Effect of IBA and growing conditions on vegetative performance of *Citrus aurantifolia* Swingle cuttings

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Abstract: The effects of indole-3-butyric acid (IBA) concentrations and three growing conditions (open area, under partial shade and under low cost polyhouse) on vegetative performance of *Citrus aurantifolia* Swingle cuttings were investigated. The experiment was conducted from March 2007 to Oct 2009. The cuttings treated with IBA @ 500 ppm, performed the best, as far as the success rate is concerned while, planting the cuttings in open area was effective in increasing the success rate of the cuttings. The vegetative growth was recorded to be the highest in the cuttings planted under polyhouse conditions. The greatest sprouted bud percent (68.50) was obtained in 500 ppm followed by 1000 ppm (53.67), while lowest (36.55) occurred in control.

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1. Introduction

Kagzi lime, which belongs to family Rutaceae, is one of the most important citrus fruits grown throughout the world. Besides having high nutritional value and table purpose use, kagzi lime is extensively used as rootstock for malta and santra (Souci, 2000, Babu, 2001).

Commercially, kagzi lime is propagated through seeds, but there is a problem of non-uniformity of progeny by this method. The second most common method of propagation of lime is through semi-hardwood cuttings, but in this method the survival percentage and success rate is less under ordinary field conditions. Under these circumstances, the use of growth regulators and slight modifications of growing conditions could lead to an increase in the success rate during the propagation of kagzi lime by cuttings. Various early workers have reported that effect of IBA on bud sprouting on different *Citrus* species. Non-significant effect of IBA on the rooting of soft-wood cuttings of kagzi lime (*Citrus aurantifolia* Swingle) was observed by Singh (1959), while, Bajwa *et al.* (1977) noticed that the cuttings of sweet lime treated with IBA, sprouted better than untreated ones. Yamashita and Astsuda (1977) also observed that increasing effect of IBA on rooting and bud sprouting of cuttings of Satsuma orange. Although, there is a lot of work done on different aspects of propagation of citrus fruits but the availability of literature on the use of auxins alongwith the modification of growing conditions under valley areas of Uttarakhand is scanty in the literature. The present investigation was therefore undertaken to study the effect of IBA and modified growing conditions on the success rate and vegetative growth characteristics of kagzi lime cuttings under valley conditions of Garhwal Himalaya.

Adventitious root formation and survivility has a lot of commercial interests because there are many plant species cutting that are difficult to root. In some plant species, adventitious root formation initiate without any treatment, while others required different growth regulators usually auxin (Syros *et al.* 2004). Auxin induces root formation by breaking root apical dominance induced by cytokinin (Cline, 2000). Indole Butyric Acid (IBA) is a synthetic rooting chemical that have been found to be reliable in the promotion of rooting in cuttings more proficiently than Indole Acetic Acid (IAA) which is a native auxin. IBA is widely used because it is nontoxic to most plants over a wide range and promotes root growth in a large number of plant species (Hartmann *et al*. 1990).

2. Material and Methods

2.1 Study area

The experiment was carried out at the Horticulture Research Centre (HRC), in Chauras campus of HNB Garhwal University, Srinagar (Garhwal). Geographically the experimental site is lying between 30° 12′ to 30° 13′ North latitude and 78° 45′ to 78° 50′ East longitude while altitudinally located at 570 m asl. The site in the valley area of Garhwal Himalaya and experience a wide range of temperature variation ranging from 0°C in winter to a

maximum of 40°C during summer. The relative humidity varies from 39.24 to 79.83 % and mean annual rainfall from 2.50 to 235.24 mm.

