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Evaluation of Stenting for the Propagation of *Syringa vulgaris* on *Ligustrum vulgare* Rootstock

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Abstract

Lilac (*Syringa vulgaris*) is a valuable shrub used in the landscape due to its beautiful leaves and flowers and pleasant fragrance. Grafting of lilac on wild privet (*Ligustrum vulgare*) was evaluated in this study to reduce the time required for propagation and the use of rootstock. Stenting or simultaneous rooting and grafting is a novel method for propagating of some woody plants. In this research, the stenting technique was applied to propagate lilac for the first time. Two stenting methods (splice and omega) and three concentrations of IBA (0, 1000 and 2000 mg L^{-1}) were evaluated. According to the results, stenting via the splice method significantly increased the percentage of rooted stentings and leafed scions and reduced the rate of dried stentings in comparison to the omega method. The percentage of rootstock callus formation, percentage of leafed scions, and fresh weight of produced roots and shoots were significantly enhanced by application of IBA. The best results were obtained with 2000 mg L^{-1}. In addition, the lowest percentage of dried stenting was observed in this treatment. Also, final success of stenting showed that the use of the splice method and IBA had the highest achievement. In conclusion, the application of 2000 mg L^{-1} IBA in comparison with splice stenting method is recommended for propagation of *Syringa vulgaris*.

Keywords: Cutting-grafting, Grafting, *Ligustrum vulgare*, Lilac, Privet, Propagation.

INTRODUCTION

Common lilac, scientifically known as *Syringa vulgaris,* is native to mountainous regions of Eastern Europe and is domesticated as an ornamental deciduous shrub or small tree (Bean, 1921). It is thus prevalent in park and landscape architecture due to its elegant flowers and leaves (Royal Horticultural Society, 2008). Moreover, the common lilac is widely produced as a cut flower that can be forced effortlessly (Jędrzejuk *et al.*, 2013). Because lilac cuttings are hard to root, the most common method of lilac propagation is to graft shoots onto wild privet (*Ligustrum vulgare*) rootstocks (Waldenmaier and Bünemann, 1991). Wild privet, also called common or European privet, is an indigenous deciduous or almost evergreen shrub in Europe, Western Asia, and Morocco (Bean, 1921). Stenting or cutting-rooting is advantageous for reducing the time required for achieving commercially grafted seedlings due to the synchronicity of rooting and grafting applied in numbers of ornamental plants and fruit trees (Nazari *et al.*, 2009; Babaie *et al.*, 2014; Izadi and Zarei, 2014; Karimi, 2011; Solgi *et al.*, 2022; Brar and Khehra, 2017). The stenting method lacks several grafting adversities; in addition, it mostly improves the degree of rooting and scion growth (Ohkawa, 1980). Plants from stenting generally have heavier root weights than cutting plants; furthermore, this technique is appropriate for studying and screening the interactions between rootstocks and scions (van de Pol and Breukelaar, 1982). Thus, it can be a valuable technique for rapid mass multiplication and year-around production of different plants to meet the increasing demand (Rawat and Kumar Das, 2020).

Auxins are phytohormones that play essential roles in nearly every facet of plant growth and development and have complex biosynthesis (Zhao, 2010). These hormones are pivotal to the growth mechanisms of leaves, buds, fruits, roots, and flowers, as well as to seed functions (Osborne and McManus, 2005). Indole-3-butyric acid (IBA) is a synthetic auxin. At the same time, it has been extracted from the leaves and seeds of some plant species (Ludwig-Müller, 2000), with which many of the plant species with hard-rooting cuttings are currently commercially rooted (Hartmann *et al.,* 2010). The grafted ornamental shrubs like the cut rose cultivars, on productive rootstocks had superior flower yield and performance relative to plants growing on their roots (Cabrera, 2002). The utilization of IBA in stenting has been studied in some commercial ornamental roses, which due to the complicated decreasing interaction between scion and rootstock, experienced weaker rooting and shooting compared to those of cutting (Pourghorban *et al.*, 2019). Although, this compound is recommended for rose growers due to the generation of heavier roots and shoots as well as higher leaves, shoots, and roots numbers (Yeshiwas *et al*., 2018). Further, indole-3-butyric acid can result in more numerous and heavier roots in *Ficus benjamina* propagated via the stenting method (Babaie *et al.*, 2014). It has a substantial positive direct impact on callus formation at the grafting union and length of the roots and shoots; moreover, the number of leaves due to the use of IBA in mulberry stenting is determined (Solgi *et al.*, 2022). As far as our knowledge, there is no study on the application of this technique in combination with IBA to propagate *Syringa vulgaris* on wild privet rootstock. The purpose of this study was to determine the performance and yield of stenting as an alternative method of cutting and to evaluate wild privet potential as a rootstock. Recently, Kiran *et al.* (2022) recommended to stenting in the month of January for the production of quality plants of rose cv. "Top Secrete". The research question is how much the stenting propagation method can be successful in overcoming the problems associated with grafting and cutting methods problems like hard rooting and being time-consuming.

