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Scientific Article

Response of black mulberry onto white mulberry rootstock to stenting (cutting-grafting) techniques and IBA concentrations

Mousa Solgi[1*], Mina Taghizadeh[1], Hossein Bagheri[1]

[1] Arak University, Faculty of Agriculture and Environmental Science, Department of Horticultural Engineering, Arak, Iran

Abstract

Ornamental black mulberry (*Morus nigra* L.) is used in landscape. Ornamental black mulberry which has high shade, tolerates air pollution and wind. White mulberry (*Morus alba* L.) is proper for kind of soils conditions. Therefore, this research was conducted to investigate the effects of stenting methods and IBA levels on some characters of black mulberry scions onto white mulberry rootstock for the first time. For this purpose, an experiment was carried out as factorial in completely randomized design with 10 replications. Two factors were including two stenting method (splice and omega) and three levels of Indole-3-butyric acid (0, 500 and 1000 mg L^{-1}). Three months after grafting, percentage of rootstock callus formation, percentage of rooting, percentage of leaf formation, number of leaf formation, the longest shoot and the longest root formation were measured. Results showed that the effect of stenting method was significant on all of evaluated parameters and splice method was superior to omega method. Also, the effects of different levels of IBA were significant on all of measured traits and they are increased by increasing the levels of IBA and the best was with 1000 mg L^{-1}. The interaction effects of these factors are significant on all of measured characteristics except for percentage of rootstock callus formation and rooting percentage. In conclusion, the stenting via splice method had higher success in comparison with omega method and the morphological traits increased by increasing the application of higher levels of IBA. The application of stenting method by splice plus 1000 mg L^{-1} IBA which was carried out for the first time is recommended for reproduction of ornamental black mulberry onto white mulberry.

Keywords: *Morus alba* L., *Morus nigra* L., callus formation, grafting, indole-3-butyric acid, vegetative propagation.

Resumo

Resposta da amoreira-preta em porta-enxerto de amoreira-branca a técnicas de stenting (enxerto de corte) e concentrações de IBA

A amoreira-preta (*Morus nigra* L.) é uma planta ornamental utilizado no paisagismo por ser uma planta que proporciona sombreamento, tolera a poluição do ar e o vento. A amoreira-branca (*Morus alba* L.) é adequada para várias condições de solo. Esta pesquisa foi conduzida para investigar os efeitos dos métodos de enxertia por garfagem e dos níveis de AIB em alguns caracteres de mudas de amora preta enxertada sobre porta-enxerto de amoreira branca. Para tanto, foi realizado um experimento em esquema fatorial em delineamento inteiramente casualizado com 10 repetições. Os fatores testados foram o método de garfagem (inglês simples e ômega) e três níveis de ácido indol-3-butírico (0, 500 e 1000 mg L^{-1}). Três meses após a enxertia, foram medidos a porcentagem de formação de calo do porta-enxerto, porcentagem de enraizamento, porcentagem de estacas com folha, número de folhas, brotação mais longa e raiz mais longa. Os resultados mostraram que o efeito do método de enxertia foi significativo em todos os parâmetros avaliados e o método de garfagem em inglês simples foi superior ao método ômega. Além disso, os efeitos dos diferentes níveis de IBA foram significativos em todas as características com a melhor dose de 1000 mg L^{-1}. Os efeitos de interação desses fatores são significativos em todas as características medidas, exceto para a porcentagem de formação de calo de porta-enxerto e porcentagem de enraizamento. Em conclusão, a enxertia pelo método de garfagem em inglês simples teve maior sucesso em comparação ao método ômega e as características morfológicas aumentaram com o aumento da aplicação de maiores níveis de AIB. A aplicação do método de enxertia por garfagem em inglês simples mais 1000 mg L^{-1} de AIB, realizado pela primeira vez, é recomendada para a produção de mudas de amoreira-preta ornamental enxertadas em amoreira-branca.

