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Vegetative propagation of Lisianthus genotypes through stem cuttings: a viable alternative to seed propagation

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ABSTRACT

Lisianthus is a potential new flower crop that has been recently introduced in India. The propagation of lisianthus through seeds has emerged as a great challenge for its commercialization. In the present study we have explored the possibility of vegetative propagation of lisianthus crop through stem cuttings. The effect of different doses of growth regulators *viz*. Indole-3-butyric acid (IBA:250&500 ppm) and naphthalene acetic acid (NAA:250&500 ppm) either alone or in combinations on rooting of stem cuttings was assessed in seven lisianthus genotypes. Treatment of lisianthus cuttings with 250 ppm IBA for 5 minutes induced the maximum rooting. The number of roots per cutting, root length and fresh and dry weight of the root was found maximum when the cuttings were treated with 250 ppm IBA for 5 minutes. The genotypes Echo Double Pink Picotee, Echo Double White and Echo Double Blue exhibited more than seventy percent rooting indicating the scope for their vegetative propagation. Among the different lisianthus genotypes, the maximum rooting was observed in Echo Double Pink Picotee. The maximum number of roots per cutting (11.6) and the maximum root length (7.9 cm) was observed in Echo Double Blue. When the lisianthus crop raised through rooted cuttings and seedling methods were compared, it was observed that the crop raised through rooted cuttings performed better than the seedling raised crop. The vegetative propagation through stem cuttings can be a viable alternative for the propagation of lisianthus.

Key words: *Eustoma grandiflorum,* auxins, stem cuttings.

INTRODUCTION

Lisianthus ([*Eustoma grandiflorum* (Raf.) Shinn], commonly known by the names 'Texas Bluebell'; 'Prairie Gentian' and 'Tulip Gentian', belongs to the family *Gentianaceae*. It is moderately a cold-tolerant annual or biennial plant native to the southern part of the United States and northern Mexico (Popa *et al*., 10). Lisianthus is relatively a new flower crop that has been introduced to the world market especially United States of America in early 1980's. In Japan, it ranked fifth in terms of the total wholesale value of cut flowers in 2017 (Ahmad *et al*., 1). In the last decade, it has emerged as one of the fastest growing segment of new flower category worldwide. It can be used as either cut flowers or as flowering pot plants and flowers are available in various colors, such as blue, purple, white, pink and bicolor. Its flowers are widely used for making bridal bouquets and many other special flower arrangements. Lisianthus has been recently introduced in India as a new specialty cut flower. The suitability of this crop as a potential new flower for mid Himalayan region has been established by Wazir (15). Although lisianthus is a newly introduced crop, it is gaining tremendous popularity among the growers all over the world, mainly due to its large and attractive flowers, long and hard stem, wide range of colours and long vase life (Roni *et al.*, 14). The non availability of quality planting material and lack of technical know how about standard cultural practices and post-harvest procedures are the major hurdles for its popularization in India.

Lisianthus is mainly propagated through seeds. However, the propagation of lisianthus through seeds have emerged as a great challenge, as it is very much complicated and difficult exercise due to slow germination and growth. Its seed are very tiny and a number of environmental factors including light, temperature, and moisture level affect the germination of seeds (Roni *et al.*, 14). A constant temperature of 20–25°C is required for germination of seeds (Roh and Lawson, 13). Seedling emergence is delayed if temperature is not maintained carefully during the germination period. High fluctuations between day and night temperatures also affect seedling growth and development, resulting in rosetting and delayed flowering (Ohkawa *et al.*, 6). Photoperiod is also an important factor for regulating germination and seedling growth because of its effect on cotyledon expansion, leaf and hypocotyl development, and photosynthesis (Roni *et al.*, 14). There is a huge time gap of 3-5 months between seed sowing and

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transplanting of seedlings when seeds are sown under open field conditions. Further, lisianthus being a crop of outcrossing nature, the seedling population exhibited a lots of variability with respect to flowering time, stem length and flower qualities besides other traits of commercial importance (Rajkumar, 12). Vegetative propagation by means of shoot tip cuttings is an efficient method for producing large numbers of plants in ornamentals. It will ensure true to the type plants with great uniformity. The genetic characteristics of a mother plant are maintained by this technique.

Adventitious rooting is a complex process and a key step in the vegetative propagation, playing an important role in the successful production of elite clones. The formation of adventitious roots is a quantitative genetic trait regulated by both environmental and endogenous factors. The auxins play a pivotal role in regulating roots development and it has been shown to be intimately involved in the process of adventitious rooting (Pop *et al.*, 9). The exogenous application of auxin to enhance rooting of cuttings has become a routine practice. The effectiveness of auxins, however, varies not only with the nature and concentration of the auxin and the plant species, but also with season. The efficacy of auxins in inducing rooting, improve the rooting percentage and survival of rooted cuttings has been shown in carnation (Kumar *et. al.*, 5), chrysanthemum (Prince *et. al.*, 11) and many other ornamentals crops.

