot length) were determined on the stentings. The highest rate of rooting in Dolce Vita cultivar was obtained at 1500 mg/L IBA and in Samurai and Utopia cultivars at 4500 mg/L IBA. Among the three cultivars of roses, highest percentage of rooting (93.75%), healing percentage (93.75%), root length (12.47 cm), fresh weight (0.84 g) and dry weight (0.07 g) of roots were observed in stentings treated with 4500 mg/L of IBA in *Rosa hybrida L*. cv. Samurai. Interaction between IBA concentrations and cultivars on chlorophyll (a, b and total) and carotenoid contents were significant in all the three cultivars of roses.

Keywords: Cutting-graft, Indole-3-butyric acid (IBA), Root, Rootstock, Roses.

Introduction

The genus Rosa comprises of about 200 species and 1800 cultivars, and belongs to the family Rosaceae (Gudin, 2003). It is one of the most important ornamental crops in the world (Senapati and Rout, 2008). Many rose bushes are planted in gardens or pots and many cut-flower roses are sold annually all over the world. Therefore, they are among the most economically important and highly demanded ornamental plants (Khosh-Khui and Teixeira DA Silva, 2006). Roses are propagated by stem cutting,

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budding, grafting, stenting (simultaneous cutting and grafting), root grafting, *in vitro* micropropagation, and in some cases by seed (Salehi and Khosh-Khui, 1997; Azadi *et al*., 2013). In the stenting method, stem containing one leaf and a dormant bud is used as a scion that is grafted onto an internode of the non-rooted rootstock. Formation of graft union and root induction on the rootstock occurs concurrently and in the case of rose hybrids within three weeks (Van de Pol and Breukel, 1982). Most studies have focused on rose productivity in response to rootstock performance (Van de Pol and Pierik, 1995). Rose propagation by the stenting method is an efficient technique with many advantages used worldwide by rose producers (Nazari *et al*., 2009). Rootstock plays an important role in successful propagation via stenting (Izadi, 2012). *Rosa manetti* and *R. canina* were successfully used as the rootstock in Colombia until 1996-97. Today, Natal Briar is used as the rootstock in the propagation of nearly all roses grown in countries like Ecuador, Colombia, Kenya, Ethiopia, and Uganda (Mercurio, 2007). The origin and classification of Natal Briar are not known, although some believe it may have originated from South Africa. Major characteristics of Rose Natal Briar include improved production in grafted varieties, longer stem, better rooting rate, faster regeneration after the cut (harvest), being able to adapt well to different cultivation environments and having high resistance to root diseases (Mercurio, 2007). Most of the cut rose cultivars are propagated via budding or are grafted onto rootstocks. They seldom grow on their own roots, which can be primarily due to the perception that flower production and plant performance in grafted plants are higher than in non-grafted (own rooted) plants, especially when they are cultivated in soil-based or soil growing media (Hanan and Grueber, 1987).

Auxins and cytokinins are compounds involved in plant growth regulation (Fishel, 2009). The important role of auxin in root formation has been documented. Although, auxin is naturally produced in plant shoots and young leaves, synthetic auxin is generally applied for successful rooting and prevention of cuttings death (Kasim and Rayya, 2009). Indole-3-butyric acid (IBA), the most well-known auxin has been widely used in stimulating rooting of cuttings in many plant species. It is non-toxic to plants over a wide range of concentrations (Hartmann *et al*., 1990). The repeated use of IBA in a concentration of 2500 to 4000 mg/L resulted in significant improvement in the rooting of the semi-hardwood cuttings in apple, plum, and olive (Hartmann *et al.*, 1997). The most suitable stems for stenting in Rose are flowering stems because of their complete levels of food and hormone (Mercurio, 2007). Use of rootstocks is a common method in the propagation of roses; however, very few studies have considered scion–rootstock graft union. The objective of this research was to increase the healing of the graft union and rooting percentage in the stenting method through the application of different concentrations of IBA on three cultivars of *Rosa hybrida* L. This method significantly reduces the propagation time for growers and breeders with the higher quality of flowers.

