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Evaluation of Propagation of Chinese Hibiscus (*Hibiscus rosa-sinensis*) through Stenting Method in Response to Different IBA Concentrations and Rootstocks

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Abstract

Stenting is a vegetative propagation method, based on cutting and grafting in one action. The question addressed in this study is whether indole-3-butyric acid (IBA) concentration is able to impress success of Chinese hibiscus propagation when grafted onto two kinds of rootstocks. The used IBA concentrations include: 0, 1000, 3000 and 5000 ppm and two cultivars of *Hibiscus syriacus* ("Blue Stain" and "Jeanne d'Arc"), which have been used as rootstock. Results indicated that, all the cultivars were superior for most of the measured parameters when IBA treatments were used compared to control plants except for average of the longest root size. The highest healing percentage resulted in stentlings (plant which has been propagated by stenting method) with 5000 ppm of IBA treatment. All IBA treatments significantly increased rooting percentage compared with the control plants. Stentlings that were treated with 3000 and 5000 ppm of IBA, produced the greatest leave number. In Chinese *hibiscus*/"Jeanne d'Arc" combination, higher root number and dry weight of roots were observed. Interactions of kinds of rootstock and IBA concentration showed that the highest healing percentage was obtained in "Blue Stain" in concentration of 5000 ppm and the highest root number were seen in stentlings with "Jeanne d'Arc" in 3000 ppm and "Jeanne d'Arc" in 5000 ppm.

Keywords

IBA, *Hibiscus rosa-sinensis*, Rootstock, Scion, Stenting

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1. Introduction

Chinese hibiscus is a well-known member of the Malvaceae family. It is native to tropical and sub-tropical regions [1]. *Hibiscus rosa-sinensis* plants are propagated by seed, stem cutting, grafting, budding, root grafting, layering and it is propagated by tissue culture as commercial method too [2]. The stenting (cutting-grafting) method as an efficient technique of propagation has many advantages as reported and is now being used worldwide by rose growers. Stenting is now being used worldwide by rose growers [3]. Though stenting is widely used in the propagation of ornamental plant, it is not known whether *Hibiscus rosa-sinensis* plant responses to stenting method. There are many physiological and environmental factors that influence adventitious root formation [4]. Many researches proved that rootstock plays an important role in the entire process of root growth and grafting take in the success of propagation through bench grafting. An investigation of kind of rootstock (*R. canina* and *R. manetti*) and scion (Avalanch, Peach Avalanch and Dolcevita) on success of stenting method of greenhouse roses showed that *Rosa canina* was the best rootstock for Avalanch cultivar [5]. Vigorous variety of hibiscus such as "Apple Blossom", "Dainty", Single Scarlet" and Euterpe that are resistant to soil pests propagated via cutting and are used as rootstock. With grafting, resistance of scion to biotic and abiotic environmental stress might be increased on the appropriate rootstock [2]. Nazari, *et al.* [3] studied propagation of four rose (*Rosa hybrida*) cultivars on the rootstock of *Rosa canina*. Result indicated that parameters of plant growth and flower quality were increased in grafted plants compared to those propagated by cuttings. The necessity of exogenous auxin application to induce root formation in cuttings has been reported in many species, e.g. *Hibiscus rosa sinensis* [1] and *Stewartia pseudocamellia* [6]. Currently, IBA is the most widely used auxin to stimulate the rooting process in cuttings because of its high ability to promote root initiation. The promoting effect of IBA on rooting is mainly the result of its conversion to IAA in plant tissue. However, IAA which is needed for the rooting process, is oxidized readily in the plant by peroxidases, whereas IAA released from IBA is not oxidized by peroxidase and remains at the base of the cutting [7]. Van de Pol and Breukelear [8] reported that stenting method is an efficient technique of rose propagation; results indicated that, the cultivars were superior for most of the studied parameters when grafted onto rootstock of *R. chnensis*, *Var. indica* treated with IBA at 5000 ppm. In *Hibiscus rosa-sinensis* optimum rooting percentage and other affecting factors on quality of cuttings were recorded at 4000 ppm concentrations of IBA [9]. This comparative study was conducted to evaluate *Hibiscus rosa-sinensis* response to IBA concentration and effect of kinds of rootstock on success of stentlings which was studied.

2. Materials and Methods

2.1. Glasshouse and Propagation Conditions

The investigation was carried out in June 2012, in fiberglass experimental glasshouse of the Gorgan University of Agricultural Sciences and Natural Resources. The greenhouse was W-E oriented and equipped with mist system as well as water cooler for adjusting balances of temperature and humidity. Misting was scheduled for two minutes spray in every 45 minutes 7:00 A.M. to 8:00 P.M. daily and twice operation around midnight to maintain average relative humidity of greenhouse in 70% ± 5%, also the mean temperature of mid-day during the study period were maintained at 29˚C ± 2˚C.

