

Effects of Indole Butyric Acid (IBA) and Stem Cuttings on Growth of Stenting-Propagated Rose in Bahir Dar, Ethiopia

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Abstract: Most rose producers in Ethiopia including those in Bahir Dar propagate roses using stem cuttings and auxins for root initiation on which scion buds are grafted simultaneously. The effects of auxins however are significantly influenced by environmental conditions, types of stem cutting and rooting media used. Therefore, this research was mainly conducted to evaluate the effects of softwood, semi-hard and hard wood cuttings and IBA concentrations of 0, 1000, 1500, 2000, 2500 and 3000ppm on growth and development of stenting-propagated roses in environmental conditions of Bahir Dar where Natal Break and Acpinc varieties were used as rootstock and scion respectively. Grafted healthy rose-cuttings with 2-3 leaves were treated with quick dip method in solutions containing the respective IBA-concentrations. After air drying, six stem cuttings were planted in pots (90x150 mm) that were filled with coco peat having the depth of 3.5 cm. The experiment was indeed factorial combined and laid down in completely randomized design (CRD) with four replications in the propagation house of Tana Flora PLC in Bahir Dar. Number of roots per cutting, root length, root dry weight, root fresh weight, shoot length, leaf number, shoot fresh weight and shoot dry weight of rose stem-cuttings were considered as performance indicator. Results deciphered that different concentrations of IBA had shown significant effects on growth parameters of grafted rose cuttings sourced from different developmental stages of rootstock stems. Especially, rose cuttings treated with 1000ppm of IBA had shown significant positive effects on most of the root and shoot parameters including root length, number of root per cutting, root fresh weight, root dry weight, shoot fresh and dry weight, leaf number and shoot length. Among the different cutting types, hardwood showed significant positive effect on growth and development of rose cuttings. The longest shoot length and the highest leaf number were recorded from hard-wood rose cuttings. However, significant interaction effects on growth parameters were not observed between cutting types and IBA concentrations.

Key words: Rooting • Plant height • Fresh weight • Rose-cuttings • Auxins

INTRODUCTION

The development of the floriculture industry especially roses production and export in Ethiopia has experienced unique and unexpected high speed in the last decade. In 2008, the land under flower cultivation was only 922 ha and it has increased to over 1,442 ha in 2012. In the same period of time the export earnings from floriculture increased from US \$ 111.7 to over US \$ 212 million by exporting 1,021 to 2,102 millions of flower stems. Hence, Ethiopia is currently become the second largest flower exporter in Africa next to Kenya. Besides, thousands of jobs have been created in and around these flower farms [1].

The number of commercial and exporting horticultural farms is increasing from year to year. According to EHPEA and EHDA [2], in Ethiopia there are 83 exporting horticultural farms having the capacity of 600 hectares of green houses, 150 hectares of open field flowers, 70 hectares with cuttings, 75 hectares of herbs and 600 hectares of vegetables and fruits. Most of the farms are concentrated around Addis Ababa at 100 kilo meter radius. However, nowadays floriculture farms are also established in other big cities like Bahir Dar located about 560 km northwestern of Addis Ababa. Despite of encouraging achievements observed in the floriculture industry of the country in recent time, it is still low compared to the existing potentials of the country for floriculture.

Among the ornamental plants produced in Ethiopia, roses are the dominant ones. Rose accounts more than 80% of the cut flowers of the country. The remaining 20% of the cut flowers is covered by chrysanthemums, poinsettia and geranium and bouquet fillers primarily Hypericum, Carnation, Gypsophila, Allium and Carthamus [3].

Roses are conventionally propagated by cutting, budding, grafting and layering methods. Grafting and cutting are indeed the common propagation methods used in commercial rose production [4]. Wild rose varieties such as *Rosa canina inermis* and *Rosa motera*, are widely used as rootstock [5, 6]. The Netherland Scientists have developed a technology for quick propagation of roses whereby cuttings of rootstock and grafting of the scion cuttings are performed in one action and such a new technique of rose propagation is known as stenting [7, 8]. With this method of rose propagation, formation of the graft union and root initiation and development from cuttings are occurred simultaneously. The stenting (simultaneous cutting and grafting) is done under mist in propagation structures (green houses) and it enables to produce planting materials within four to six weeks and flowers only after four months. Thus stenting can be performed all year round [9-11]. The success however depends upon many factors like season of cutting, age and portion of the branch, growing media, moisture level, nutrient status and temperature [4, 12, 13]. Provision of optimal growing conditions and proper timing may enhance the establishment and growth of the cutting.