2.2 Methodology

The experiment was conducted from March 2007 to Oct 2009. 4-5 gunny bags of sandy loam soil were taken from HRC field, exposed to Sun for killing the insects, spores of pathogens and the weeds. Stones, gravels and weeds were removed manually. After 2-3 hours drying in Sun in the month of June, 1 part of FYM was mixed thoroughly with the 2 parts of well dried sandy loam soil. This prepared media was filed in perforated polythene bags of about 1kg capacity (20-22 cm height x 8-10 cm diameter). IBA solution of 500, 1000 and 1500 ppm were prepared and kept in 1 L beakers. Healthy, uniform and mature tree (8-10 years) were used to obtain the stem cuttings. Cutting of 25cm long having 6-8 nodes with 0.6 - 1.2 cm diameter were prepared from central and basal parts of the branch. Cuttings were defoliated for reducing the transpiration rate and allowing the closer spacing in the bags. The cuttings were arranged into the 4 bundles each with 81 cuttings. Three bundles of cuttings were treated with different concentrations of IBA viz., 500 ppm, 1000 ppm and 1500 ppm respectively. The basal parts (2-3 cm) of all the cuttings were dipped in different concentrations of IBA for 5 second, concentrated solution dip method, (Hartmann *et al*. 2002) at room temperature of 20 $\pm$ 2 ^{0}C (Hartmann *et al*. 2002). Fourth bundle of cuttings was used as control (simply dipped for 5 sec in plain tap water). The treated cuttings were planted in the 3 different growing conditions, viz, open sunny area (M_1), partial shade of big tree throughout the day (M_2) and polyhouse conditions (M_3) of 3m (l) x 2m (b) x 2m (h) size.

For bud initiation and development studies, the histological analysis was carried out using protocol by Johansen (Johansen, 1940). Standard methodology was used to record the observations on root characteristics (Hartmann *et al.* 2002). The experiment was laid out in the factorial randomised block design (FRBD) with 3 replications having 9 cuttings in each replication within each treatment combination. The analysis of the data was done as per the standard methods (Cochran and Cox, 1992).

3. Results and Discussion

Mean values in Table 1 indicate that the maximum number of sprouted cuttings after 130 days was recorded in the treatment C_1 (500 ppm IBA) with 68.50% followed by C_2 (1000 ppm IBA) treatment with 51.83 %. While, least number of sprouted cuttings i.e. 37.00% were recorded in C_0 control. Present findings are in line with the some earlier reports in the literature (Verma, *et al.* 2005) but contidictory to the findings made by (Singh and Singh, 2005), who noticed maximum sprouting percentage in higher concentration (3000 ppm IBA). The maximum mean sprouting percentage (60.50%) was observed under the treatment M_1 (open area) closely followed by the treatment M_2 (partial shade) with 48.54%. The best treatment combination was found C_1M_3 (500ppm IBA with polyhouse condition) with 83.33% of sprouted cuttings. This may be due to favourable climatic conditions to the survival of cuttings under polyhouse condition as well as the effect of rooting hormones in lower doses.

Some callus developed at the basal end of cutting. The basal end was swollen and the colour was changed from green to whitish green. The formation of callus still continued to the following days until the root primordial emerged from the callus. Although the root primordial seems to be emerged from the callus, closer examination from histological analysis showed that the root was emerging from an area near the vascular bundle and not from the callus itself.

Data pertaining to the percentage of unsprouted cuttings shows significant effects of various concentrations of IBA and different growing conditions. The maximum percentage of unsprouted cuttings (16.67%) were found under the treatment C_0 (control) followed by C_1 (500 ppm IBA) treatment with 12.94%. While growing of cuttings under M_2 (partial shade) treatment resulted maximum percentage of unsprouted cuttings (26.33%) among all the growing conditions. The interaction between various IBA concentrations and different growing conditions was also found to be significant. The maximum percentage of unsprouted cuttings (50.00%) were observed under the treatment C_0M_2 (control with partial shade) followed by C_1M_2 (500 ppm IBA with partial shade) treatment with 38.83%. The higher percentage of unsprouted cuttings observed in the above said treatment combinations might be due to the selection of poor quality cuttings, high temperature and low humidity.