Keywords: *Morus alba* L., *Morus nigra* L., formação de calos, enxertia, ácido indol-butírico, propagação vegetativa.

*Corresponding Author: m-solgi@araku.ac.ir

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Introduction

White mulberry (*Morus alba* L.) and black mulberry (*Morus nigra* L.) belong to the genus Morus of Moraceae family. These species are native to Asia such as Iran, Pakistan, Japan, India, China and Korea with subtropical or mild temperate climate in the northern hemisphere (Łochyńska and Oleszak, 2011; Mohiuddin et al., 2011; Hussain et al., 2017; Hashemi and Khadivi, 2019). Both white and black mulberries are used as silkworm production, nutrition, flavor, biomedical activities and landscape designing (Mohiuddin et al., 2011; Lucia et al., 2016; Hussain et al., 2017; Hashemi and Khadivi, 2019; Rodrigues et al., 2019). Persian name of black mulberry is "Shah Tut" in Iran and are used for sericulture, nutrition value and landscape (Mohiuddin et al., 2011; Hosseini et al., 2018; Khodaiyan and Parastouei, 2020; Gómez-Mejía et al., 2021). Mulberry species are adapted to different and diverse harmful environmental conditions (Mohiuddin et al., 2011). In Asia, Southern Europe and in Southern U.S.A., mulberry trees are utilized for landscaping. Their resistance to pruning and their low water requirements make them very suitable plants for urban conditions, house gardens, street shade and city embellishment (Tipton, 1994). Nowadays, black mulberry cultivation has been greatly increased and it is mainly being used as an ornamental plant in gardens (Desai et al., 2018). In Iran, black and white mulberries are cultivated in many regions for landscape using (Taghizadeh and Kazemi, 2019). Mulberry organs have the unique ability to uptake relatively large quantities of heavy metals and hold aerosol pollutants from the air of contaminated areas which is suitable for landscape uses (Nikolova, 2015). White mulberry is a shade tolerant tree and black mulberry can tolerate atmospheric pollution which is suitable for air polluted areas (Mohiuddin et al., 2011; Kobus-Cisowska et al., 2020). Mulberry trees are commonly propagated by cutting, grafting and tissue culture (Hussain et al., 2017; Aref Hawramee et al., 2019; Zenginbal and Demir, 2018).

Stenting (cutting-grafting) is an effective vegetative method for quick propagation of horticultural crops. Cutting and grafting is accomplished simultaneously in this technique (Karimi, 2011; Babaie et al., 2014; Pourghorban et al., 2019). It is a valuable technique in propagating of many plant species such as conifers, apple, plum, pear (Hartman et al., 2002), pomegranate (Karimi, 2011), roses (Park and Jeong, 2012; Izadi et al., 2013; Pourghorban et al., 2019), Chinese hibiscus (Izadi and Zarei, 2014), weeping fig (Babaie et al., 2014). However, it is not known about mulberry plants response to different stenting methods.

On the other hand, adventitious root initiation and formation through stenting method depends of several factors, including environmental conditions and the physiological condition of rootstocks (Park and Jeong, 2012; Izadi and Zarei, 2014; Karimi and Nowrozy, 2017). The effect of exogenous auxins (particularly IBA) has been proven for root induction and root formation in many cutting of plants (Hartman et al., 2002; Babaie et al., 2014; Chater et al., 2017). Currently, there is no study on the rooting of cuttings and stenting method propagated mulberries. Nonetheless, Van de Pol and Breukelaar (1982) indicated that, the rose cultivars were superior for most of the studied parameters when stented onto rootstock of *Rosa chinensis*, var. indica treated with IBA at 5000 ppm. Tizazu et al. (2017) indicated that the performance of rose cuttings treated with 1500 ppm of IBA was the best in all growth parameters including shoot length, root length, root number. Also, in *Hibiscus rosa-sinensis* the best rooting percentage and other affecting factors on quality of cuttings were recorded at 4000 ppm concentrations of IBA (Izadi and Zarei, 2014).