Growth promoting hormones promote rooting of cuttings of many ornamental plants including roses [14, 15] by influencing the growth and development of plant cells. The speed of root development in cuttings depends on the cutting types, the rooting medium and the type and concentration of hormones used. The rooting effects of auxins are nowadays used commercially in the propagation industries of horticultural crops especially in the floriculture sector. Among the auxins, Indole Butyric acid (IBA) and Naphtalin Acetic acid (NAA) are typically used for rooting of cuttings of the majority of plant species including roses. Their effect however depends on the concentration applied and the age of cuttings [16-19]. The concentrations of these auxins recommended by the manufacturers are quite general and not specific to roses and it may also differ with environmental conditions [20, 21]. Hence, investigating the optimal concentration of IBA under specific environmental conditions for specific crop is necessary. The present study was therefore mainly

conducted to assess the effects of different concentrations of IBA and cutting types on growth and development of stenting-propagated roses in Bahir Dar, Ethiopia.

MATERIALS AND METHODS

Description of the Study Area: The study was conducted in the propagation house of Tana Flora PLC in Bahir Dar north western Ethiopia from December 2013 to January 2014. The study area has an altitude of 1850 meter above sea level and located at 11.71°N latitude and 37.30° E longitude. The average annual rainfall of the study area is about 1250mm and the minimum and maximum temperatures are about 10.5°C and 27°C, respectively.

The propagation house used for the study had a misting facility arranged to mist intermittently for 30 seconds at every five minutes interval between 9:00am and 6:00pm depending on daily temperature. The average temperature and relative humidity in the propagation house were 35°C and 85%, respectively. The light intensity in the propagation house was adjusted automatically to 50% (diffused light). The house was provided with good air circulation through automated side and open top vents.

Experimental Material: In this experiment, rose varieties of Natal Break and Acpinc were used as rootstock and scion, respectively which are commonly used in Ethiopia. As a source of IBA, Hormande brand water soluble Hortus imported from the Netherlands was used. Soft, semi-hard and hardwood cuttings having six cm length were taken from healthy branch of the mother rootstock plants. As propagation medium, coco peat produced from waste product of coconut palm (*Cocos nucifera*) imported from Israel was used. Propagation plastic pots with the size of 90x150 mm were used for the study.

Experimental Treatments and Design: Different types of cuttings at different portion of rootstock rose plants, namely softwood, semi-hardwood and hardwood cuttings, having two to three leaves and free from any defects were used for the experiment. The flower buds from the scion plants were prepared and grafted on rootstock cuttings using with simple whip grafting (splice grafting) method. The top cut of the rootstocks and the bottom end cut of the scions were cut at angle of 45° so as to fit the cut surfaces of one partner exactly with that of the other to facilitate the joining process. For best cambial contact of

scion and rootstock, the two partners were fixed with clipper. The bottom ends of grafted cuttings were then scrap off (wound making, vertical cut) with knife to make wound and facilitate root induction.

Five level of IBA (0, 1000, 1500, 2000, 2500 and 3000ppm) were prepared by weighing the respective quantity of IBA and by dissolving it in water. The application of IBA was done by a five-second quick-dip of the basal portion of grafted stem cuttings prepared above in the respective hormone concentration to the depth of 2 cm, whereas the control was dipped in water without IBA. After drying of the treated portion, six treated stem cuttings of the same types were then planted in plastic pots which were filled with coco peat as rooting medium. To neutralize the pH value of coco peat, 2kg calcium nitrate (CaNO_3) was added. The treatments were laid in completely randomized design (CRD) with four replications (pots). Each pot consisted of six stem cuttings that were then put on propagation bench.

Management of Stem Cuttings: For successful rooting, stem cuttings should be kept moist throughout the experiment [22]. Therefore, they were misted for 30 seconds at five minutes interval between 9 am and 6 pm depending on the daily temperature. The propagation house was ventilated through side and top ventilators. Whenever the temperature rose above 35°C , the side and top ventilators were left open to maintain the temperature and relative humidity of the propagation house. After 35 days, all seedlings were transferred to hardening room and watering was provided three times a day by showering without misting. During hardening process, shade net put over the top of cuttings in the greenhouse was removed during the day time from 10:00am up to 5:00pm to expose seedlings to full sunlight. After 5:00pm, shade net was placed again on the top of the seedlings. The required macro and micro-nutrients were supplied through irrigation water (fertigation).

