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Effect of bio-regulator treatment, wounding and growing media on survival and vegetative growth of stem cutting in lemon (*Citrus limon* Burm.)

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Abstract
The present investigation was carried out to know the effect of bio-regulator treatment, wounding and growing media on survival and vegetative growth of stem cutting in lemon (*Citrus limon* Burm). The results of the propagation study revealed that stem cuttings of lemon when treated with wounding + 4000 ppm IBA and grown in soil + sawdust + vermi-compost had recorded significantly earliest sprouting (6.92 days) and highest sprouting percent (79.38%), survival percent (76.38%), plant height (19.80 cm), number of shoots/cutting (7.34), number of leaves/cutting (12.88), stem girth (2.15 cm). The results also revealed that 'wounding' (without IBA treatment) was found to be least significant among chemical and mechanical treatments in respect of above parameters of stem cutting.

Keywords: *Citrus limon*, stem cutting, IBA treatments, wounding, rooting media, survival

Introduction
Lemon (*Citrus limon* Burm.) is a leading premier citrus crop which occupies an important place in citrus industry throughout the world. It belongs to Rutaceae family with East Indies as its primary centre of origin. It is mainly grown in tropical and sub-tropical regions the world. In the world, India is the 5th largest producer of lime and lemon which produces about 2.78 million tons of Lemon from an area of 0.26 million hectares with the productivity of 11.2 tons/hectare. Andhra Pradesh is the leading producer of Lemon in India followed by Telangana and Gujarat (Anonymous, 2016-17) [2, 3]. It is used for preparation of lemonade, refreshing drinks and number of culinary like pies, cakes, fish, meat and salad. It is a good source of citric acid, which is used for pharmaceutical purpose and for aerated waters. The lemon oil is a stimulant and carminative when given internally as medicine. Lemon juice along with common salt is recommended as a remedy for dysentery, dry bleach, putrid, sore throat and for correcting foetid breath. Lemon squash and pickles are the fine preserves used in India. Among the various cultivars of lemon grown in Tarai region, Pant Lemon-1 has been found most promising. The cultivar is becoming popular among the orchardists all over the country due to regular bearing habit and tolerance to citrus canker, tristiza, and dieback. Quality planting material is always prime need to the fruit growers. Therefore, it is necessary to produce quality planting material for higher production of quality fruits. For this purpose, a rapid method for multiplication of planting material is needed to obtain good quality plants (Frey *et al.* 2006) [9]. Various techniques of vegetative propagation (*i.e.* air layering, patch budding and stem cutting) have been suggested for multiplication of lemon plants. Among these stem cutting is more popular because it provides many numbers of plants in short period with large success ratio.

It has been reported that success of stem cutting depends mainly upon type of wood, the stage of growth used in making the cuttings, the time of year in which the cuttings are taken, rooting media and several other factors can be very important in securing satisfactory rooting of plants. Although propagation by cutting is seldom practiced commercially because survival of plant raised by stem cutting is poor. In some fruit plants, propagated by stem cutting has become more feasible with the development of auxin treatment. For general use in rooting stem cuttings of the majority of plant species, NAA and IBA, particularly the latter is recommended, to determine the best materials and optimum concentration for rooting any

particular species under a given set of conditions (Hartmann and Kester, 1976) [10]. In addition to auxin treatment, root production on stem cutting can be promoted by wounding the base of cutting in a number of plant species. Basal wounding is beneficial in rooting cutting of certain species especially cuttings with older wood at the base. Following wounding, callus production and root development frequently are heavier along the margins of the wound. Evidently wounded tissues are stimulated in to cell division and production of root primordia (Hartmann and Kester, 1976) [10]. Generally rooting medium has been adjudged to be the most critical factor determining seedling quality in the nursery acting as a reservoir for nutrients and moisture. It is an important input for better seedling production. It directly influences the growth, development, ramification and functioning of rooting system. An ideal rooting medium should be loose, porous with high water holding capacity. Rooting medium holds cutting in place, holding moisture content for new emerged roots, congenial conditions for respiration and maintains optimum temperature for the root initiation. The nature of roots arising from the cuttings is also influenced by the type of rooting medium e.g., cutting when planting in pure sand produced long, unbranched, coarse and brittle roots but those planted in a mixture of sand, soil and peat, produce well developed branches (Chattopadhaya, 1994) [6]. The research findings on the above aspects i.e. rooting media, wounding and auxin treatments of stem cutting in lemon are rather meager and inadequate. Moreover, the success rate in case of stem cutting without pre-planting treatments is not as good as observed with other techniques of propagation in lemon. Therefore, it was realized to ascertain the performance of stem cutting of lemon with auxin, wounding and rooting media treatments.

