



Effect of different growing media, hormonal treatment and growing season on shoot and root characters of lemon (*Citrus limon* L.) cuttings

V. P. Singh¹, P. K. Nimbolkar*², S. K. Singh¹, N. K. Mishra¹, C. Awchare² and A. Tripathi¹

¹Department of Horticulture, College of Agriculture, G.B. Pant University of Agriculture & Technology, Pantnagar, India

²Division of Fruit Crops, Indian Institute of Horticultural Research, Bengaluru, India

*e-mail: prashantnimbolkar111@gmail.com

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Abstract: The present study was conducted to evaluate the effect of growing media, hormonal treatments and growing seasons on the shoot and root characters and survival percentage of cuttings of lemon cv. Pant Lemon-1. Pooled data of two season (rainy and spring) has shown that soil+sand+FYM growing medium resulted maximum length of sprouts and average number of leaves per cutting. Whereas, average diameter of shoot and average length of longest roots were maximum in soil+cocopeat based medium. Average diameter of root was higher in soil+cocopeat and soil+FYM medium, but did not differ significantly. Maximum survival percentage of cuttings was noted with soil+sand+FYM. IBA (500 ppm) was found superior regarding for all parameters except on average diameter or roots per cutting with IBA+NAA (500 ppm each). Taken as a whole, rainy season was found superior over spring season in respect to all shoot and root characteristics.

Key words: Cuttings, Growing media, Growth hormones, Shoot and root parameter

Introduction

Lemon (*Citrus limon* L.) belongs to the family Rutaceae. It is a native of the East Indies and has spread all over the world in tropical and sub-tropical regions and considered as an important species of *Citrus* family. Citrus is one of the major fruit crops, and it cultivated in 1042.49 thousand ha area with the production of 10089.73 thousand tons, among which lemon and lime contribute about 255.21 thousand ha in area 2523.51 thousand tons on production basis (Anonymus, 2013). Lemons are either used as fresh or in preserved form. *Tarai* region of Uttarakhand is unique in climate and characterized by hot and humid summer and extremely cold winters. Plant growth regulators, such as indole-3-butyric acid (IBA), are frequently used to promote rooting (Hartmann *et al.*, 2002). Cultivar difference and weather condition also affect rooting in various fruit plant cuttings (Mishra and Singh, 1984). Lemon is usually propagated by seeds which are commonly polyembryonic in nature, resulting heterogeneous population and high chance of viral disease contamination by this method (Babu, 2001). Cutting is considered an alternative technique, providing genetic purity and uniformity in planting material, especially as plants with limited seed availability, such as low seed producing cultivars, and recalcitrant nature of seed. Furthermore, propagation through stem cuttings is inexpensive, rapid and simple technique as compared to other vegetative methods.

The stimulation of adventitious root formation in stem cuttings treated with auxin is well known (Blazich, 1988). Bhatt and Tomar

(2010) studied different levels of IBA (viz., 500, 1000, 1500 ppm) on rooting of Swingle (*Citrus auriantifolia*). They concluded that, the concentration of 500 ppm IBA performed the best in all aspects, as root formation, length of root, thickening of root and leaf sprouting in shoot. The effect of various concentrations and treatment time of IBA and NAA on root formation of stem cuttings of Tahiti lime (*Citrus latifolia*) were investigated by Prati *et al.* (1999). Rooting of Tahiti lime cuttings was high, above 50% reaching up to 96% in some cases, with no effect of application of any plant growth regulator. Mohammed, (1999) studied the effect of IBA on rooting of guava cuttings. She found that the highest rooting percentage was obtained with concentration of 4000 ppm Compared with 2000 and 6000 ppm. Modawei, (2002) studied the effect of IBA on rooting of rose cuttings. She reported that 4000 ppm of IBA was superior over 2000 ppm and 1000 ppm IBA over all seasons. Al-saqri and Alderson (1996) studied the effect of cuttings type on rooting of *Rosa centifolia*. They found that medium and basal cuttings gave a better rooting response than apical and sub-apical cuttings. Kumar *et al.* (1995), they noted, cuttings of lemon (cv. Baramasi) taken in July had higher rooting success, required fewer days for sprouting and showed better shoot and root growth than those taken in February. IBA at 2000 ppm gave the highest rooting percentage.