MATERIALS AND METHOD

In this study, both types of plant materials (lilac scions and privet rootstocks) were collected from the Arak University landscape. Scions were prepared from mature shoots of *Syringa vulgaris* (Fig. 1).

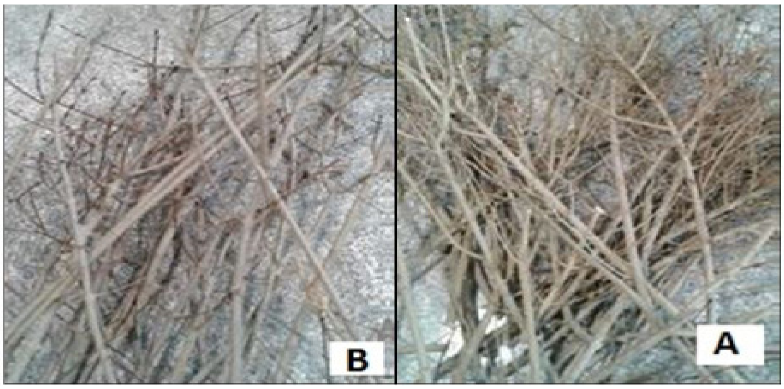

Fig. 1. Wild privet shoots as rootstocks (A) and common lilac shoots as the scions (B).

Treatments and stenting methods

Two factors including two stenting methods (splice and omega) and IBA as a rooting hormone at three levels (0, 1000, and 2000 mg L^{-1}) were used (Fig. 2). Shoots selected from the middle were sliced into pieces with at least two buds and prepared for splice grafting and omega grafting method (Fig. 2). The cuts were made 3 cm below the lowest bud of the scions and 2 cm above the uppermost rootstock buds using a clipper for the splice method. Also, with the cuts were made for the omega technique using a grafting tool (Fig. 3). Immediately after cutting the scions, necessary rootstock procedures were performed. The rootstocks and scions, which had almost the same diameter, overlapped to conjoin the vascular cambium layers in the best way (Fig. 4). The grafted cuttings were then immersed into the IBA treatment for 5 s. Light-color parafilm tapes were applied to furl the grafting union (Fig. 5). Afterward, the plants were placed in a growth medium containing 70% sand and 30% perlite. Moreover, Benomyl (0.3 % V/V) was used to disinfect the propagation medium.

Fig. 2. Applied omega grafting tool for this study.

Fig. 3. Cutting types for splice (left) and omega (right) stenting.

Fig. 4. Overlapping of the scions and rootstocks using a splice (left) and an omega grafting tool (right).

Fig. 5. Covering the grafting union with parafilm tapes.

Plant maintenance conditions

The greenhouse equipment for environmental control includes convective heating, evaporative cooling pads, and exhaust fans. The mean mid-day temperature and relative humidity during the study period were maintained at 25 °C ± 2 °C and 70 % ± 5 %, respectively. Also, due to the plants' requirement of a relative humidity of 95-100%, particularly during the first week after cutting the grafting, a polyethylene coating was thrown over the box of the grafted plants.

Evaluated characteristics

Three months after grafting, the cutting grafts were removed from the medium box and washed with water. Some morphological characteristics, such as the percentage of rooted rootstocks, percentage of leafed scions, percentage of dried grafted plants (did not show any symptoms like as callus formation in junction and/or rootstock and rooting formation after three months), percentage of callus formation from rootstocks, fresh weight of produced roots and leaves, and length of the longest produced root and shoot, were recorded. Following the evaluation of the characteristics, the grafted plants were planted in pots with (10 cm diameter) having the beds made of sand and agricultural soil (1:1). Two months next to the cultivation of cutting grafts (five months after stenting), the final grafting success rate was evaluated.