2.2. Plant Materials and Stenting Procedure

Semi-hard wood cuttings of two variety of *Hibiscus syriacus* ("Blue Stain" and "Jeanne d'Arc") with at least two nodes and 5 mm diameter used as rootstock. One species of *Hibiscus*, known as *Hibiscus rosa-sinensis* was used as scion. Each scion was consisted of a peace of stem with two nodes and one leaf. The scions were selected according to the thickness of the rootstock. Scions then were grafted with omega grafting methods onto 4.0 cm length cutting of rootstocks. Omega grafting tool was used to perform omega grafting. Scions and rootstocks with an appropriate smooth cut could be grafted together with maximum overlap of cambium layer. Electrical tap was used for wrapping the graft union. Immediately after grafting, all cut surfaces were thoroughly covered by horticultural grafting wax. Basal ends of rootstock was treated with IBA at the rated of 1000, 3000 and 5000 ppm (quick dip method). Control plants were treated with basic alcoholic solution (with no treated IBA). Stenting was rooted following insertion in perlite medium.

2.3. Data Collection and Analysis

70 days after planting, stentlings were taken out from beds and data were recorded for some morphological traits such as root number, root length, leaf number, rooting and grafting take percentage, per stentlings. The experiment was conducted as completely randomized design with three replications. The data were analyzed using SAS software and means were compared through Duncan test ($p < 0.05$).

3. Results and Discussion

3.1. Healing Percentage & Root Indices

There were clearly differences in rooting response and grafting take between the IBA-treated and untreated samples. The maximum number of roots per stentlings (5.66 and 5.60) was observed for the treatment with 5000 and 3000 ppm IBA while untreated offshoots produced fewer roots (4.24) per rooted offshoot (**Table 1**).

Our results were corresponding with results like that already observed in *Aesculus indica*. Vegetative propagation of *Aesculus indica* through stem cuttings treated with plant growth regulators showed that the highest rooting rate (50%) was recorded in the cuttings with the application of IBA at 4000 ppm. The cuttings treated with IBA at 2000 ppm had 25% rooting rate [10]. Naier *et al.* [6] investigated effect of auxin concentration on rooting of *Stewartia pseudocamellia* and announced that Cuttings treated with rooting hormones had higher rooting percentages (71.9% to 93.6%) as compared with the control (53%). According to Hartmann *et al.* [2], one of the best rooting promoters is the IBA due to its fast auxin activity and an enzymatic system of fairly slow destruction. Strydum and Hartman [11] compared respiration level of treated cuttings with exogenous auxin and control plants; he found that positive effect of auxin is related to increase of level of respiration, high level of amino acid storage at the base of cuttings 24 hours after treatment with auxin. These processes occur simultaneously with increased nitrogen material content at the base of cuttings that move toward upper part of cutting as asparagine does. Al-Salem and Karam [12] reported that concentration of auxin had a significant effect on rooting regardless of auxin type or chemical form. The greatest rooting percentage, root number, length, and fresh and dry weights were exhibited by basal cuttings treated with 24 mM IBA. Investigation of the relationship between adventitious root formation and ethylene synthesis following IBA treatment by Sun and Bassuk [13] on the "Royalty" rose cuttings showed that, IBA induced root formation in rose cuttings was correlated with increased ethylene concentration. The endogenous ethylene concentration was correlated significantly with the number of roots formed on cuttings. Compatibility is dependent on the presence of a particular factor or absence of the factors in both rootstock and scion. The possibility of vary simple genetic control mechanism or of a threshold effect were raised. However, significant differences among clones within the compatibility status groups suggested that other factors probably are involved [14]. Auxin can be effective to rooting cuttings in a certain concentration, depending on the crop and cultivar [2]. In our condition "Jeanne d'Arc" *3000 ppm and "Jeanne d'Arc" *5000 ppm were the best treatments, with highest (5.30 and 5.27 respectively) root number. "Blue Stain" *3000 ppm, "Blue Stain" *1000 ppm and "Blue Stain" *control (4.01, 3.70 and 3.38 respectively) had less root number (**Table 3**). Investigation of effect of kind of rootstock (*Rosa canina and Rosa manetti*) on success of stenting method of greenhouse roses proved that rootstock can affected root number; Avalanch/R. canina caused more root number [5]. Hartman *et al.* [5] announced that high level of auxin may have negative

Table 1. The effect of IBA treatments on growth parameters and graft healing of *Hibiscus rosa sinensis* stentlings.