Although, a lot of work has been done on different aspects of propagation of citrus fruits, however, research on adopting rooting of cuttings as propagation technique is limited, in addition to that, the use of growth regulators in propagation of lemon, has not been

studied nor practiced, except in few experiments. Therefore, this study was intended to evaluate best growing media and rooting hormone in relation to seasonal variation.

Materials and Methods

The experiments were conducted during the year 2013-2014 under open condition in the Horticulture Research Centre of G.B. Pant University of Agriculture and technology, Pantnagar is situated at 29°N latitude, 79.3°E longitude and at an altitude of 243.8 m above the mean sea level in the Tarai region of Shivalik range of Himalaya. The experimental material consisted of 20 cm long stem cuttings collected from the middle portion of one year old shoots of cv. Pant lemon-1. The cuttings were planted during two different seasons (rainy and spring season) and maintained under uniform cultural practices. The treatment combination in this experiment consisted of seven different type of media mixtures consisting, Soil (M_1), Soil + FYM (M_2), Soil+Vermicompost (M_3), Soil+Cocopeat (M_4), Soil+Sand+FYM (M_5), Soil+Sand+Vermicompost (M_6) and Soil +Sand + Cocopeat (M_7). Concentration of Growth Regulators viz. Control 0 ppm (G_0), IBA 500 ppm (G_1), NAA 500 ppm (G_2) and IBA+NAA 500 ppm each (G_3) applied at the basal portion of the cutting as a quick dip method with two different season (rainy and spring). The experiment was laid out in factorial Randomized Block Design. Each treatment combination was applied to 10 cuttings and replicated thrice. Two third portion of each cutting was inserted in the media. All cuttings maintained under uniform cultural schedule during the whole period of investigation. Length of longest shoot was measured with the help of measuring scale in each treatment at weekly interval and the mean length of longest shoot was worked out. The diameter of five randomly selected sprouts was measured in mm by vernier caliper at weekly interval and average was worked out. Length of roots measured with the help of measuring scale and mean value was calculated. The sample of 3 cutting from each replication was taken for the study. The diameter of thickest root of each cutting is measured with the help of vernier caliper, just below the root shoot juncture. The survival of cutting was recorded in each treatment at after 8 week at the termination of the experiments and percent survival of cuttings was calculated by dividing number of survive cuttings to total number of cuttings and multiplied with hundred. Data recorded during course of investigation were subjected to statistical analysis under two factor RBD design.

Results and Discussion

The data presented on average length of sprout per cutting revealed that there was significant effect of growing media, growth regulators and growing seasons on average length of sprouts per cutting (Table-1). In the rainy season, average length of sprout per cutting in M_7 (soil+sand+cocopeat) medium was found to be higher (8.69 cm) while minimum (5.79 cm) average length of sprout per cutting was found in M_1 (soil) medium. The positive effects of cocopeat on sprout length might be due to better root growth in cocopeat medium which led to the increase in the growth of sprouts. Among the growth regulators cuttings treated with IBA, 500 ppm gave maximum (8.66 cm) average length of sprout per cutting while minimum (5.04 cm) average length of sprout per cutting was