Experimental design and statistical analysis

This experiment was conducted in a factorial arrangement based on a completely randomized design with three replications, each consisting of 10 samples. Statistical significance between mean values was assessed using analysis of variance (ANOVA) and the conventional Duncan's multiple range test (DMRT) at $P < 0.05$ using SAS (9.1).

RESULTS

According to ANOVA results, the effect of stenting method on the percentages of rooted rootstocks ($P < 0.05$), dried grafted plants characteristics ($P < 0.05$), and percentage of leafed scions ($P < 0.01$) were significant. However, other traits did not show significant differences. The various IBA concentrations significantly influenced the percentage of leafed scions and the length of the tallest shoot at a 5% probability, and the percentages of callus formation from rootstocks, dried grafted plants, and fresh weight of produced roots and leaves were significant at a 1% probability. In contrast, it did not have any significant effect on the percentage of rooted rootstocks and the length of tallest roots. The interaction effect of these factors only affected the percentage of leafed scions at a 1% probability (Table 1).

Table 1. Analysis of variance of three levels of IBA and two methods of stenting's impact on various traits after 3 months.

		MS							
S.o.V	**df**	**Longest root length**	**Longest shoot length**	**Shoot fresh weight**	**Root fresh weight**	**Dried stenting**	**Produced leaves**	**Produced roots**	**Callus formation**
Type of Stenting (A)	1	0.320^{ns}	2.420^{ns}	0.031^{ns}	0.00001^{ns}	16.155^{*}	21.505^{**}	1.008^{*}	0.016^{ns}
IBA Concentration (B)	2	1.416^{ns}	4.402^{*}	0.231^{**}	0.08^{**}	18.722^{**}	12.518^{*}	0.211^{ns}	2.57^{**}
A × B	2	0.0003^{ns}	0.715^{ns}	0.083^{ns}	0.006^{ns}	7.055^{ns}	8.845^{*}	0.141^{ns}	0.191^{ns}
Error	12	0.616	0.678	0.03	0.01	2.0	2.121	0.123	0.212
CV (%)		30.62	24.46	16.17	11.67	24.71	25.23	29.95	24.46

*, ** and ^{ns}: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the DMRT test, respectively.

The comparison between the stenting methods indicated that the percentage of rooted stenting was affected by the stenting method, and the splice method had a better rooting percentage (17%) than the omega method (5%) after three months (Fig. 6a).

Based on the mean comparisons, the influence of the stenting method was significant for a significant percentage of dried grafted plants, and the splice method produced lower dried grafts (48%) compared with the omega method (67%) (Fig. 6b). These outputs are similar to those of previous root stenting.

The splice method significantly influenced the leaf production of stenting. Also, the interaction effect of stenting methods and IBA concentrations significantly impacts on the leaf production. The splice stenting method produced more leaves (48%) than the omega method (27%) (Fig. 6c).

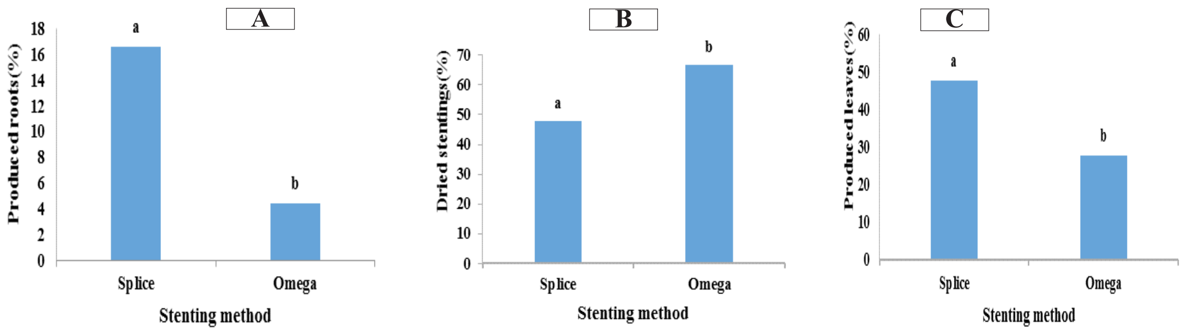

Fig. 6. The influence of the two methods of stenting on the produced roots percentage (a), on the dried stenting percentage (b), and produced leaves percentage (c).

Moreover, the application of IBA especially 2000 mg L^{-1} increased the leaf formation. The comparison effect revealed that IBA levels had less influence on leaf formation grafted via the splice method than the omega method (Fig. 7a). Therefore, the best result was achieved using the omega method with 2000 mg L^{-1} of IBA (53.3%).