Understanding the response of ornamental black mulberry onto white mulberry rootstock to stenting techniques and IBA concentrations is necessary. There is no report on the using stenting methods and application of exogenous auxin on mulberries so far. Therefore, this study was conducted to evaluate qualitative and quantitative characters of ornamental black mulberry onto white mulberry rootstock by stenting methods accompanied by different IBA concentrations.

Materials and Methods

Greenhouse and Propagation Conditions

This study was carried out in a polyethylene greenhouse. The greenhouse was N-S oriented and equipped with mist system. The greenhouse equipment for environmental control including consisting of convective heating, evaporative cooling pads and exhaust fans. The mean temperature and relative humidity of mid-day during the study period were maintained at 25°C ± 2°C and 70% ± 5%, respectively.

Plant Materials

The dormant hard wood rootstock cuttings of *Morus alba* L. and cutting scions from unisexual male tree of *Morus nigra* L. (ornamental black mulberry) with at least two nodes and 5 mm diameter were used. The rootstocks and scions were taken from shoots to be 5 to 10 cm in length and a 5 mm in diameter. Scions then were grafted with stenting methods onto rootstocks. Bottom of the rootstocks were treated with IBA plant growth regulator at different concentrations and then were placed in the medium of perlite.

Stenting Procedure and Treatments

The cutting scions were selected based on the thickness of the cutting rootstocks. Scions then were grafted with omega and splice methods onto rootstocks. Omega grafting tool and clipper were used to perform omega and splice receptive. Scions and rootstocks with an appropriate smooth cut could be grafted together with the maximum overlap of the cambium layer. The bottom of the rootstocks and the grafting unions were treated with IBA plant growth regulator at different concentrations by quick dip method. Parafilm tape was used for wrapping the graft unions. Immediately after grafting, all stentings were placed in the medium of perlite. Stentings were sprayed with fungicides every 30 days to prevent of fungal infection. The grafted plants were grown for 90 days under the stated greenhouse conditions.

By the end of experiment grafted plants were taken out of medium and some morphological characteristics such as percentage of rootstock callus formation, percentage of rooting, percentage of leaf formation, number of leaf formation, the longest shoot and the longest root formation were recorded.

Data Collection and Analysis

The experiment was designed as a completely randomized design in factorial arrangement with two factors in three replications. The first factor included stenting methods (Omega and splice) and the second factor was the different IBA concentrations (0, 500 and 1000 mg L^{-1}). The observations were recorded by means of three replications, each comprising 10 stentings. The IBA dilution was in alcohol. Statistical significance between means (percentage of rooting and rootstock callus formation, number and percentage of leaf formation, the longest shoot and root formation) values was assessed using analysis of variance (ANOVA) and a conventional Duncan's Multiple Range Test (DMRT) at $p \leq 0.05$ using SAS (9.1) statistical software.

Results

Percentage of rootstock callus formation

According to ANOVA results, the interaction between stenting methods and IBA concentrations on mean of percentage of rootstock callus formation was not significant (Table 1). However, the effect of stenting methods and different concentrations of IBA were significant. The splice stenting method had higher percentage of callus formation (71%) in comparison of omega method (49%). The results showed that percentage of callus formation increased by increasing the level of IBA application. The highest callus formation in rootstocks was obtained in 1000 mg L^{-1} IBA (73%) (Figure 1).