found in cuttings treated with distilled water. Similarly during the spring season, maximum (5.51 cm) average length of sprout per cutting and minimum (3.73cm) average length of sprout per cutting were observed M_7 (soil+sand+cocopeat) medium and M_1 (soil) medium respectively. Treatment with IBA, 500 ppm gave maximum (5.47 cm) average length of sprout per cutting while minimum (2.94 cm) average length of sprout per cutting was observed in distilled water. Close findings on effect of IBA were also reported by Reddy *et al.* (2005) in scented geranium (*Pelargonium graveolens*) cuttings. Overall during both the seasons, M_7 (soil+sand+cocopeat) medium and IBA 500 ppm gave 7.10 cm and 7.06 cm length of sprout per cutting respectively. Whereas, pooled mean of length of sprouts per cutting was minimum (4.76cm) in M_1 (soil) and 3.99 cm in distilled water. Among the growing seasons higher shoot length (7.48 cm) was found in the cuttings planted in rainy season in compare to spring season (4.60 cm). This might due to better moisture availability and higher temperature in rainy season which increased the nutrient absorption capacity of the cuttings and ultimately leading to better growth.

The data on average diameter of sprout per cutting presented in table-1 revealed that there was significant effect of growing media, growth regulators and growing seasons on average diameter of sprout per cutting. In the rainy season, average diameter of sprout per cutting was found to be higher (2.39 mm) in the cuttings planted in M_4 (soil+cocopeat) medium while lower (1.84 mm) average diameter of sprout per cutting was found in M_1 medium. Among the growth regulators treated with cuttings IBA, 500 ppm gave maximum (2.27 mm) average diameter of sprout per cutting while minimum (1.79 mm) average diameter of sprout per cutting was found in cutting treated with distilled water (control). During spring season, maximum (1.77 mm) average diameter of sprout per cutting was obtained in M_7 (soil+ sand +cocopeat) medium while minimum (1.39 mm) average diameter of sprout per cutting was found in M_1 (soil) medium. Treatment with IBA, 500 ppm resulted in maximum (1.76 mm) average diameter of sprout per cutting while minimum (1.42 mm) average diameter of sprout per cutting was observed in cutting treated with distilled water (control) Similar results were also obtained by Pandey *et al.* (2001)., while examined the effects of auxins including IBA and NAA on root of cuttings of *Ginkgo biloba*. In pooled data of both the seasons, mean average diameter of sprout per cutting was found maximum (2.05 mm) in M_4 (soil+cocopeat) medium while minimum (1.62 mm) mean average diameter of sprout per cutting was noted in M_1 (soil) medium. Present findings are also corroborated with the results of Dvin *et al.*, 2011 in apple. The cuttings treated with 500 ppm IBA gave maximum (2.01 mm) average diameter of sprout per cutting while minimum (1.61 mm) mean average diameter of sprout per cutting was found in distilled water. Better moisture availability and higher auxin activity during rainy season might have led to higher growth of sprout. The results shows higher (2.01 mm) diameter of sprout per cuttings in rainy season while lower (1.63 mm) diameter of sprouts was observed in spring season.

Average length of longest root per cutting was significantly differing among treatments of growing media, growth regulator concentrations in growing seasons (Table-1). In rainy season,

Table-1: Effect of growing media, growth regulator and growing season on average length of shoots/sprouts per cutting, average diameter of shoot (mm) per cuttings and average length of longest roots per cutting (cm)