Till date, limited efforts have been made to multiply this crop vegetatively through stem cuttings. Earlier studies by Roh and Lawson (13), reported delayed flowering and fewer number of flower buds in rooted cuttings as compared to the seedlings. Hence, the earlier studies fail to prove the effectiveness of stem cuttings as an alternative approach for commercial propagation of lisianthus. Though several reports are available on *in vitro* propagation of this crop through tissue culture (Popa *et al.*, 10; Rajkumar, 12; Roni *et al.*, 14), however, such techniques requires skilled manpower and sophisticated infrastructure that is not feasible at farmers field or nursery level. Hence, to overcome the propagation hurdles, we have studied the possibility for vegetative propagation through stem cuttings in this crop. In the present study, we have determined the effects of growth regulators on rooting of stem cuttings in different lisianthus genotypes. Further, we have also compared the plants raised through rooted cuttings and seedlings for flowering related traits in order to prove the effectiveness of stem cutting in lisianthus.

MATERIALS AND METHODS

Seven lisianthus genotypes namely Echo Double Yellow (EDY), Echo Double Champagne (EDC), Echo Double White (EDW), Echo Double Lavender (EDL), Echo Double Pink Picottee (EDPP) Echo Double Blue (EDB) and Echo Double Pink (EDP) were used in the present study. The seeds of these were procured from Bharat Seeds, Kolkata, India. This series was found promising for cut flower production under mid Himalayan condition. The mother plants were raised under low cost polytunnels in the research farm, Indian Agricultural Research Institute, Regional station Katrain, Kullu, Himachal Pradesh, India (32°12'N; 77°13'E ; 1,560 m asl), during the year 2015-17. Healthy mother plants from the ratooning crop of the previous year were selected. The terminal cuttings (5 cm in length) with 2-3 pairs of leaves were taken from healthy mother plants during months of February. They were treated with 0.2 percent Carbendazim (Biostadt India Limited) for 30 minutes. The basal end of the cuttings were then treated for five minutes with different doses of Indole-3- butyric acid (IBA; Merck, Mumbai, India) and Naphthalene acetic acid (NAA; Merck, Mumbai, India) either alone or in combinations. The required amount of the growth regulators was first dissolved in minimum amount of IN KOH solution and then volume was made with the help of distilled water. The different growth regulator treatments used were : T_1 (Control: distilled water) T_2 (IBA:250ppm), T_3 (IBA:500ppm); T_4 (NAA:250ppm), T_5 (NAA:500ppm); T_6 (IBA+NAA: 250ppm). The cuttings after treatment were planted in plastic pots (15 cm) filled with a mixture of cocopeat, perlite and vermiculite (3:1:1). Fifteen cuttings were planted per pot and three pots were used per replication/treatment and each treatment was replicated thrice. After planting, cuttings were irrigated with the help of rose can and the plastic pots were kept under the glasshouse. The temperature was maintained at 18-23°C, and relative humidity at 80-85 % within the help of the humidifiers. The rooting media was drenched with 0.2% Captan (India Agro Sciences**)** at weekly interval to control fungal infection. After 45 days of planting, observations were recorded on different rooting parameters of the cuttings viz., percent rooting, number of roots per cutting, root length and fresh and dry weight of the roots. Per cent rooting was determined by counting the number of rooted cuttings and dividing this by the total number of cuttings per replication. For the other rooting parameters, five cuttings per replication were randomly chosen for recording the observation and average was calculated. For the number of roots per cutting, all the roots originating from the cuttings were counted from five randomly selected cuttings and average was worked out. The root length of all the roots produced per cutting was

measured; the sum of the length was then divided by the total number of roots to calculate average root length. The weight of five freshly harvested roots was determined and average fresh weight per rooted cutting was recorded. The freshly harvested roots of rooted cuttings were then dried in an oven at 60°C for 48 hours to a constant weight, and weight of dried roots per rooted cutting was taken as the dry weight of root.

We also assessed the performance of the plants raised through two propagation techniques viz., seed propagation and vegetative propagation through stem cuttings in different lisianthus genotypes. The seedlings and rooted cuttings were planted in the black polythene bags (10 cm) during the month of April. After transplanting the plants were shifted under the low cost polytunnels. The plants were weekly sprayed with liquid fertilizers (N: P: K = 19:19:19 and 13:0:45). The average maximum temperature ranged from 20-35ºC and the minimum temperature ranged from 10-15 ºC during the active plant growth and flowering periods. Five seedlings/rooted cutting were planted per replication and observations on various flowering parameters were recorded.