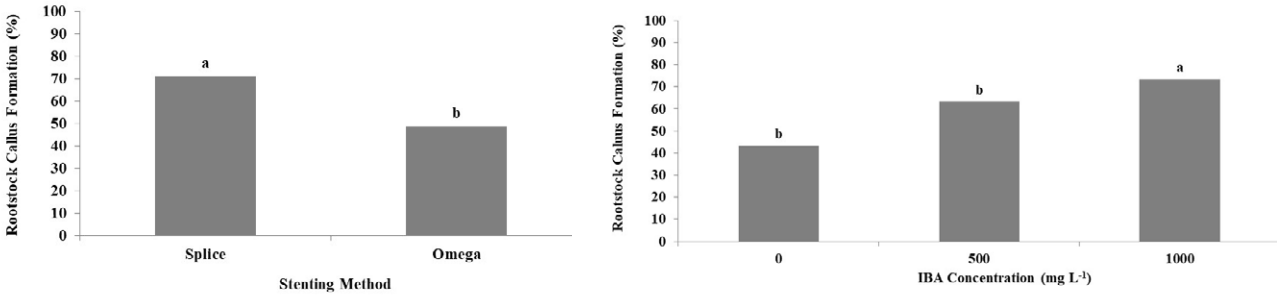

Figure. 1. Effect of two stenting methods (left) and four IBA concentrations (right) of rootstock callus formation after three months.

Percentage of rooting

The effect of stenting methods and different concentrations of IBA on rooting percentage were also significant. Although, it's interaction was not significant (Table 1). The comparison between stenting methods indicated that, rooting percentage was affected by stenting method and splice method had also greater rooting percentage (57%) than omega method (39%).

Like callus formation, percentage of rooting increased by increasing the IBA concentration. The highest callus formation in rootstocks was observed in 1000 mg L^{-1} IBA (62%) and the lowest was in control without IBA (Figure 2).

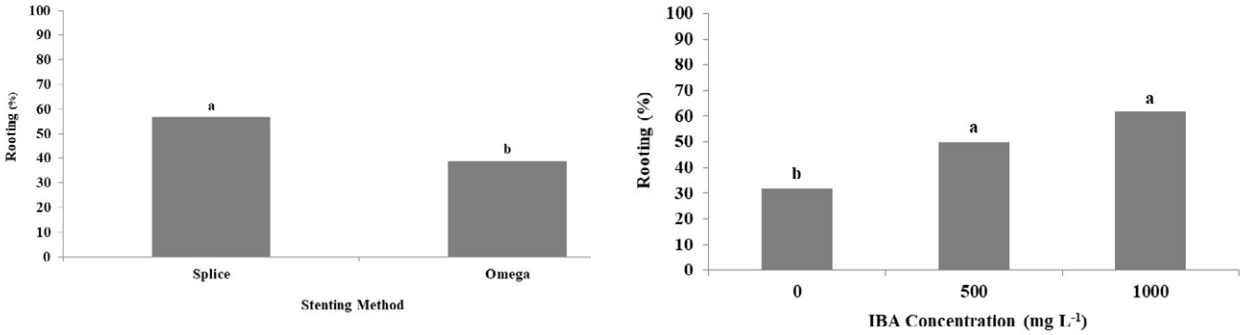

Figure 2. Effect of two stenting methods (left) and four IBA concentrations (right) on rooting percentage after three months

Table 1. Variance analysis of two stenting methods and three levels of IBA on some characteristics of ornamental black mulberry onto white mulberry rootstock after three months.

S.O.V	DF	Mean Squares					
		Percentage of rootstock callus formation	Percentage of rooting	Percentage of leaf formation	Number of leaf formation	The longest shoot formation	The longest root formation
Stenting Method	1	2222.22 **	14.22*	55.86**	533.55**	13.40**	213.55**
IBA Concentration	2	1400.00**	1372.22**	7.72**	194.38**	2.81**	104.22**
Stenting Method × IBA Concentration	2	22.22^{ns}	172.22^{ns}	2.73**	139.38**	1.38**	40.22*
Error	12	127.77	183.33	1.39	3.38	0.185	8.00
CV (%)		18.83	28.33	38.99	30.12	25.33	24.47

*: $p \leq 0.05$; **: $p \leq 0.01$; ns: not significant

Percentage of leaf formation

Analysis of variance indicated that percentage of leaf formation on cutting stentings was affected by interaction between propagation methods and IBA levels and was significant in all stenting treatments (Table 1). The highest leaf formation was observed in splice stenting (26%) which is similar to rooting and callus formation percentage. Based on mean comparison of IBA concentrations, higher level of IBA increased in this trait and the highest parameter was obtained in 1000 mg L^{-1} IBA treatment (Table 2).