Treatments	Length of sprout/ cutting			Average diameter of shoot (mm)			Average length of longest roots/cutting (cm)		
	S ₁ (Rainy season)	S ₂ (Spring season)	Pooled mean	S ₁ (Rainy season)	S ₂ (Spring season)	Pooled mean	S ₁ (Rainy season)	S ₂ (Spring season)	Pooled mean
M ₁ (soil)	5.79	3.73	4.76	1.84	1.39	1.62	6.03	3.53	4.78
M ₂ (soil+FYM)	5.89	3.83	4.86	1.82	1.74	1.78	6.34	5.34	5.84
M ₃ (soil+vermicompost)	7.29	4.29	5.79	1.90	1.56	1.73	6.69	4.97	5.83
M ₄ (soil+cocopeat)	7.93	5.35	6.64	2.39	1.70	2.05	9.20	5.93	7.57
M ₅ (soil+sand+FYM)	8.51	4.78	6.64	2.20	1.63	1.92	7.27	6.55	6.91
M ₆ (soil+sand+vermicompost)	8.22	4.76	6.49	1.84	1.67	1.75	7.37	5.48	6.43
M ₇ (soil+sand+cocopeat)	8.69	5.51	7.10	2.14	1.77	1.95	7.28	8.33	7.81
G ₀ (Control)	5.04	2.94	3.99	1.79	1.42	1.61	5.60	4.53	5.07
G ₁ (IBA 500 ppm)	8.66	5.47	7.06	2.27	1.76	2.01	8.52	6.40	7.46
G ₂ (NAA 500ppm)	7.82	4.59	6.21	2.08	1.71	1.89	7.81	5.51	6.66
G ₃ (IBA+NAA 500+500 ppm)	8.38	5.43	6.90	1.93	1.66	1.80	6.73	6.49	6.61
Overall mean (season)	7.48	4.60	2.02	2.02	1.64	7.17	6.45		
	F-test	S.Em (±)	C.D. (P=0.05)	F-test	S.Em (±)	C.D. (P=0.05)	F-test	S.Em (±)	C.D. (P=0.05)
Growing media (M)	S	0.23	0.63	S	0.05	0.15	S	0.66	0.23
Growth hormones (G)	S	0.17	0.48	S	0.04	0.11	S	0.50	0.17
Season (S)	S	0.12	1.26	S	0.03	0.29	S	0.35	0.12

Table-2: Effect of growing media, growth regulator and growing season on average diameter of roots/cuttings (mm) and survival percentage of lemon cutting

Treatments	Average diameter of roots/cuttings (mm)			Survival percentage of lemon cutting		
	S ₁ (Rainy season)	S ₂ (Spring season)	Pooled mean	S ₁ (Rainy season)	S ₂ (Spring season)	Pooled mean
M ₁ (soil)	1.14	0.86	1.00	53.11	64.57	58.84
M ₂ (soil+FYM)	1.35	1.13	1.24	74.19	73.75	73.97
M ₃ (soil+vermicompost)	0.99	0.98	0.98	87.18	50.00	68.59
M ₄ (soil+cocopeat)	1.23	1.26	1.24	74.14	72.40	73.27
M ₅ (soil+sand+FYM)	1.05	1.11	1.08	79.45	85.00	82.23
M ₆ (soil+sand+vermicompost)	1.48	0.93	1.20	88.81	67.08	77.95
M ₇ (soil+sand+cocopeat)	1.15	1.25	1.2	84.69	70.68	77.69
G ₀ (Control)	1.05	0.85	0.95	67.86	49.29	58.57
G ₁ (IBA 500 ppm)	1.27	1.15	1.21	82.37	80.99	81.68
G ₂ (NAA 500ppm)	1.21	1.12	1.17	80.84	75.84	78.34
G ₃ (IBA+NAA 500+500 ppm)	1.26	1.18	1.22	78.39	70.15	74.27
Overall mean (season)	1.20	1.15	77.36	69.06		
	F-test	S.Em (±)	C.D. (P=0.05)	F-test	S.Em (±)	C.D. (P=0.05)
Growing media (M)	S	0.02	0.55	S	0.05	1.45
Growth hormones (G)	S	0.02	0.416	S	0.04	1.10
Season (S)	S	0.01	0.29	S	0.28	0.78

maximum (9.20 cm) average length of longest root per cutting was found in M₄ (soil+cocopeat) medium while minimum (6.03 cm) average length of longest root per cutting was observed in M₁ (soil) medium. Better texture and porosity of coco-peat which facilitated easy penetration of roots (Singh *et al.*, 2002). Longest (8.52 cm) average length of roots per cuttings was observed with 500 ppm IBA while minimum (5.60 cm) average length of longest root per cutting was observed in distilled water. According to Villar, (1997) auxin treatment help in accumulation of metabolites at the site of application, synthesis of new protein, callus formation, cell division and cell enlargement thereby leading to better root formation. The results of present investigation are strengthened by Verma *et al.* (2005) who reported maximum root length in citrus cuttings with application of IBA. In spring season, maximum (8.33 cm) average length of longest root per cutting was found in M₇