Materials and Methods

This study was carried out in May 2017. Plant materials used in this study were sourced from a commercial rose greenhouse (Markazi Province, Arak, Iran). The greenhouse was equipped with a mist system to help adjust moisture levels. During the study cycle, daily average temperature and relative humidity were maintained at 20 ± 5 °C and 85 ± 5%, respectively (Pourghorban *et al*., 2019). Flowering stems were collected early in the morning and kept under cool and moist conditions until being transported to the work area. Flowering stems include full-grown leaves with open flowers.

After harvesting, every individual stem was kept apart and cut into sections with a five-leaflet leaf and a dormant bud. *Rosa hybrida* L. cultivars including 'Dolce Vita' (flower color: Pink lip), 'Samurai' (flower color: dark red) and 'Utopia' (flower color: fire color) were grafted onto cuttings of *R. hybrid* L. 'Natal Briar' as the rootstock based on our previous study on *R. hybrida* L. cv. 'Dolce Vita' (Pourghorban *et al*., 2019).

A piece of a single internode without a bud was used for each rootstock. All leaves and buds were removed from the rootstock cuttings. Bud removing was done to promote better rooting and preventing sucker growth from the rootstock.

The top of the rootstock internodes and the basal part of the scions were held together to be simultaneously cut for ease of stenting. Scion and rootstock were combined by splice grafting method. For proper growth of the graft, the scion and rootstock were kept in close contact with each other by wrapping them with a piece of grafting tape. The bottom of rootstocks were dipped for 10 seconds in different concentrations of IBA (1500, 3000 and 4500 mg/L). The base of the control rootstocks were treated with distilled water. Stentings were planted in a mixture of coco peat and perlite medium (in 1:2 ratio) and placed in a greenhouse under a mist system. The beds were disinfected using fungicides ‘Captan’ at 0.2 % concentration every two weeks to prevent fungal infection. Stentings were grown for 60 days under the above-mentioned greenhouse conditions. At the end of the study, the plants were removed from the culture medium and their morphological and biochemical traits including the percentage of grafting success, rooting percentage, root number, longest root length, fresh and dry weights of roots, shoot traits (including leaf number, shoot number, and longest shoot length), chlorophyll (a, b and total) and carotenoid contents were evaluated. To measure the root dry weight, first, the fresh weight was obtained, and then the roots were placed in paper envelopes and dried in an oven set at 60 °C for 24 h.

Chlorophyll (a, b, and total) and carotenoid contents of fresh leaves were determined by spectrophotometric method, and absorbance were read at 663, 645, and 470 nm against the solvent blank (80% acetone), respectively based on the method described by Lichtenthaler (1987). The experiment was performed as a factorial in a completely randomized design with three replications. The collected data (three cultivars of *R. hybrida* L.) was statistically analyzed, and the results were compared using Duncan’s new multiple range test at the 5% level of probability using the SAS program (version 9.2).

Results

Results of the analysis of cultivars indicated that the growth and development of shoots and roots of stentings significantly increased when IBA was applied. The most significant effect was recorded for stentings treated at a concentration of 4500 mg/L of IBA in *R. hybrida* L. cv. Samurai. Different stages of rooting and healing in *R. hybrida* L. cv. Samurai via the stenting method is shown in Figure 1 (a-f).

Rooting and healing percentage

Based on the results of this experiment, the cultivars and IBA concentrations significantly ($P \leq 0.01$) affected the rooting and healing percentage (Table 1). The highest rooting and healing percentage (93.75%) were observed in *R. hybrida* L. cv. Samurai treated with 4500 mg/L of IBA. The maximum rooting (81.25% and 79.16%) and healing percentages (81.25% and 68.75%) in Dolce Vita and Utopia cultivars were observed when treated by 1500 and 4500 mg/L IBA, respectively (Table 1). An example of rooting and healing in three *R. hybrida* L. cultivars at different concentrations of IBA are shown in Figures 2, 3, and 4 (a-d).

Fig. 1. Different steps of stenting in *Rosa hybrida* L. cv. Samurai a). Rootstock and scion connection, b). Activated lateral bud, c). Callus step, d). Emergence of primary root and shoot, e). Full growth of stenting, f). Transmission and complete growth in the greenhouse.

Table 1. Effect of different IBA concentrations on stenting rooting of three cultivars of *Rosa hybrida* L.