Data Collection and Analysis: After 45 days of planting, various growth parameters of stem cuttings were evaluated against the treatments. For that purpose, seedlings were carefully uprooted and media particles adhered to roots were removed with great care by hand and the following parameters were recorded:

Number of Roots per Cutting: The numbers of roots grown in four sample seedlings were counted and the average number was taken.

Root Length (cm): The length of emerged roots was measured from the point of emergency to the tip using linear meter and the average length from four sampled seedlings was taken for further analysis.

Root Fresh Weight (g): The fresh weight of initiated roots was measured using electrical sensitive balance and the average weight from four sampled seedlings used.

Root Dry Weight (g): After drying of the roots from four sampled seedlings in oven drier for 24 hours at 60°C , the weight of dried roots was measured using electrical sensitive balance and the average weight was taken for further analysis.

Length of the Shoots (cm): The length of newly grown shoots of four sampled seedlings was measured from the point of emergency to the tip using linear meter and the average length was taken.

Shoot Fresh Weight (cm): The weight of newly grown shoots from four sampled seedlings was measured by using electrical sensitive balance and the average weight was taken.

Shoot Dry Weight (g): After drying the newly grown shoots of four sample seedlings in oven drier for 24 hours at 60°C , the weight of dried shoots was measured using electrical sensitive balance and the average dry weight was taken for further analysis.

Leaf Number: The newly grown leaves from four sampled seedlings were counted and the average leaf number was taken.

The collected data were subjected to analysis of variance (ANOVA) using SAS soft ware computer program version 9.0. Mean comparison was performed by using Least Significant Difference Test (LSD) at 1% or 5% significant level.

RESULTS AND DISCUSSIONS

Effects of IBA Concentrations on Growth Parameters of Rose Cuttings: The effects of different IBA concentrations on different growth parameters of rose stem cuttings are presented in Table 1. Different concentrations of IBA had a significant effect on root length, number of leaves & root, root & shoot fresh weight and root & shoot dry weight. These indicated that

Table 1: Effects of IBA concentrations on growth parameters of rose cuttings

IBA concentrations	NRPC	RFW (g)	RL (cm)	RDW (g)	SL (cm)	LFN	SFW (g)	SDW (g)
0ppm	31.85b	0.32b	5.79c	0.04b	8.97c	34.04b	1.003c	0.17c
1000ppm	54.2a	0.79a	11.29a	0.21a	14.4ab	56.50a	2.05a	0.61a
1500ppm	44.14a	0.90a	9.5b	0.17a	13.56ab	54.7a	1.49b	0.47b
2000ppm	54.3a	0.85a	9.60b	0.16a	13.7ab	55.3a	1.77ab	0.48b
2500ppm	50.4a	0.78a	9.10b	0.16a	15.47a	58.50a	2.01a	0.58ab
3000ppm	48.6a	0.89a	10.4ab	0.16a	12.91b	53.3a	1.89ab	0.50ab
P-values	** 0.0017	** 0.0041	** 0.0001	** 0.0001	** 0.0001	** 0.0001	** 0.0001	** 0.0001

**=highly significant, ns=non-significant; NRPC =Number of roots per cutting, RL=Root length, RFW=Root fresh weight, RDW=Root dry weight, SL=Shoot length, LFN=Leaf number, SFW=Shoot fresh weight, SDW=Shoot dry weight, P-value=probability value. Means with the different letters in column are significant.

Table 2: Effects of stem-cutting types on growth parameters of rose cuttings

Cutting type	NRPC	RFW (g)	RL (cm)	RDW (g)	SL (cm)	LFN	SFW (g)	SDW (g)
Soft wood	47.95	9.26	0.74	0.14	11.6b	45.29c	1.53	0.55
Semi-hardwood	49.43	9.49	0.84	0.18	13.2a	52.4b	1.75	0.57
Hardwood	44.4	9.13	0.69	0.13	14.6a	58.4a	1.82	0.71
P-values	ns 0.44	ns 0.74	ns 0.40	ns 0.06	** 0.0019	** 0.0003	ns 0.14	ns 0.06

**=highly significant, ns=non-significant; NRPC =Number of roots per cutting, RL=Root length, RFW=Root fresh weight, RDW=Root dry weight, SL=Shoot length, LFN=Leaf number, SFW=Shoot fresh weight, SDW=Shoot dry weight, P-value=probability value. Means with the different letters in column are significant.

the growth and development of shoots and roots of rose-cuttings were significantly promoted by application of IBA. However, the most significant effect was recorded on stem cuttings treated with a concentration of 1000 mg/l of IBA (Table 1). The maximum numbers of roots (54.2), root length (11.3 cm), root dry weight (0.21 g), shoot dry (0.61 g) and fresh weight (2.05 g) of rose cuttings were observed from cuttings treated with 1000ppm of IBA. However, the maximum root fresh weight (0.9 g) was shown from rose-cuttings treated by 1500ppm of IBA. The maximum number of leaves (58.5) and the highest shoot length (15.47 cm) were obtained from cuttings treated with 2500ppm of IBA but not statistically significant compared to stem cuttings treated with 1000 mg/l of IBA. The untreated control was the least in all above mentioned growth parameters of roots and shoots.