(soil+sand+cocopeat) medium while minimum (3.53 cm) average length of longest root per cutting was observed in M₁ (soil) medium. Aminah *et al.*, 2012 reported significant increase in root length in cocopeat based medium in *Neolamarckia cadamba* which are in agreement to present findings. Cuttings treated with the combination of IBA and NAA at 500 ppm each gave maximum (6.49 cm) average length of longest root per cutting while minimum average length of longest root per cutting (4.53 cm) was observed in distilled water treated cuttings. Coir pith enhanced root initiation, root elongation, root biomass and rooting percentage. Being a well drained media it promoted better root characters (Singh *et al.*, 2002). During both the seasons, mean average length of longest root per cutting was found maximum (7.81 cm) in M₇ (soil+sand+cocopeat) medium while minimum (4.78 cm) mean average length of longest root per cutting was observed in M₁ (soil) medium. Beneficial effect of

cocopeat was higher due to higher moisture holding capacity of cocopeat based medium provide better condition for rooting of the cuttings. Auxins plays main role in the process of root formation. For successful root induction either applied or endogenous auxins are important. Treatment of 500 ppm IBA produced maximum (7.46 cm) mean average length of longest root per cutting while minimum (5.07 cm) mean average length of longest root per cutting was observed in distilled water. Similar results have been reported by Dvin *et al.*, 2011 which recorded the highest root length (9.83 cm) in cocopeat+perlite medium with 1500 ppm IBA in apple. The data on longest root per cutting shows that in rainy season longer roots (7.16 cm) were obtained while shorter (5.73 cm) length of roots produced in spring.

Significant effect of growing media, growth regulator concentrations and growing seasons were noticed on average diameter of root per cutting (Table-2). In rainy season, maximum (1.48 mm) average diameter of root per cutting was found in M₆ (soil+sand+vermicompost) medium while minimum (0.99 mm) average diameter of roots per cutting was observed M₃ (soil+vermicompost). Vermicompost was found to be the best medium for rooting and growth of carnation stem cuttings because rooting of stem cuttings in sand and soil can be improved by the addition of vermicompost (Mahale *et al.*, 2002). In present study treatment of cuttings with 500 ppm IBA gave maximum (1.27 mm) average diameter of thickest roots per cutting while minimum (1.05 mm) average diameter of thickest root per cutting was observed in distilled water. Similarly Bhatt and Tomar (2010) found that treatment of 500 ppm IBA performed the best for root thickness and other parameters in Kagzi-lime. In spring season, maximum (1.26 mm) average diameter of thickest root per cutting was found in M₄ (soil+cocopeat) while minimum (0.86 mm) average diameter of thickest root per cutting was observed in M₁ (soil) medium. Rubasingh *et al.* (2009) observed similar findings in *Chirita moonii* cuttings. Data displayed that Cuttings treated with the combination of IBA and NAA at 500 ppm each gave maximum (1.18 mm) average diameter of thickest root per cutting while minimum (0.85 mm) average diameter of thickest root per cutting was observed in distilled water (control). Combination of NAA and IBA in the most of treatment had better according to use of each one of these hormones lonely. During both the season, mean average diameter of thickest root per cutting was found maximum (1.24 mm) in M₄ (soil+cocopeat) medium and M₂ (Soil+FYM) medium each, while minimum (0.98 mm) mean average diameter of thickest root per cutting was observed in M₃ (soil+vermicompost) medium. This is due to media contain cocopeat provides sufficient anchorage or support to the plant, serves as reservoir for nutrients and water, with addition of sand in cocopeat it allow oxygen diffusion to the roots and permit gaseous exchange between the roots and atmosphere outside the root substrate which lead to increase diameter of roots (Argo, 1998 and Abad *et al.*, 2002). Cuttings treated with the combination of IBA and NAA at 500 ppm each produced maximum (1.22 mm) mean average diameter of thickest root per cutting while minimum (0.95 mm) mean average diameter of thickest root per cutting was observed in distilled water. Among the growing season rainy season proves better for