Materials and Methods

The present investigation entitled "Effect of bio-regulator treatment, wounding and growing media on survival and vegetative growth of stem cutting in lemon (*Citrus limon* Burm.)", was conducted at Sardar Vallabhbhai Patel University of Agriculture and Technology, Meerut, 250 110 (UP), during 2017-18. The climate of this region is sub-tropical with maximum temperature of about 42°C during summer (April to June) and a minimum temperature of about 7°C during winter (December to February). The average annual rainfall in the region is about 862.7 mm and the annual relative humidity varies from 67 to 83 percent. The experiment was laid out in Factorial RBD with 4 replications in each treatment. There were 8 treatments with 2 stem cuttings in each replication of all 16-treatment combinations. The details of treatments i.e. Control (stem cuttings without wounding and without bio-regulator treatments), Stem cuttings with wounding, Stem cuttings + 2000 ppm IBA, Stem cuttings + 4000 ppm IBA, Stem cuttings + 6000 ppm IBA, Stem cuttings with wounding + 2000 ppm IBA, Stem cuttings with wounding + 4000 ppm IBA and Stem cuttings with wounding + 6000 ppm IBA. For obtaining stem cuttings, 9-10 month old shoots of Pant Lemon-1 cultivar of lemon was selected for conducting experiment. Two rooting media were prepared and filled in polybags. About 1-1.5 kg growing medium was filled in each polybag. The details of growing media used for the study are as follows: soil + sawdust + vermi-compost @ 1:1:1 and soil + sawdust + FYM @ 1:1:1. The propagation study was carried out under net house in which there was a provision of 50 percent shade. The stock solution of 12000 ppm IBA (Indole Butyric Acid) was prepared as per the procedure described by Prakash (1984) [19]. The 18 cm long and pencil thickness sized cuttings were made and treated with prepared IBA solution according to different doses of IBA. Thereafter, cuttings were planted in poly-bags and placed in shade net-house during third week of July month. The various observations like days taken to bud sprouting, sprouting percentage, survival percentage, plant height, number of shoots per cutting, number of leaves per cutting, stem diameter were recorded with proceeding of experiment. The recorded data were statistically analyzed by using Factorial RBD as suggested by Panse and Sukhatme (1985) [18].

Result and Discussion

In the present study the performance of cutting was greatly influenced by rooting media, rooting hormone like IBA in different concentrations and wounding. Among the different treatments applied to stem cuttings, 4000 ppm IBA + wounding and rooting medium soil + sawdust + vermi-compost was found to be the best treatment in respect of days taken to bud sprouting, percent sprouting, percent survival, plant height, number of shoots sprouted per cutting, number of leaves developed per cutting, stem diameter in cultivar of Pant Lemon-1.

The influence of bio-regulator, wounding and rooting media on bud sprouting in stem cutting of lemon was investigated under the present study. During the study it was observed that stem cuttings of lemon when treated with wounding + 4000 ppm IBA and planted in 'soil + sawdust + vermi-compost' rooting medium had earliest sprouting (6.92 days), while stem cuttings of control treatment when planted in 'soil + sawdust + FYM' had taken maximum days to sprout (20.38 days). Of the rooting media, 'soil + sawdust + vermi-compost' was found to be significantly better over 'soil + sawdust + FYM' in terms of bud sprouting irrespective of treatments. The findings of the study are in accordance with the findings of Thankamani *et al.,* (2005) [22] and Kaur and Malhi, (2006) [12]. Other researchers have also reported the significant effect of rooting hormone and growing media on sprouting in stem cutting of lemon (Pandey *et al.,* 2003) [17]. Earliest sprouting in cuttings treated with 4000 ppm IBA + wounding in the current study may be due to prevention of down-ward translocation of carbohydrate and accumulation of higher level of endogenous auxins in the ringed, lower portion of treated cuttings during the period of root initiation which might have resulted in earliest completion of physiological process involved in rooting and sprouting (Kaur and Malhi, 2006 and Hartmann and Kester, 1986) [12]. The results of the study are further supported by the fact that among the synthetic auxins, IBA is more effective in inducing adventitious root formation in stem cutting (Hartmann and Kester, 1986) [11]. It has been confirmed that auxin is a requirement for initiation of adventitious roots on stem cuttings (Hartmann and Kester, 1986) [11] which has resulted in early sprouting in cuttings treated with IBA. Another possible reason for early sprouting in wounded cutting may be because of the fact that following wounding, callus production and root development increase along the margins of the wound. Due to wounding natural accumulation of auxins and carbohydrates in the wounded area is increased. Beside this, ethylene production is also increased which promote adventitious root formation in stem cutting resulting in early intake of buds in cuttings (Hartmann and Kester, 1986) [11]. The promotive effect of wounding on bud sprouting as observed in the present study may also be due to the fact