Cultivars of rose	IBA concentrations (mg/L)	Rooting (%)	Healing percentage (%)	Root number	Root length (cm)	Root fresh weight (g)	Root dry weight (g)
Dolce Vita	0	27.08f	35.41ef	6.76fg	5.15e-g	0.29de	0.01d
	1500	81.25b	81.25b	21.58a	8.48bc	0.84a	0.06ab
	3000	60.41c	47.91de	14.94b	7.73cd	0.67ab	0.043b
	4500	29.16f	29.16f	11.18cd	6.03ef	0.61b	0.046b
Samurai	0	56.25c	37.50d-f	5.05gh	6.41de	0.51bc	0.04bc
	1500	60.33c	39.58d-f	9.16de	7.66cd	0.63ab	0.05b
	3000	60.25c	50d	10.68cd	8.34bc	0.66ab	0.05b
	4500	93.75a	93.75a	11.61c	12.47a	0.84a	0.07a
Utopia	0	35.41ef	37.5d-f	3.47h	3.66g	0.15e	0.01d
	1500	43.75de	35.41ef	7.1e-g	4.51gf	0.17e	0.01d
	3000	45d	41.66d-f	8.44ef	4.7gf	0.39cd	0.02cd
	4500	79.16b	68.75c	11.01cd	9.73b	0.65ab	0.05ab

Means in each column followed by different letters are statistically different using Duncan test ($p < 0.05$).

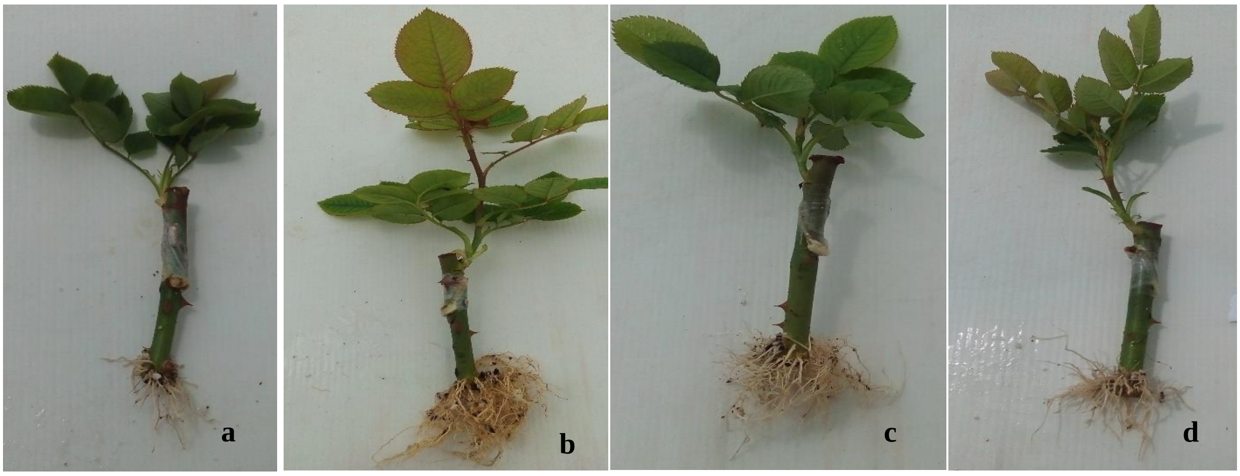

Fig. 2. Effect of different concentrations of IBA on rooting of *Rosa hybrida* L. cv. Dolce Vita propagated by stenting method. Control plant (without hormone) (a), 1500 mg/L IBA (b), 3000 mg/L IBA (c) and 4500 mg/L IBA (d).