Table 2. Mean comparison of two stenting methods and three levels of IBA on some characteristics of ornamental black mulberry onto white mulberry rootstock after three months

	Leaf formation (%)		Number of leaf formation		The longest shoot formation (cm)		The longest root formation (cm)	
IBA Stenting	Splice	Omega	Splice	Omega	Splice	Omega	Splice	Omega
0 mg L^{-1}	10cA*	0aB	2.33cA	0aB	2cA	0aB	8.66cA	6aB
500 mg L^{-1}	26.66bA	3.33aB	9.33bA	0.33aB	5.33bA	0aB	14.33bA	9aB
1000 mg L^{-1}	40aA	3.33aB	23aA	1.66aB	14aA	1aB	22aA	9.33aB
CV	38.99		30.12		25.33		24.47	

*Averages followed by the same lowercase letter in the column and uppercase in the row do not differ by Duncan test, 5% error probability.

Number of leaf formation

Moreover, the effect of stenting methods and different concentrations of IBA on number of leaf formation were significant and its interaction was significant too (Table 1). The splice stenting method had higher number of leaf formation (12 leaves) in comparison of omega method (1 leaf). Furthermore, number of leaf formation improved by increasing the level of IBA application and the highest characteristic was obtained in 1000 mg L^{-1} IBA like above mentioned traits (Table 2).

The longest shoot formation

Table 1 indicated that, the longest shoot formation affected by stenting methods and different concentrations of IBA and its interaction was significantly as well. Splice stenting method had higher shoot length (7 cm) compared with omega stenting method (1 cm). This result is in agreement with another above stated results in stenting method. Application of IBA levels increased the length of the longest shoot significantly. Obviously, the highest shoot length was obtained in 1000 m gL^{-1} IBA (7.5 cm) in comparison with no-treated stenting (1 cm) (Table 2).

The longest root formation

The effect of stenting methods and different levels of IBA on the longest root formation are shown in Table 1. The result of this parameter was similar to the longest shoot formation trait. Splice stenting method achieved the best effect (15 cm) and the 1000 mg L^{-1} IBA treatment caused the highest root length (16 cm) in comparison to no-treated by IBA control (7 cm) (Table 2).

Discussion

According to the results of this study, stenting methods and IBA concentrations had significant effects on the morphological traits and the success of black mulberry onto white mulberry stenting. The auxin concentration of 1000 mg L^{-1} and splice stenting techniquewere the best treatments to the propagation of ornamental *Morus nigra* on *Morus alba* rootstock (Figure 3).

Figure 3. Effects of three levels of IBA (from the left to right; 0, 500 and 1000 mg L^{-1}) and two stenting methods (Top; splice and Bottom; omega) on some characteristics of ornamental black mulberry onto white mulberry rootstock after three months

Principally, grafting success is affected by method which used. Various techniques such as cleft, side, notch, wedge, and splice are approved for different woody and herbaceous plant species (Hartman et al., 2002; Solgi et al., 2012). The select of grafting and stenting type within plant species could also be related to climatic conditions, scion and rootstock vigor and their size (Izadi et al., 2013). Generally, the activation of rootstock and scion cambium is required for grafting success. Undifferentiated parenchyma cells (namely callus) proliferate from cambia layers in the grafting union. Callus formation in addition to their joining together with the maximum overlap is one of the most major steps in achievement (Hartman et al., 2002; Solgi et al., 2012). Subsequently, new cambial layers differentiate and making a constant vascular connection between scion and rootstock (Izadi et al., 2013). The activation of cambia and subsequent callus formation and vascular connection depends on several factors such as environmental conditions (for example temperature and humidity), season, plant growth regulators, plant species and plant materials characteristics (Hartman et al., 2002; Izadi et al., 2013; Solgi et al., 2012).