that wounded cuttings absorb more water from the medium than unwounded cuttings. Wounding also permits greater absorption of applied growth regulators by the tissues at the base of the cuttings (Hartmann and Kester, 1986) [11]. In context to growing media, soil + sawdust + vermi-compost caused earliest sprouting, longer and thicker sprouts due to optimum nutrient uptake and enhanced availability of nutrients and growth promoting substances. Another possible reason for earliest sprouting in stem cuttings grown in soil + sawdust + vermi-compost in the present study might be because of optimum availability of water and nitrogen in the rhizosphere due to the presence of vermi-compost in the rooting medium which might have facilitated better uptake of nitrogen, the chief constituent of amino acid and co-enzymes that are of biological importance. Optimum N uptake might have reduced the C: N ratio and thereby inducing buds at earlier days (Kumar and Ponnuswami, 2013) [14]. This may also be due to higher net assimilation on account of better growth leading to the production of endogenous metabolites earlier in optimum level enabling early bud initiation and thereby early sprouting. Further, vermi-compost in comparison to FYM used for polybag mixture in the present study contained good amount of plant available nutrients. Therefore, due to quick and rapid availability of nutrients to the cuttings grown in the vermi-compost containing rooting medium, the buds sprouted earliest in stem cutting (Adak *et al.*, 2014) [1]. The comparatively lesser time taken by cuttings to sprout due to bio-regulator, wounding and growing media treatments in the present study might also be due to rapid sap flow in cuttings resulting in early callus formation and initiation of subsequent growth. Incorporation of sawdust and vermi-compost in the growing medium create favorable condition for optimum growth of cutting due to better moisture retaining capacity and better aeration, drainage and porous quality of sawdust and high nutritive and growth promoting values of vermi-compost. It has been reported that vermi-compost is at least 4 times more nutritive than conventional cattle dung compost (Suhane, 2007 and Munroe, 2007) [21, 15]. Success rate of stem cutting of lemon was significantly affected in the present study due to bio-regulator treatment, wounding and rooting media. Significantly higher percent survival was observed when stem cuttings were treated with 4000 ppm IBA and wounding. Among the rooting media, soil + sawdust + vermi-compost had better effect on percent survival of cutting than rooting medium containing FYM. The finding of the present study also revealed that survival rate was decreased at highest concentration of IBA (6000 ppm). Significantly higher success rate of stem cutting due to IBA + wounding treatment was also reported by Chayanika *et al*, 2011 [7]. This may also be due to higher net assimilation rate on account of better growth leading to the production of endogenous metabolites earlier in optimum level enabling early bud initiation and thereby better survival. Further, vermi-compost in comparison to FYM used for polybag mixture contained higher level of nutrients. Therefore, due to quick and rapid availability of nutrients to the cuttings grown in vermi-compost containing rooting medium, the survival percentage was improved (Sinha, *et al.*, 2014) [20]. On the other hand minimum survival percent recorded with control treatment in the present study might be because control cuttings had neither been treated with rooting hormone nor been wounded resulting in lesser absorption of nutrient, hormone, water and metabolites in cutting. As a result, control cuttings had minimum percent success. Sawdust was incorporated in the rooting media in the present study because of the fact that sawdust has unique characteristics i.e. high water holding capacity, better aeration, drainage and high porosity along with enhanced microbial activity due to the presence of vermi-compost in the polybag mixture which helped in better nutrient uptake by the plants (Kumar and Ponnuswami. 2013) [14]. It is further supported by the fact that vermi-compost retains nutrients for longer time, while conventional compost fails to deliver the required amount of macro and micro nutrients including the vital NPK (nitrogen, potassium and phosphorus) to plants in shorter time, the vermi-compost does. As reported by Canellas, *et al.*, 2002 [4], the humic acid in vermi-compost stimulates plant growth even in small amount.