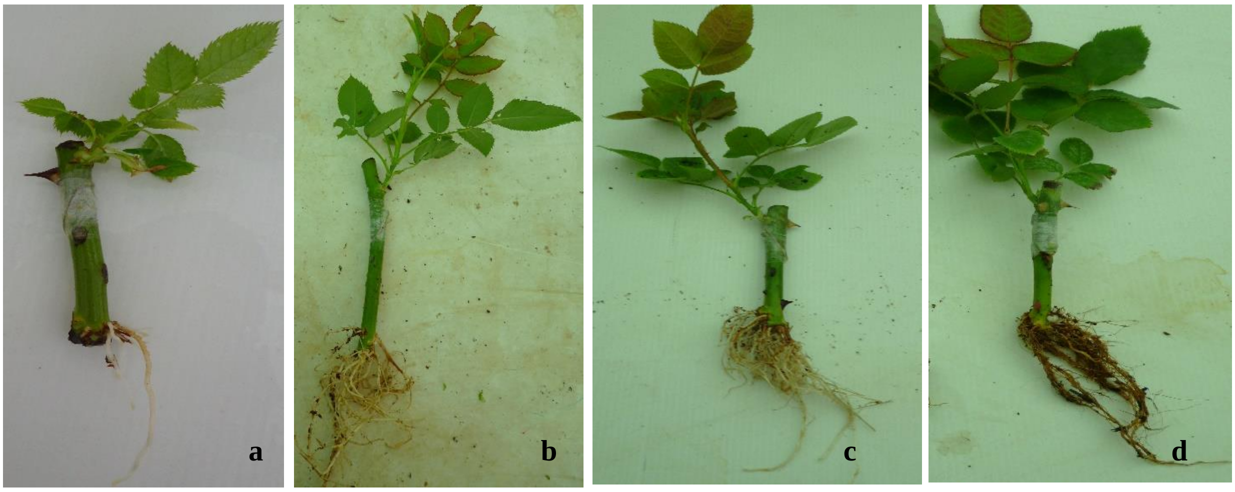

Fig. 3. Effect of different concentrations of IBA on rooting of *Rosa hybrida* L. cv. Samurai stentings. Control plant (without hormone) (a), 1500 mg/L IBA (b), 3000 mg/L IBA (c) and 4500 mg/L IBA (d).

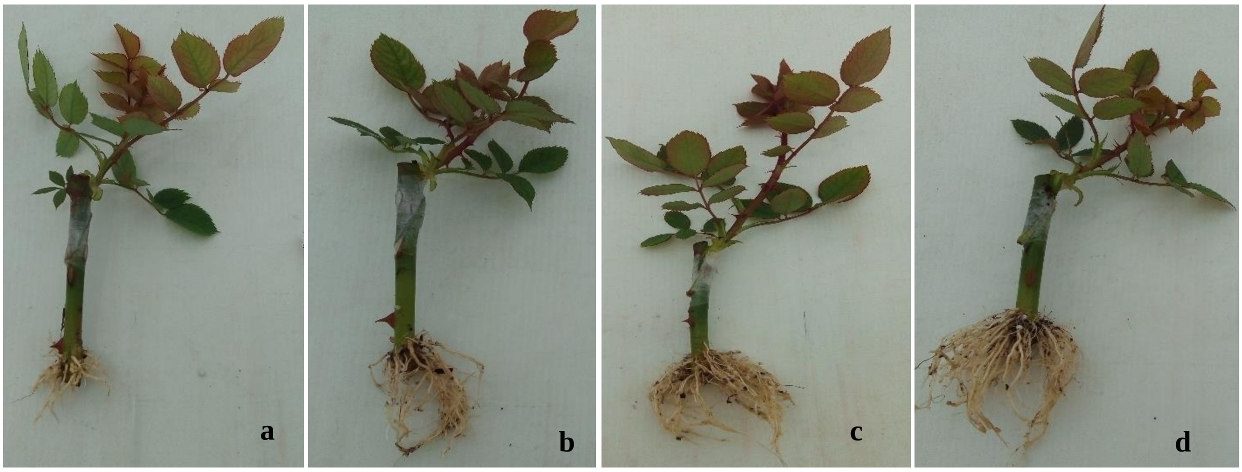

Fig. 4. Effect of different concentrations of IBA on rooting of *Rosa hybrida* L. cv. Utopia propagated by stenting method. Control plant (without hormone) (a), 1500 mg/L IBA (b), 3000 mg/L IBA (c) and 4500 mg/L IBA (d).