The best shoot growth performances obtained in this study were in agreement with the findings of Ramtin *et al.* [23]. They found that the concentration of 1000 mg/l IBA produced the best result for root length, number of bracts, number of cyathium, number of leaves and size of bract on poinsettia. These results are also in accordance with the findings of Susaj *et al.* [20] and Younis [24] where they found a concentration of 1000ppm of IBA gave the best result in the propagation of rose plants by cutting. As mentioned earlier the best result in most growth parameter of stem cuttings was observed by the lowest IBA concentration (1000ppm) used in this experiment. This is probably due to high daily average temperature prevailed

(about 35°C) in the propagation house at the time of the experiment that increases the growth and developmental activities of plant cells so that low concentration of hormone was enough for the growth and development of plants. This implies the optimum response curve of IBA depends with intensity of temperatures prevailed in the propagation house. This finding is also in agreement with the finding of Taghvaei *et al.* [25] where they concluded that higher the temperature the lower is the optimum IBA-hormone concentration required for the successful vegetative propagation of stem cuttings.

Effects of Stem Cutting Types on Growth Parameters of Rose Cuttings: The effects of different types of rose stem cuttings on different growth parameters are presented in Table 2 and discussed below. The results of this study showed that, among different growth parameters only shoot length and leaf number of cuttings were significantly influenced by stem cutting types of roses. Shoot length and number of leaves of roses from hardwood cuttings were significantly higher than those from softwood cuttings. Shoot length of roses from hardwood cuttings was on an average of about 14.6 cm while the shoot length from softwood-cuttings was about 11.6 cm and that of semi-hardwood-cuttings was intermediate (13.2 cm). The maximum number of leaves produced by hardwood-cuttings was about 58.4 followed by semi-hardwood cuttings 52.4 and softwood cuttings 45.3. The other growth parameters such as number of roots, root length root fresh and dry weight were non-significantly affected by rose stem cuttings.

Table 3: Interaction effects of stem cutting types and IBA concentrations on growth parameters of stenting-propagated rose

CT	IBA Con.	NRPC	RL (cm)	RFW (g)	RDW (g)	SL (cm)	LFN	SFW (g)	SDW (g)
SW	0ppm	30.06	5.84	0.24	0.04	9.00	31.75	0.91	0.16
SW	1000ppm	56.60	13.00	0.94	0.17	14.00	51.00	2.20	0.62
SW	1500ppm	45.06	7.93	1.06	0.18	11.10	48.10	1.31	0.43
SW	2000ppm	55.81	9.31	0.93	0.16	11.40	46.40	1.40	0.42
SW	2500ppm	50.70	9.30	0.65	0.14	14.06	51.18	1.82	0.49
SW	3000ppm	43.81	10.60	1.11	0.13	9.93	43.18	1.52	0.40
SHW	0ppm	34.68	6.03	0.51	0.08	9.22	33.50	1.26	0.21
SHW	1000ppm	48.68	10.18	0.82	0.30	12.93	58.50	1.84	0.54
SHW	1500ppm	41.31	10.81	1.04	0.17	14.87	53.06	1.55	0.54
SHW	2000ppm	52.81	9.62	0.81	0.15	13.43	53.12	1.73	0.42
SHW	2500ppm	66.43	10.06	0.93	0.20	15.62	60.70	2.11	0.63
SHW	3000ppm	52.68	10.25	0.91	0.17	13.68	55.87	2.01	0.55
SHW	0ppm	30.80	5.84	0.24	0.02	8.71	36.87	0.83	0.14
HW	1000ppm	57.56	10.68	0.61	0.15	16.25	59.93	2.11	0.68
HW	1500ppm	46.06	9.87	0.60	0.15	14.62	62.87	1.60	0.45
HW	2000ppm	54.43	9.87	0.82	0.16	16.37	66.62	2.16	0.61
HW	2500ppm	34.06	7.87	0.77	0.14	16.75	63.56	2.10	0.61
HW	3000ppm	43.81	10.62	1.11	0.18	15.12	61.06	2.14	0.56
CV P-values	29.29	17.78	50.32	49.46	21.18	20.30	30.72	27.50	
	ns 0.32	ns 0.11	ns 0.53	ns 0.64	ns 0.56	ns 0.94	ns 0.70	ns 0.34	