In the present study vegetative growth of stem cutting in respect of plant height, number of shoots sprouted per cutting, number of leaves emerged per cutting and stem diameter was significantly affected due to the effect of rooting hormone, wounding and growing media. Among the treatments, 'wounding + 4000 ppm IBA' had significantly increased the maximum vegetative growth in terms of plant height (18.78 cm), number of shoots per cutting (6.77), number of leaves per cutting (12.42) and stem diameter (2.09 cm) followed by wounding + 6000 ppm IBA. However, minimum vegetative growth in respect of plant height (5.11 cm), number of shoots per cutting (1.42), number of leaves per cutting (2.80) and stem diameter (1.04 cm) was recorded in stem cuttings which were not wounded and treated with IBA. Of the rooting media, the performance of soil + sawdust + vermi-compost was found to be significantly better over soil + sawdust + FYM in respect of growth of stem cuttings. Significant increase in vegetative growth of stem cuttings due to IBA treatment has also been reported by Fochesato (2007) [8] and Oliveira (2014) [16]. The maximum vegetative growth recorded in stem cuttings when treated with 4000 ppm IBA + wounding and grown in soil + sawdust + vermi-compost rooting medium might be because of the fact that wounded cutting when treated with moderate concentration of IBA absorb greater amount of nutrients, hormones and metabolites. As a result, endogenous level of nutrient and hormone is increased in the cuttings which might have increased the vegetative growth of stem cutting. The significant improvement in vegetative growth in wounded cuttings when treated with moderate concentration of IBA has also been observed by Kaur and Malhi (2006) [12]. Another possible reason for better growth of stem cutting when treated with IBA and planted in vermi-compost containing rooting medium might be due to activation of auxin content in vegetative part and also due to nutrient rich rooting medium resulting in more number of leaves per cutting. These findings are in agreement with the results of earlier workers, namely, Kumar *et al.*, (2004) [13]. The higher values of growth parameters of cutting i.e. plant height, stem diameter, number of shoot and leaves recorded in cuttings which grown in vermi-compost containing rooting medium in the present study might be because of the fact that cuttings grew vigorously under growing medium containing vermi-compost and sawdust due to their unique characteristics. The favorable moisture level in the rooting media throughout the growth period of cutting might have stimulated the physiological process of cell elongation and cell division which might have contributed to elongation of stem on both directions (Kumar and Ponnuswami., 2013) [14]. The increase in stem diameter could also be attributed to the increase in uptake of nutrients due to the presence of vermi-compost in the rooting medium which contained good amount of nutrients.

Table 1: Effect of bio-regulator treatment, wounding and rooting media on days taken to bud sprouting, sprouting percent, survival percent and plant height in stem cuttings of lemon

Treatments	Days taken to bud sprouting			Sprouting %			Survival %			Plant height (cm)		
	Rooting media			Rooting media			Rooting media			Rooting media		
	Soil + sawdust + vermi-compost	Soil + sawdust + FYM	Mean	Soil + sawdust + vermi-compost	Soil + sawdust + FYM	Mean	Soil + sawdust + vermi-compost	Soil + sawdust + FYM	Mean	Soil + sawdust + vermi-compost	Soil + sawdust + FYM	Mean
Control (stem cuttings without wounding and without bio-regulator treatments)	17.06	20.38	18.72	37.34	35.20	36.27	33.11	28.33	30.72	5.99	4.24	5.11
Stem cuttings with wounding	15.71	19.31	17.50	44.44	40.75	42.59	40.50	35.13	37.81	7.42	5.03	6.22
Stem cuttings + 2000 ppm IBA	12.23	15.28	13.75	58.09	55.66	56.87	55.80	51.08	53.44	10.31	7.39	8.85
Stem cuttings + 4000 ppm IBA	10.65	11.35	11.00	68.85	65.45	67.15	64.04	58.19	61.11	12.42	10.33	11.37
Stem cuttings + 6000 ppm IBA	11.27	14.11	12.69	67.13	61.34	64.23	62.18	56.14	59.16	11.28	9.79	10.53
Stem cuttings with wounding + 2000 ppm IBA	9.22	11.11	10.16	71.17	69.66	70.41	70.95	65.81	68.38	14.20	13.30	13.75
Stem cuttings with wounding + 4000 ppm IBA	6.92	8.19	7.55	79.38	78.12	78.75	76.38	74.62	75.50	19.80	17.76	18.78
Stem cuttings with wounding + 6000 ppm IBA	8.19	9.47	8.83	77.10	74.14	75.62	74.48	70.88	72.68	16.10	15.59	15.84
Overall Mean	11.40	13.65	12.52	62.94	60.04	61.48	59.68	55.02	57.35	12.19	10.43	11.31
CD (P = 0.05)				CD (P = 0.05)			CD (P = 0.05)			CD (P = 0.05)		
Chemical & Mechanical treatments		0.84			1.58			1.88			0.74	
Rooting media		0.42			0.79			0.94			0.37	
Chemical & Mechanical treatments × Rooting media		0.18			2.24			2.66			1.04	