Root number and length

Mean comparison for different concentrations of IBA and cultivars revealed that root number and root length were statistically significant (P≤ 0.01). Among the three cultivars, Dolce Vita exhibited the maximum mean value of 21.58 for root number at 1500 mg/L IBA, which was significantly different from the means of other varieties and concentrations of IBA. The highest root length (12.47cm) was obtained in Samurai cultivar treated with 4500 mg/L IBA. The maximum root number (11.01) and root length (9.73cm) in Utopia cultivar were observed at 4500 mg/L IBA (Table 1).

Fresh and dry weights of root

According to the results, all IBA treatments significantly (P≤ 0.01 & 0.05) increased the fresh and dry weights of roots. The maximum root fresh weight (0.84 g) was observed in Dolce Vita and Samurai cultivars treated with 1500 and 4500 mg/L IBA, respectively. While maximum root dry weight (0.07g) was obtained at 4500 mg/L IBA in Samurai cultivar (Table 1).

Leaf number, percentage of shoot induction and shoot length

Maximum leaf number (8.85), maximum percentage of shoot induction (79.16%), and longest shoot length (14.05 cm) were obtained in Samurai cultivar treated with 4500 mg/L IBA. In Utopia cultivar, the highest leaf number (5.75), percentage of shoot induction (50%), and shoot length (13.45cm) were observed at 4500 mg/L. While in Dolce Vita cultivar, IBA concentration had no significant effect on the leaf number, but the highest percentage of shoot induction (77.08%) and shoot length (7.5cm) were observed at 1500 mg/L IBA (Table 2).

Chlorophyll and carotenoid content

The results indicated that the interaction between IBA concentrations and cultivars on chlorophyll (a, b, and total) and carotenoid contents were significant in all the three studied cultivars of rose. The maximum content of chlorophyll a (1.19 mg/g), total chlorophyll (1.77 mg/g), and content of carotenoid (0.89 mg/g) were observed in Utopia cultivar when 4500 mg/L IBA was applied. Furthermore, all IBA treatments significantly improved the content of chlorophyll b in all three studied cultivars. The maximum content of chlorophyll b (0.58 mg/g) was observed in the Dolce Vita cultivar when 1500 mg/L of IBA was applied (Table 3).

Table 2. Effect of different IBA concentrations on stenting shoot growth of three cultivars of *Rosa hybrida* L.

Cultivars of rose	IBA concentrations (mg/L)	Leaf number	Shoot (%)	shoot length (cm)
Dolce Vita	0	2.48d	33.33d-f	6.77c
	1500	3.6cd	77.08a	7.5bc
	3000	3.63cd	47.91bc	7.11bc
	4500	3.63cd	21.91f	7.36bc
Samurai	0	3.23cd	37.50c-e	7.25bc
	1500	3.83cd	41.66b-d	7.86bc
	3000	4.36bc	47.91bc	8.82b
	4500	8.85a	79.16a	14.05a
Utopia	0	2.7cd	29.16ef	6.51c
	1500	3.2cd	29.16ef	6.5c
	3000	3.23cd	39.58b-e	7.1bc
	4500	5.75b	50b	13.45a

Means in each column followed by different letters are statistically different using Duncan test ($p < 0.05$).

Table 3. Effect of different IBA concentrations on stenting chlorophyll and carotenoid content of three cultivars of *Rosa hybrida* L.

Cultivars of rose	IBA concentrations (mg/L)	Chlorophyll a (mg/g)	Chlorophyll b (mg/g)	Total chlorophyll (mg/g)	Carotenoid (mg/g)
Dolce Vita	0	0.58g	0.26e	0.86g	0.44h
	1500	1.03c	0.58a	1.62b	0.77c
	3000	0.61g	0.37b-d	0.98f	0.51g
	4500	0.6g	0.33d	0.94fg	0.49g
Samurai	0	0.77f	0.35cd	1.15e	0.56f
	1500	0.9de	0.43b	1.33c	0.66e
	3000	0.94d	0.43b	1.36c	0.70d
	4500	1.13b	0.56a	1.7ab	0.82b
Utopia	0	0.76f	0.42b	1.19de	0.58f
	1500	0.84e	0.40bc	1.26cd	0.65e
	3000	0.92d	0.43b	1.35c	0.69de
	4500	1.19a	0.57a	1.77a	0.89a

Means in each column followed by different letters are statistically different using Duncan test ($p < 0.05$).