The impact of various concentrations of IBA on the percentage of rootstock callus formation was significant. The highest percentage of callus formation was in 2000 mg L^{-1} (63.3%), which is comparable with the lowest percentage observed for control treatment (15%) (Fig. 7b).

The mean IBA level significantly influenced the percentage of dried stenting percentage (Fig. 7c). Increasing the IBA concentration reduced the proportion of dried grafted plants. The highest and lowest dried cutting graft yields were observed for the control treatment (73%) and 2000 mg L^{-1} IBA (38%) treatments, respectively. These results were similar to previous characteristics, including the percentage of rootstock callus formation and leaf production, which were enhanced at higher IBA concentrations (Fig. 7c).

According to the mean comparison of root fresh weight, this trait increased with increasing application of higher IBA concentrations (Fig. 7d). Thus, the most considerable root fresh weight was recorded in 2000 mg L^{-1} (0.55 g), and the least was for the control treatment (0.14 g). The result of this parameter was similar to the above mentioned characters like percentage of rootstock callus formation and leaf production.

Like the roots fresh weight, the shoot fresh weight and length of the longest produced shoot were significantly enhanced by using IBA. Thus, the largest fresh weight and longest shoot were produced because of 2000 mg L^{-1} IBA and the least amount were obtained in the control treatment (Fig. 7e and 7f).

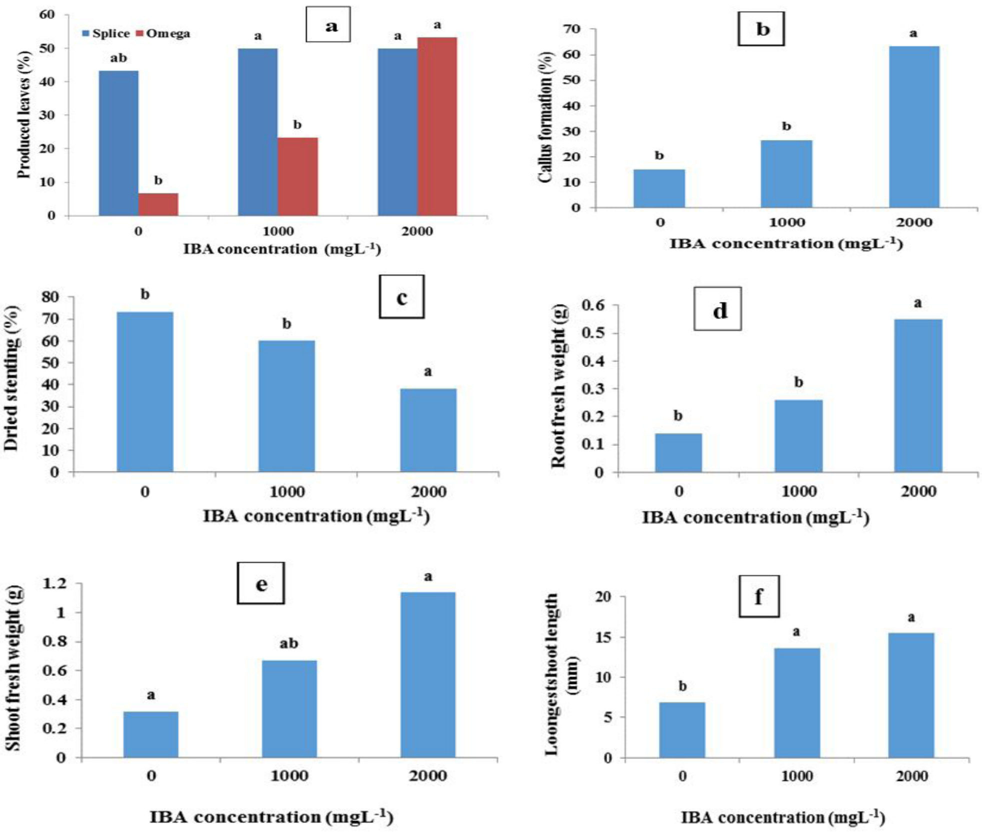

Fig. 7. The effects of two stenting methods and different IBA concentrations on produced leaves percentage (a), on callus formation percentage (b); on the proportion of dried stenting percentage (c), root fresh weight (d), fresh weight (e), and length of the longest shoot (f).