Table 2: Effect of bio-regulator treatment, wounding and rooting media on number of shoots/live cutting, number of leaves/stem cutting and stem diameter in stem cuttings of lemon

Treatments	Number of shoots/live cutting			Number of leaves/stem cutting			Stem diameter (cm)		
	Rooting media			Rooting media			Rooting media		
	Soil + sawdust + vermi-compost	Soil + sawdust + FYM	Mean	Soil + sawdust + vermi-compost	Soil + sawdust + FYM	Mean	Soil + sawdust + vermi-compost	Soil + sawdust + FYM	Mean
Control (stem cuttings without wounding and without bio-regulator treatments)	1.79	1.05	1.42	3.30	2.30	2.80	1.06	1.02	1.04
Stem cuttings with wounding	2.89	1.39	2.14	4.68	3.75	4.21	1.11	1.07	1.09
Stem cuttings + 2000 ppm IBA	3.56	2.31	2.93	6.25	5.00	5.62	1.21	1.18	1.19
Stem cuttings + 4000 ppm IBA	5.18	4.34	4.76	8.00	7.00	7.50	1.46	1.40	1.43
Stem cuttings + 6000 ppm IBA	4.55	3.62	4.08	7.23	5.89	6.56	1.32	1.28	1.30
Stem cuttings with wounding + 2000 ppm IBA	5.56	4.77	5.16	10.13	9.00	9.56	1.59	1.51	1.55
Stem cuttings with wounding + 4000 ppm IBA	7.34	6.20	6.77	12.88	11.96	12.42	2.15	2.03	2.09
Stem cuttings with wounding + 6000 ppm IBA	6.07	5.11	5.59	11.63	10.75	11.19	1.73	1.63	1.68
Overall Mean	4.62	3.60	4.11	8.01	6.96	7.48	1.45	1.39	1.42
CD (P = 0.05)				CD (P = 0.05)			CD (P = 0.05)		
Chemical & Mechanical treatments		0.43			0.73			0.15	
Rooting media		0.21			0.36			0.09	
Chemical & Mechanical treatments × Rooting media		0.61			1.03			0.21	

The findings of present study also supported by the fact that improvement in stem diameter might be due to increased cell wall plasticity ascribed to the stimulatory action of nutrients and amino acid**.** Leaf is the prime attribute of vegetative growth of cutting which is considered an important functional unit of plant which contributes to the formation of assimilates. Number of leaves per cutting plays an impressive role in the photosynthetic efficiency of plant. A plant should produce sufficient number of leaves to harness light energy and synthesize adequate photo assimilates for biomass production (Kumar and Ponnuswami, 2013) [14]. The emergence of more number of leaves in stem cuttings when treated with 4000 ppm IBA + wounding and planted in soil + sawdust + vermi-compost in the present study might also be because of early sprouting in cuttings. Due to early sprouting in treated cutting, the leaves emerged early in cuttings. As a result, more number of leaves was produced in cuttings. The finding of present study is further supported by the fact that sap flow in cuttings might be rapid due to early sprouting (Chandel *et al.*, 1998) [5]. High water holding capacity, better aeration and drainage and high porosity of sawdust along with enhanced microbial activity due to the presence of vermi-compost in the polybag mixture helped in better nutrient uptake by the cutting (Kumar and Ponnuswami., 2013) [14].

Conclusion

On the basis of results obtained on different parameters of stem cutting as recorded above, it can be concluded that among the chemical and mechanical treatments applied to stem cutting of lemon, application of wounding + 4000 ppm IBA was found to be significantly most effective, while the performance of vermi-compost containing rooting medium (i.e. soil + sawdust + vermi-compost) was observed to be significantly superior over FYM containing rooting medium (i.e. soil + sawdust + FYM) in causing earliest sprouting and significantly maximum survival, vegetative and root growth of stem cutting in Lemon cv. Pant Lemon-1.

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