Table 1: Effect of different concentrations of IBA and various growing conditions on the success related parameters of kagzi lime cuttings

IBA Conc.	Percentage of sprouted cuttings (%)				Percentage of unsprouted cuttings (%)				Percentage of dead cuttings without sprouting (%)			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
Control (Water)	61.00 (51.38)	33.33 (35.25)	15.33 (23.01)	36.55 (36.55)	0.00 (0.00)	50.00 (45.00)	0.00 (0.00)	16.67 (15.00)	27.66 (31.72)	16.66 (4.05)	66.66 (4.75)	36.99 (6.84)
500 ppm	72.16 (58.21)	50.00 (45.00)	83.33 (66.26)	68.50 (56.49)	0.00 (0.00)	38.83 (38.54)	0.00 (0.00)	12.94 (12.85)	16.66 (24.02)	5.50 (3.53)	11.00 (9.26)	11.05 (8.94)
1,000 ppm	50.00 (45.00)	55.50 (48.16)	55.50 (48.17)	53.67 (47.11)	0.00 (0.00)	5.50 (13.41)	0.00 (0.00)	1.83 (4.71)	16.66 (24.05)	27.66 (1.70)	11.00 (9.34)	18.44 (5.03)
1,500 ppm	58.83 (50.15)	55.33 (48.07)	38.83 (38.53)	51.00 (45.50)	0.00 (0.00)	11.00 (19.29)	0.00 (0.00)	3.67 (6.43)	16.66 (24.05)	5.33 (13.14)	27.66 (1.71)	16.55 (2.97)
Mean	60.50 (51.19)	48.54 (44.12)	48.25 (43.99)		0.00 (0.00)	26.33 (29.06)	0.00 (0.00)		19.41 (25.96)	13.97 (20.60)	29.08 (31.27)	
$CD_{0.05}$												
IBA Conc. (C)				3.07				1.05				1.65
Growning conditions (M)				2.66				0.91				1.65
C x M				5.32				1.82				3.29

*Figures in the parenthesis are arc sine transformed values.

M_1= Open sunny area, M_2= complete shade throughout the day under a big tree, M_3= Polyhouse conditions

Data recorded in respect of percentage of dead cuttings without sprouting reveals that different IBA concentrations and interactions showed significant effect. The maximum percentage of dead cuttings without sprouting (36.99%) were noticed under C_0 (control) treatment followed by C_2 i. e.1000 ppm IBA (18.44%), while the minimum percentage of dead cuttings without sprouting (11.05%) was observed under C_1 (500 ppm IBA). The treatment M_3 (polyhouse condition) showed the maximum percentage of dead cuttings without sprouting (29.08%), whereas the minimum percentage of dead cuttings without sprouting (13.97%) were recorded under M_2 (partial shade) treatment. The highest and lowest mortality percentage of cuttings without sprouting in polyhouse and partial shade conditions might be due to the high and low temperature. The maximum percentage of dead cuttings without sprouting (66.66%) was found under the treatment C_0M_3 (control with polyhouse) followed by C_0M_1 (control with open area), C_2M_2 (1000 ppm IBA with partial shade) and C_3M_3 (1500 ppm IBA with polyhouse) treatments with same figure (27.66%), while C_3M_2 (1500 ppm IBA with partial shade) was found best treatment combination in term of minimum percentage of dead cuttings without sprouting (5.33%). Data in table 1 indicates the overall superiority of IBA @ 500 ppm and planting in open condition over other treatment combinations for better success of the semi-hardwood cuttings of kagzi lime.

Maximum length of longest sprout (6.59 cm) was observed under C_1 (500ppm IBA) treatment. This was closely followed by C_3 (1500 ppm IBA) with 6.45 cm long sprout. Treatment M_3 (polyhouse condition) was found to be the best for producing maximum length of the sprouts (9.38cm) which was significantly better than all other treatments. Maximum length of the longest sprout (12.33cm) was noticed under C_1M_3 (500 ppm IBA with polyhouse condition) treatment combination and was closely followed by C_3M_3 (1500 ppm IBA with polyhouse condition) with 11.47cm long sprout. This might be due to maximum temperature, high humidity and production of more photosynthetes due to more number of leaves under polyhouse in comparison to outside climatic conditions. Verma *et* al. (2005) also reported the maximum mean sprout length (19.79cm) in kagzi lime, propagated through cuttings when compared with the budded plants and nucellar seedlings.