Two months since the remained cutting grafts were established from the previous step and planted in the pots, the final stenting success was evaluated. Analysis of variance revealed that there is a significant effect of the stenting method at 1% and various IBA levels at 5% (Table 2).

Table 2. Analysis of variance of three IBA levels and two stenting methods on the final percentage of stenting success after 5 months.

S.o.V	df	MS
		The final percentage of stenting success
Type of Stenting (A)	1	24.07**
IBA Concentration (B)	2	15.35*
A × B	2	1.37ns
Error	12	1.93
CV (%)		30.12

*, ** and ns: Significant at $P < 0.05$, $P < 0.01$ and insignificant based on the DMRT test, respectively.

The comparison of the success of the two stenting methods on cutting grafts revealed that the splice method significantly has a superior success rate (32%) compared with the omega method (13%) after 5 months (Fig. 8a). These outcomes correspond to other characteristics during the prime step (after three months). Also, the final success of stenting was increased with superior amounts of IBA. 2000 mgL^{-1} treatment caused the highest success rate (32%), compared with the control treatment with only 8% success (Fig. 8b).

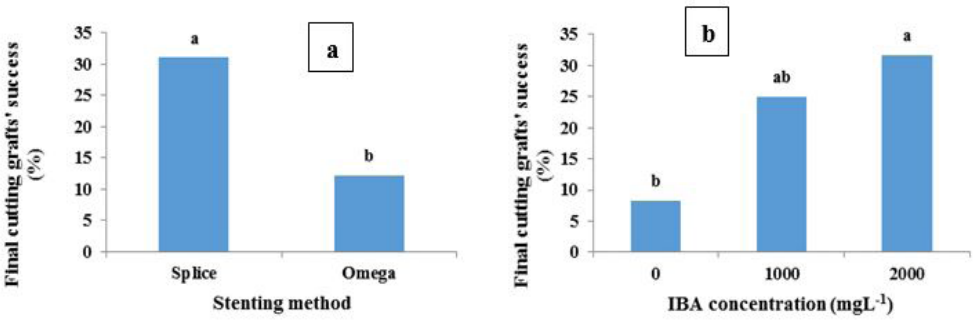

Fig. 8. The influence of the two stenting methods on the final cutting-grafting success after 5 months (a) and the influence of different IBA concentrations on the final cutting-grafting success after 5 months (b).

DISCUSSION

There is no report to investigate the stenting of common lilac. Our study used IBA levels for rooting wild privet as rootstock and two stenting methods on grafting of lilac, simultaneously. According to the present findings, the stenting splice method produced more rooted grafted plants and leaves. Further, the splice technique generated fewer dried stenting. Besides, after five months, the final cut-graft achievement was higher for the splice method. The current study indicated that the splice stenting method has higher final success, survival, and growth factor percentages than the omega stenting method. Generally, a connection between the scion chamber and rootstock is prerequisite for successful grafting. So, failure to create a stable connection between the scion and rootstock vessels and consequently the insertion of the cambium in an abnormal position causes unsuccessful grafting and finally lack of grafting survival and subsequent growth (Ramos, 1998; Solgi *et al.*, 2012; Solgi *et al.*, 2022). Some grafting methods have a minor connection between scion and rootstock cambiums, lowering the grafting success rate (Nowrozi *et al.*, 2016).

In this study, the stenting splice method had a higher success rate owing to earlier root creation and early establishment on rootstock, causing superior vegetative growth of the grafting combination. There is no report related to any stenting method for this shrub to date, and the reported researches are mostly on fruit trees and rose production. For instance, Khalili *et al.* (2011) suggested the application of the omega stenting method due to its desired impact on rose commercial production. In another study, greenhouse rose varieties were grafted onto *R. manetti* rootstock. Results indicated that the omega stenting method was more successful than the tongue method. The "Peach Avalanch" rose cultivar on *R. manetti* rootstock has the highest number of roots, stems, and leaves and produces the longest roots (Izadi *et al.*, 2013). Contrary to our previously mentioned results, the omega stenting method was found suitable for rose propagation.