Measured Parameter				IBA (ppm)
	control	1000	3000	5000
Number of roots	4.24[b]	4.25[b]	5.60[a]	5.66[a]
Average of longest root (cm)	2.12[a]	1.60[a]	1.96[a]	1.82[a]
Average of leaves number	2.05[b]	2.86[a]	3.02[a]	3.21[a]
Healing percentage (%)	66.66[b]	68.79[b]	73.33[b]	80.76[a]
Dry weight of roots (g)	0.726[a]	0.733[a]	0.729[a]	0.728[a]

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

effect on root length. In current study although there was not significant different between average of "longest root size" of treatments and control (**Tables 1-3**) but inverse relationship was observed between length of root and root number samples treated with IBA (**Table 1**). Probably more nutritional materials and other factors which influencing rooting were used for more root number therefore; root length have been reduced [5].

3.2. Leaves Number & Dry Weight of Roots

Highest leave number (3.21, 3.02 and 2.86) observed in treated samples with IBA than control plant (2.05) although there was not significant different between leave numbers in different IBA concentration (**Table 1**). On the other hand, highest number of root observed in treated plants with IBA, Cytokinins are believed to be synthesized in roots and then exported in the xylem sap to shoots. Evidence for this comes from the presence of cytokinin in the xylem extrude of de-rooted plants and the modulation of cytokinin production in bleeding sap when root growth vigor was affected by environmental factors such as water stress, flooding, and root temperature. In addition; the presence of cytokinins in root tissue has been demonstrated directly. One of the cytokinin functions are stimulating morphogenesis phenomenon like shoots initiation or bud formation in plants [15]. Cline *et al.* [16] investigate hormonal control of second flushing in Douglas-fir shoots and reported that cytokinin play important promotive role, in the control of second flushing in the terminal spring-flushing Douglas-fir shoot.

In current experiment highest leaf number was observed in stentlings with higher root number. Chalapathi *et al.* [17] reported that cuttings treated with IBA 500 ppm was found to be superior with respect to shoot length, number of branches, number of leaves and root length, survival percentage and sprouting percentage. Kind of rootstock had not significant effect on the leave number (**Table 2**) but interaction between IBA concentration and kind of rootstock, "Blue Stain" *5000 ppm and "Jeanne d'Arc" *3000 ppm caused highest leave number (**Table 3**). Our last investigation with different rootstock, grafting technique and scions showed, kind of rootstock had no significant effect on the new generated leave number, however interaction between treatments

Table 2. The effect of rootstock's cultivars on growth parameters and graft healing of *Hibiscus rosa sinensis* stentlings.

Measured Parameter	Rootstock's cultivar	
	"Blue Stain"	"Jeanne d'Arc"
Number of roots	3.817^{b}	5.184^{a}
Average of longest root (cm)	1.64^{a}	2.10^{a}
Average of leaves number	2.93^{a}	2.64^{a}
Healing percentage (%)	72.47^{a}	72.29^{a}
Dry weight of roots (g)	0.730^{a}	0.738^{a}

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

Table 3. The effect of interaction between rootstock's cultivars and IBA treatments on growth parameters and graft healing of Hibiscus rosa sinensis stentlings.

	"Blue Stain"				"Jeanne d'Arc"			
IBA (PPM)	0	1000	3000	5000	0	1000	3000	5000
Number of roots	3.38^{f}	3.70^{f}	4.01^{f}	4.16^{bcde}	5.23^{abcd}	4.92^{abcde}	5.30^{ab}	5.27^{abc}
Average of longest root (cm)	1.41^{a}	1.48^{a}	1.844^{a}	1.84^{a}	2.83^{a}	1.71^{a}	2.07^{a}	1.80^{a}
Mean of leaves number	2.56^{bcdef}	2.55^{bcdefg}	2.85^{abcd}	3.77^{a}	1.56^{bcdefg}	3.17^{abc}	3.20^{ab}	2.65^{abcde}
Healing percentage (%)	66.60^{e}	64.28^{f}	66.66^{e}	92.3^{a}	66.66^{e}	73.30^{c}	80.00^{b}	69.23^{d}
Dry weight of roots (g)	0.710^{fg}	0.720^{ef}	0.714^{fg}	0.724^{de}	0.730^{bc}	0.745^{a}	0.742^{ab}	0.728^{cd}