Maximum diameter of thickest sprout (0.32 cm) was recorded under the treatment C_1 (500 ppm IBA) closely followed by C_2 (1000 ppm IBA) and C_3 (1500 ppm IBA) with 0.31cm and 0.30cm diameter, respectively. The treatment M_3 (polyhouse condition) was observed to be the best treatment to produce maximum diameter of thickest sprout (0.34 cm). Maximum diameter of thickest sprout (0.40cm) was obtained under C_1M_3 (500 ppm IBA with polyhouse condition) treatment followed by C_2M_2 (1000 ppm IBA with partial shade) treatment with 0.36cm diameter.

Data recorded with respect to maximum number of leaves on new sprouts revealed that the highest value (9.11) was found under C_1 (500 ppm

IBA) followed by the treatment C_3 (1500 ppm IBA) with 8.77. Treatment M_3 (polyhouse condition) produced maximum no. of leaves on new sprout (12.08). Treatment C_1M_3 (500 ppm IBA with polyhouse condition) produced the maximum no. of leaves on new sprout (14.33), closely followed by C_3M_3 (1500 ppm IBA with polyhouse condition) treatment with a mean value of 14.00.

Table 2: Effect of different concentrations of IBA and various growing conditions on the vegetative growth characters of kagzi lime cuttings

IBA Conc.	Length of longest sprout (cm)				Diameter of thickest sprout (cm)				Max. number of leaves on new sprout			
	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean	M_1	M_2	M_3	Mean
Control (Water)	4.03	2.40	6.66	4.36	0.33	0.23	0.30	0.28	5.66	4.33	10.33	6.77
500 ppm	3.13	4.33	12.33	6.59	0.33	0.23	0.40	0.32	6.00	7.00	14.33	9.11
1,000 ppm	2.60	3.73	7.07	4.46	0.26	0.36	0.33	0.31	5.66	5.33	9.66	6.88
1,500 ppm	3.83	4.07	11.47	6.45	0.26	0.33	0.33	0.30	6.33	6.00	14.00	8.77
Mean	3.39	3.63	9.38		0.29	0.28	0.34		5.91	5.66	12.08	
$CD_{0.05}$												
IBA Conc. (C)				1.24				NS				1.17
Growning conditions (M)				1.08				0.028				1.01
C x M				2.150				0.055				2.02

M_1= Open sunny area, M_2= complete shade throughout the day under a big tree, M_3= Polyhouse conditions

4. Conclusion

The results of investigation clearly reveal that the IBA 500 ppm is most effective in the stimulation of callus system and bud initiation from cutting and their development of *Citrus auriantifolia* cutting, and can be used for mass scale multiplication. It was interesting to observe that open area growing condition alone gives good results but moreover, IBA 500 ppm gives good results with combination of polyhouse growing condition. The results of this investigation are expected to pave the way for substantially augmenting natural regeneration through seeds; in addition, this has the advantage clonal or true to type propagation of elite trees.

The bud development percentage increased as the IBA concentrations increased because application of IBA on cutting increased the bud development percentage. The number buds increased as the IBA concentration increased in experiment in all growing condition of cuttings. According to Krishnankutty 2005, the application of IBA can increase the bud initiation and development.

Conclusively it rejuvenate that growth regulators treatment of the semi-hardwood cuttings of kagzi lime with 500 ppm IBA was found to be better as compare to all other hormonal concentrations with respect to achieving better success and producing better vegetative growth, while among the different growing conditions planting of cuttings in open area gave better success and that in polyhouse condition gained better vegetative growth.

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