Based on our results, the application of higher concentrations of IBA, there were significant impacts on rootstocks callus formation, scion leaf production, and fresh weight of roots and shoots. The maximum effect was observed at a concentration of 2000 mg L^{-1}. In addition, the lowest dried cutting graft proportion was observed at this concentration of IBA. Solgi *et al*. (2022) investigated the propagation of black mulberry into white mulberry by stenting. They demonstrated that 62% of white mulberry rootstocks produced rooting at 1000 mg L^{-1} IBA. Whereas, no significant effects were observed using a combination of IBA and stenting as a new technique for propagating lilac in this study. Babaie *et al.* (2014) reported that *Ficus benjamina* produced 50% rooting by the omega stenting method without IBA. In contrast, in our study, just 4.5% of the cutting grafts formed roots via the omega grafting method without IBA consumption. Also, according to a stenting survey of black mulberry onto white mulberry by Solgi *et al.* (2022), 39% rooting percentage was observed, compared to 4.5% in this study. Furthermore, 6.66% of lilac leaves were produced in this research in relation to 0% leaf formation in black mulberry (Solgi *et al.*, 2022). Pourghorban *et al.* (2020) 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^{-1} IBA.

In general, successful rooting during vegetative propagation via cutting grafting depends on diverse physiological conditions from which the cuttings originated, plant genotype, and environmental conditions. Some of the most severe operatives in cutting-grafts rooting are node condition, leaf number, cutting time, light intensity, temperature, humidity, cultivation bed type, and phytohormones (Izadi *et al.*, 2013; Park and Jeong, 2012; Solgi *et al.*, 2022; Hartman *et al.*, 2010).

On the other hand, prominent factors on grafting success can be divided into internal and external. External factors like grafting time, method, and environmental conditions such as temperature and relative humidity influence grafting success. Besides, internal compounds like phytohormones, phenols, and vegetative activity of scion and rootstock are the most influential factors in terms of internal factors influencing the final grafting achievement and success (Nowrozi *et al.*, 2016; Solgi *et al.*, 2012; Hartmann *et al.*, 1990). The suggested and applied stenting method is typically linked to plant species and genetic differences. Genetic differences among plant cultivars influence on internal grafting factors like tissues water content, soluble carbohydrates, starch, C/N ratio, phenolic compounds, and hormone content in scion tissues. These factors can cause differences in cultivar grafting success (Pinghai and Rongting, 1993; Stanisavljevic and Mitrovic, 1997). Also, choosing proper rootstocks is vital to improving future plant scion growth. Nazari *et al.* (2009) reported that propagation of *R. canina* via stenting produces smaller flower shoots than that achieved via cutting.

Furthermore, the stenting success rate ascended in the next five months because IBA was applied at higher levels. Rootstock rooting can be an influential factor in the success of cutting-grafting. It has been observed in some of the cutting grafts that there was grafting success; at the same time, roots were not developed in rootstocks, causing some grafted plants not to attain the compatibility circumstances at the next step and waste. It is suggested to use the optimum conditions like rooting hormones, appropriate beds, and appropriate environmental conditions to avoid this challenge (Izadi *et al*., 2013).

The splice grafting method increased the proportion of rooted cutting grafts, grafting success, and growth ability of plants analogous to the omega technique. Next to the primary evaluation (after three months), the percentage of rooted cutting grafts was lower than that in the final assessment (after five months). The reason is the lack of adequate roots and weak rooting at earlier grafting times. Sometimes when the roots are insufficient and weak, grafting success and scion growth reduction occur due to the lack of water and nutrient absorption and transfer (Hartmann *et al*., 2010; Nowrozi *et al*., 2016).These findings prove that the grafting method may be a prerequisite to successful grafting (Izadi *et al*., 2013; Hartmann *et al.,* 1990). The lilac cuttings on wild privet rootstocks experienced low grafting success percentages (8% for omega without IBA application and 32% for 2000 mg L^{-1} IBA) compared to those of *Ficus benjamina* (75.83% for omega without IBA application and 87.61% for 2000 mg L^{-1} IBA) according to (Babaie *et al.,* 2014).

CONCLUSION

This study was conducted to find an alternative method of lilac propagation that would be faster, easier, and more justifiable economically. However, the evaluation of the elements indicated that stenting for the propagation of common lilac on wild privet is not commercially justifiable due to its low success rate and significantly weak growth factors. Based on these results, further studies on the effects of changing IBA concentrations, grafting time, and quality of scions and rootstocks are suggested for the improvement and success of stenting in *Syringa vulgaris*.

AUTHOR CONTRIBUTIONS

Mousa Solgi has received research grants from Arak University and rolled in planning, analyzing, calculating, comparing, and writing the article in collaboration with Mohammad Sajad Asheghi. Hossein Bagheri was in charge of plant cultivation and measurement.

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CONFLICT OF INTEREST

On behalf of all authors, the corresponding author states that there is no conflict of interest.

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