Ns=non-significant NRPC=Number of roots per cutting, RL=Root length, RFW =Root fresh weight, RDW=Root dry weight, SL= Shoot length, LFN=Leaf number, SFW=Shoot fresh weight, SDW=Shoot dry weight, CT=Cutting type, SW=softwood, SHW=Semi-hardwood, HW=hardwood, IBA Con. =IBA concentration, CV=Coefficient of variation, P-value=probability value.

The results of this experiment demonstrated that stem cuttings has influenced the shoot development of rose stem cuttings. Hardwood cutting has showed the best shoot performance. The reason may be due to the fact that hardwood cuttings contain stored foods such as hydrocarbons, nucleic acids, proteins and natural hormones (IAA and/or, cytokinins) that can be used for shoot growth and development. The status of the stored foods is an important feature for rooting, growth and development capacity of stem cuttings which are more in quantity in hardwood than in soft and semi-hardwood cuttings that enable the hardwood cuttings to grow and develop quickly than other types of cuttings [26].

The results obtained in this experiment were also in line with the results of Balakrishnamurthy *et al.* [27]. They observed the rooting and survival capacity of softwood, semi-hard and hardwood cuttings of rose cultivar 'Bourbon hybrid and Edward' treated with 1000ppm IBA. According to their results hardwood-cuttings gave 73.3% rooting and the highest plant survival rate assessed 30 days after treatment. Besides, the presence of leaves on hardwood cuttings exerts a strong stimulating effect on root initiation. In addition leaves and buds are also known to be sources of auxin [28]. The vigorous roots initiated from hardwood enabled the cuttings to absorb more nutrients and produce more leaves.

The study conducted by Ramtin *et al.* [23] showed that lower cuttings or hardwood cuttings have produced the longest root and highest number of bracts, number of leaves and number of buds and size of bract on poinsettia which is in line with the results of the experiments. Okunlola [28] also studied the vegetative propagation of *Duranta repens* in the nursery and finally concluded *D. repens* can be better propagated using semi-hard and hardwood cuttings.

Interaction Effects of Stem Cuttings and IBA Concentrations on Growth Parameters of Rose Cuttings: The results of the analysis of variance showed that there were no significant interaction effects of hormones and cutting types on the growth parameters of rose-cuttings (Table 3). However, semi-hardwood cuttings treated with 2500ppm produced the highest number of roots (66.3) followed by hardwood cuttings (57.6) treated with 1000ppm IBA while the control showed the lowest root number in all cases. In case of root length, softwood cuttings produced the longest root (13 cm) followed by semi-hardwood cuttings. Shoot length of cuttings from hardwood treated with 2500ppm IBA was about 16.75 cm followed by cuttings from semi-hardwood cuttings treated with 2500ppm. The untreated controls have produced in all cases the shortest shoot length. In the case of leaf number, the highest number of leaves was produced from hardwood cuttings treated with 2000ppm IBA followed by semi-hardwood cuttings treated with 2500ppm IBA.

CONCLUSION AND RECOMMENDATIONS

The results of this study showed that the application of IBA hormone and using different stem cuttings significantly affect rooting capacity and shoot characters of stenting-propagated rose. Among tested IBA concentrations, stem cuttings that received 1000ppm of IBA were ascribed with better rooting capacity and shoot system under the environmental conditions of the study area. The untreated stem cuttings have shown the least root and shoot performance. Among the three stem cutting tested, semi-hardwood and hardwood cuttings of rose showed good result regarding to shoot characters. However, significant effect on shoot length and leaf number was obtained from hardwood cutting. Results from semi-hardwood cuttings showed an intermediate value in most growth parameter tested. On the contrary softwood cuttings showed the lowest results in most root and shoot characters. There were no interaction effects between IBA concentrations and stem cutting types on the rooting and shoot characters of the cuttings used in this experiment.

From the results of this research findings pre-treating of rose stem cutting with 1000ppm of IBA improved the rooting capacity, growth and development of stenting-propagated roses. Besides, hardwood cuttings followed by semi-hardwood cuttings are the appropriate cutting types to be used in stenting-propagated roses under the condition of the study area. Further research on the effects of IBA below 1000ppm on rose hardwood cuttings and using different growth media is also recommended.

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