According to our findings, stented black mulberry onto white one plants by splice method substantially improved all of evaluated traits compared to that omega method (Table 1, Table 2, Figure 3). Some authors reported different stenting methods in horticultural crops (Hartman et al., 2002; Karimi, 2011; Park and Jeong, 2012; Izadi et al., 2013; Babaie et al., 2014; Izadi and Zarei, 2014). However, there is no report on the application of stenting method for mulberries. The results presented here are in opposite with the results of Izadi et al. (2013) who reported that omega grafted plants of Avalanch/*R. manetti*, produced more number of shoots and roots as well as leaves as compared to splice grafted. The similar thickness of scions and rootstocks subsequent the optimum contact between their cambium layers is a prerequisite for stenting accomplishment. It seems, splice stenting method which was carried out by horticultural clipper produced even cut surfaces and higher cambia contacts in comparison with omega stenting method in this research.

According to the present findings, there were evidently differences in all of parameters (particularly rooting characteristics) and stenting take between the IBA-treated and untreated stentings. The application of IBA treatments (especially 1000 mg L^{-1}) considerably increased the rate of traits (Table 1, Table 2, Figure 3). Our results were conforming to results like that already observed in *Hibiscus rosa-sinensis*. Izadi and Zarei (2014) showed that dry weight of produced roots in stentings of Chinese hibiscus improved by applying concentration of exogenous IBA. Furthermore, Karimi (2011) indicated that the stenting propagation of *Punica granatum* treated with IBA had a higher root's fresh weight than the control. Babaie et al. (2014) obtained the longest root length of *Ficus benjamin* var. Starlight by using 4000 mg L^{-1} IBA in stenting propagation too. In ornamental plants, auxins, especially IBA, were widely used for accelerating the formation of adventitious roots in certain cultivars under both in vitro and in vivo conditions (Nguyen et al., 2020).

It has been proved that the natural auxin (IAA) affect most aspects of plant physiology comprising root initiation and cell division. It is generally accepted that auxins have a certain role in the rooting initiation and therefore leads to control growth and development in plants, including lateral root initiation, root gravity response and other vegetative growth parameters (Aref Hawramee et al., 2019). IAA are synthesized in buds and leaves and moved to the bottom of cuttings and grafting unions (Hartman et al. 2002; Izadi et al., 2013). By the application of auxin, the hydrolysis and movement of carbohydrates and nitrogenous substances take place at the base of cuttings and grafting unions lead to cell division and cell elongation (Babaie et al. 2014). IAA is oxidized readily by peroxidases in the plant, whereas IBA is not oxidized by peroxidase easily. Thus IBA is one of the best rooting agents (Hartman et al., 2002; Izadi and Zarei, 2014).

On the other hand, the amount of food storage in the cutting scions is another important factor which might have affects stenting success. Stored carbohydrates are hydrolyzed to simple ones and transported to the grafting union. Rongting and Pinghai (1993) showed that high amounts of starch, soluble sugars and C/N ratio (carbohydrate/ nitrogen) cause increasing success (Solgi et al., 2012).

According to the present findings, the IBA treatment increased the stenting success and improved root characters percentage compared to the control. This may be associated with cell division stimulated by the auxin at the stenting unions and root formation.

Conclusions

In conclusion, splice stenting method has potential for vegetative propagation of black mulberry onto white mulberry than omega stenting method. Splice stenting method produced even cut surfaces and higher cambia contacts in comparison with omega stenting method. Additionally, the application of 1000 mg L^{-1} IBA considerably increased the morphological characteristics, particularly rooting. Subsequently black mulberry is heterozygous and is suitable for landscape using and for air contaminated areas and, the using of splice stenting method plus IBA is suggested for its propagation.

Author Contribution

MS: experimental activities, writing of the manuscript, data analysis; **MT**: manuscript writing revision and corrections, supervised the data analysis**. HS**: supervised the research, designed the experiment.

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