development of root with maximum (1.19 mm) mean diameter of roots followed by spring season (1.11 mm). Optimum environmental conditions along with plant growth regulators might have resulted in maximum diameter of roots in rainy season planting.

It is evident from the data (Table-2) that growing media, growth regulators and growing seasons had significant effect on survival percentage of cuttings. In rainy season, highest (88.81 %) survival percentage of cuttings was found in the cuttings planted in M₆ (soil+sand+vermicompost) medium while minimum (53.11 %) survival percentage of cuttings was observed in M₁ (soil) medium. The findings of Gopale and Zunjoroo (2011) in *Jatropha* are in accordance with the present results. In the present investigation among the growth regulators IBA at 500 ppm resulted in maximum (82.37 %) survival percentage of cuttings while minimum survival percentage of cuttings (67.86 %) was found in cuttings treated with distilled water which was taken as control. Significant effect of growth regulators on survival percentage was due to root induction capacity of auxin which help in production of adventitious roots and thereby increase in the survival of plants. Sharma *et al.* (2009) also found increase in the survival percentage of pomegranate cuttings treated with 500 ppm IBA in combination with 1% Borax 300 ppm IBA with Borax 2% and IBA alone at 5000 ppm. Seasonal timing also played an important role in rooting of cuttings. During spring season, maximum (85.00 %) survival percentage of cuttings was observed in M₅ (soil+sand+FYM) medium while lowest survival percentage of cuttings (50.00%) was found in M₃ (soil+vermicompost) medium. The positive effect of high organic matter and moisture retention capacity of FYM might have led to effective rooting of cuttings. The findings of Bashir *et al.* (2007) in *jobba* cuttings support the results of present investigation. Among different growth regulators IBA at 500 ppm was found to be most effective and gave maximum (80.99 %) survival percentage of cuttings while minimum (49.29 %) survival percentage of cuttings was observed in distilled water (control). Pooled data on of both season maximum (82.23 %) survival percentage in M₅ (soil+sand+FYM) medium while minimum (58.80 %) mean survival percentage of cuttings was observed in M₁ (soil) medium. Kumar and Syamal, (2005) also observed similar result with soil+FYM medium in guava air layering. The effect of IBA at 500 ppm gave maximum (81.68 %) mean survival percentage of cuttings while minimum (58.57 %) mean survival percentage of cuttings was observed with cuttings treated with distilled water (control). These finding are in line with the finding of Patil *et al.* (2003) who observed maximum survival percentage with IBA at 300 ppm in grape cuttings. Superior rooting of cuttings with higher success (77.36 %) was obtained in spring season as compared to monsoon seasons (69.07 %). This might be due to the excessive rainfall during rainy season which adversely affect the growth and mobilization of metabolites in plants.

Based on above findings it can be concluded that vermicompost and cocopeat based medium was found to be best for rooting. However, time of the year at which cuttings were taken had influenced effects on rooting ability of the cuttings. The highest percentage of rooted cuttings of lemon was obtained in autumn (August). Indole Butyric Acid alone at 500 ppm found superior over

NAA at 500 ppm. Furthermore, these results have clearly indicated that physiological stage of the plant, external application of synthetic auxin and environmental conditions such as temperature, low relative humidity and diseases have significant major effects on the success of propagation of lemon by cuttings.

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