Discussion

This study showed the positive effect of IBA on the successful rooting of three cultivars of *R. hybrida* L. propagated by the stenting method. In recent comparative studies on rooting of *R. damascena* stem cuttings, it was found that IBA had a significant effect on the rooting (Khan et al., 2004; Kazankaya et al., 2005; Dawa et al., 2013). The different levels of endogenous auxin in cutting and genotype characteristics might be a reason for variation in rooting efficiency of cuttings among genotypes (Nasri et al., 2015). The role of auxin in callus induction, stimulation of cell division, cambium layer formation, and differentiation of vascular tissue is well established (Kazankaya et al., 1997). Auxin presence is essential for the induction of the root starter cells (Hartmann et al., 2002). Babaie et al. (2014) reported that a concentration of 4000 mg/L of IBA caused best results for the propagation of *Ficus benjamina*. Natural or synthetic auxin is essential for root formation. Auxin also stimulates the formation of adventitious roots in many species through facilitating the transfer of carbohydrates and nitrogen compounds to the cutting base that stimulating root primordia induction. Furthermore, auxin facilitates the transfer of leaf carbohydrate and nitrogen to the roots resulting in an increase in the root dry weight (Hartman et al., 2002). Auxin has an important role in root induction through cell growth, cell expansion, and initiation of cell division (Akinyele, 2010). The role of grafting technique on the success of stenting propagation of two rose varieties showed that application of IBA at the rate of 5000 ppm induced root emergence (Izadi et al., 2013). In the propagation of *Aesculusindica* treating stem cuttings with plant growth hormone, the maximum rooting rate (50%) was achieved when IBA at a concentration of 4000 mg/L was applied. The cuttings treated with IBA at 2000 mg/L had 25% rooting rate (Majeed et al., 2009). Both environmental factors and physiology of plants can affect the healing of rootstock and scion in the stentings. The suitable environmental conditions helped in the rapid flow of plant sap in rootstock and scion, leading to the formation of the cambium layer, vascular tissue and grafting success (Islam et al., 2004; Sharma and Verma, 2011). Environmental conditions in the greenhouse can also be effective for rooting in stentings (Naier et al., 2008). Some factors involved in the response are related to the growth rate, age of tissue, leaf area in the rootstock, as well as temperature and humidity conditions (Bekhradi et al., 2011). One of the most important requirements of splice grafting is that scion and rootstock should be of the same thickness, similar angle, straight and smooth cut surfaces when placed against each other (Hartmann et al., 2002).

The wounds resulted from cutting stimulates cell division in phloem, xylem, and parenchymal, causing callus formation (Soleimani et al., 2010). Physiologically, stenting is more complicated than cutting propagation because the formation of the graft union and rooting should occur simultaneously, and there are influenced by the photosynthesis, bud development, and root formation (Van de Pol et al., 1986). All stages of the graft union formation (lining up of vascular cambia of the rootstock and scion, wound healing response, callus bridge formation, cambium formation, and vascular tissue formation) are affected by auxin (Hartmann et al., 2010).

The results of the present study indicated that IBA concentration significantly affected root and shoot characteristics in stenting and that the effect is cultivar dependent in rose. The highest rooting and healing percentages, root length, fresh and dry weights of root, leaf number, shoot percentage, and shoot length were observed in Samurai cultivars treated with 4500 mg/L IBA. Although the best root and shoot characteristics of the Utopia cultivar was similar to those of the Samurai cultivar at 4500 mg/L concentrations of IBA, other results were weaker than the Samurai cultivar, except for chlorophyll (a, b and total) and carotenoid contents that were higher in the Utopia cultivar. However, the best concentration of IBA for Dolce Vita cultivar was found to be 1500 mg/L. High concentrations of IBA produced toxicity for the stenting of this cultivar. The root and shoot characteristics in all concentrations of IBA were higher than those in control plants. Using this method, the growers could propagate roses in a significantly shorter time compared with the cutting method. Moreover, higher quality of flowers could obtain using the stenting method.

Acknowledgments

The authors appreciate Mr. Feizian for providing greenhouse facility and Mr. Rostami for his assistance during this experiment.

Conflict of interest

The authors declare no conflict of interest for this study.

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