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

showed that M1R1S1 (splice grafting, *Rosa canina*, Avalanch cultivar respectively) was the best treatment, with highest leaf number. The healing progress of the graft union area may vary according to propagation techniques and environmental conditions during and fallowing grafting, growth activity of stock plant, insect and disease contamination, use of plant regulators, and stock-scion combination [18]. Healing percentage in *Hibiscus rosa-sinensis* was significantly improved by IBA treatment while 80.76% was induced by 5000 ppm of IBA (the highest percentage among the treatments), however 66.66% of healing percentage was observed in the control plants (**Table 1**). Interaction between auxin concentration and rootstock's cultivar on healing percentage was significant. Highest healing percentage (92.30%) were found in samples with "Blue Stain" *5000 ppm and lowest healing percentage (64.28%) was observed in "Blue Stain" *1000 ppm (**Table 3**). It seems auxin treatment helps to increase root number per stentlings. As root is considered as main source of cytokinin, it may help producing higher leave number. Moreover leaf is a source of auxin and carbohydrate. Carbohydrates have been considering optimal markers since they are the main energetic resource during the rooting process. The levels of total carbohydrates and starch in the cuttings are positively related to the rooting but not through one cause-effect relationship [19]. In another research, effect of IBA treatment on healing percentage of stentlings of rose was investigated. Results showed that treatment of graft portion with IBA, increases callus formation, 37 days after grafting. Approximately 68% of samples that were treated with IBA in grafting portion, their healing process were completed whereas at this time only 25% of control plants were healed. Vascular formation between rootstock and scion is the main important stage for success of grafting work. It seems using exogenous auxin in grafting portion helps vascular formation [20]. Auxin is often considered as the main phytohormon involved in the regulation of cambial activity and its promontory effect on cambial cell division. Auxin is mainly present in zones where cambial activity is necessary such as in young growing stems. Mitoses occurred in cambial cells when the auxin level is higher than a threshold value and cell production is correlated to auxin concentration. Cambium sensitivity to auxin appears directly linked to the ability of cambial cell to polar transport of the regulator [21]. In present study no exogenous auxin was used in grafting portion but It seems, vascular contact occurred in IBA treated samples earlier than control, whereas scions containing leaf, auxin and carbohydrate produced in leaf, moves to grafting portion and base of stock. It helps unity of grafting and interaction of endogenous IAA with exogenous applied IBA in the base of the rootstock for the emergence of root primordial. The effect of IBA concentration and kind of scion on dry weight of root was not significant, but interaction between them had significant effect on the dry weight of produced roots. Highest dry weight of root recorded on interaction between "Jeanne d'Arc" *1000 ppm and "Jeanne d'Arc" *3000 ppm (0.745 and 0.742 respectively) and lowest dry weight of root were obtained on "Blue Stain" *control and "Blue Stain" *3000 ppm. Other observations did not lead to prominent result as it varied with kind of cultivar and IBA concentration (**Table 3**). Karimi [22] investigated effect of kind of rootstock and IBA concentration on successful propagation of *Punica granatum* and the results also indicated that R1 rootstock ("Gorj-e-Dadashi") which was treated with 500 ppm IBA produced more successful callus formation at the graft union. Our data showed that dry weight of produced roots in stentlings can be substantially improved by applying concentration of exogenous IBA. Similar result already was observed in *Arbutus andrachne* [12]. Simultaneously, stimulation of rooting process with auxin, carbohydrate transportation from leave to root increases, therefore it causes increasing of dry weight of root [2]. Also Karimi [22] in his experiment about stenting propagation of *Punica granatum* showed that the cutting-grafting combinations treated with IBA had a higher root's fresh weight than the control. The highest root fresh and dry weight was found in R1C2 and R2C1 treatment combinations, respectively (R1: "Gorj-e-Dadashi", R2: "Gorj-e-Shahvar", C1: without IBA treatment and C2: 500 ppm IBA).

4. Conclusion

In conclusion, current study demonstrated that the application of auxin plays an important role in success of *Hibiscus rosa-sinensis* propagation through stenting method. The highest root number, dry weight of root, healing percentage and leave number were obtained with auxin treatment (3000 ppm and 5000 ppm). Although effect of rootstock's cultivar just on root number was significant but its interaction with auxin was significant on the produced root number, root dry weigh, mean of healing percentage and leave number. One of the best combination was "Jeanne d'Arc" *3000, therefore the application of auxin with 3000 ppm is advisable for stenting propagation of *Hibiscus rosa-sinensis* when grafted on "Jeanne d'Arc" rootstock. However "Blue Stain" treatment's result was not consistent but using 5000 ppm of IBA caused good achievements at least in a few meas-

ured parameters.

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