The experiment was laid out in Completely Randomized Design, with three replications. Effects of six growth regulators on rooting of seven lisianthus genotypes was studied. All the experimental data was analyzed using analysis of variance (ANOVA) with SPSS-16 program for Windows. Significant differences between means were assessed by Least significant difference (LSD) at P = 0.05 for CRD. Duncan's multiple ranges test was also employed to find significant differences among means of traits

RESULTS AND DISCUSSION

Application of auxins significantly improved the rooting efficacy of cuttings in all the lisianthus genotypes over the Control (Table 1). The treatments of lisianthus cuttings with IBA 250 ppm resulted in highest rooting (79.6%) followed by NAA 250ppm (78.5%). Very poor rooting (14.8%) was observed in control devoid of any rooting hormones. Among the various lisianthus genotypes, the maximum rooting was exhibited by Echo Double Pink Picotee (76.5%). The rooting percent in the genotypes Echo Double Pink Picotee, Echo Double Blue and Echo Double White was more than 70 percent indicating the scope for vegetative propagation of these genotypes. However, poor rooting was observed in genotypes Echo Double Champagne (46.3%) and Echo Double Lavender (56.9%). The interaction between genotypes and rooting hormone was significant as revealed by the F value (data not shown). The highest percent rooting was observed in Echo Double Pink Picotee when treated with 250 ppm NAA (98.9%), followed by Echo Double White with 500 ppm NAA (96.1%). Adventitious rooting is a multifactorial response that leads to new roots at the base of stem cuttings, and the establishment of a complete and autonomous plant. The rooting process comprises of two main phases i.e. root induction and root formation and the auxins requirement is higher during the induction phase, whereas, they are reported to play inhibitory role during the root formation phase (da Costa *et al*., 3). Auxin is one of the major endogenous hormones known for its role to induce the process of adventitious rooting. The physiological stages of rooting are correlated with changes in endogenous auxin concentrations. High endogenous auxin concentration is normally associated with a high rooting rate at the beginning of the rooting process (Pop *et al*., 9).

The increase in percentage of rooting in the hormone treated cuttings may be due to the fact that rooting hormones helps in mobilization of reserve food materials, elongation of meristematic cells and differentiation of cambial initials into root primordial.

Table 1: Effect of growth regulators on percent rooting in different lisianthus genotypes.

Treatments/ Variety	Percent Rooting (%)							
	EDC	EDY	EDW	EDL	EDPP	EDB	EDP	Mean
T1: (Control: (Distilled Water)	13.9±6.8	21.1±7.8	17.4±6.4	9.4±2.5	10.7±5.6	12.9±5.5	17.9±7.5	14.8±7.0d
T2: (IBA: 250ppm)	79.2±11.5	65.6±13.6	82.8±5.9	75.9±7.6	86.9±5.1	83.3±3.7	83.3±11.6	79.6±10.7a
T3: (IBA:500ppm)	92.4±6.8	34.4±7.8	71.6±8.7	58.2±11.7	85.1±5.2	75.6±7.6	63.2±6.7	68.7±19.4c
T4: (NAA:250ppm)	69.7±17.9	56.7±5.6	65.5±19.9	79.0±5.3	98.9±2.7	92.5±2.4	87.4±8.2	78.5±17.6a
T5: (NAA:500ppm)	85.6±10.7	47.8±6.5	96.1±4.3	62.4±9.7	85.0±5.1	81.4±8.9	72.2±7.8	75.8±16.9ab
T6: (IBA:250ppm +NAA:250ppm)	51.4±9.7	52.2±7.7	92.4±6.8	56.6±10.9	92.5±2.4	75.9±8.3	83.4±8.1	72.1±18.8bc
Mean	65.3±28.7c	46.3±16.9e	70.9±28.1b	56.9±24.5d	76.5±30.6a	70.3±27.3b	67.9±25.4bc	

Our results on role of auxins in induction of rooting in lisianthus corroborates with the earlier findings of Grewal *et al.* (4) in chrysanthemum. The efficacy of auxins in inducing early and profuse rooting has been earlier reported by Kumar *et al.* (5) in carnation. The genotypic difference for root induction may be due to the fact that the physiological and biochemical quality of the mother plants, in addition to their genetic make-up, also determine the rooting of cuttings (Osterc, 7). The endogenous auxin, carbohydrate content, mineral nutrients, and other bio- chemical components, such as phenolics that act as rooting co-factors or auxin transport modulators also influence root induction of cuttings. The varietal response to exogenous application of growth regulators depends on the capacity of the individual genotypes to readily transport them to the sites of utilization where they initiate the formation of adventitious roots (da Costa *et al.*, 3).The genotypic variations in root induction with the varied doses of growth regulators have earlier been reported by Prince *et al.* (11) in carnation.

Treatment of lisianthus cuttings with different doses of the growth regulators significant improved the number of roots per cutting in all the genotypes (Table 2; Fig. 1). The maximum number of roots (10.6) per cutting was recorded with IBA (250 ppm), however, very few roots were induced in control (distilled water). The number of roots induced per cutting was significantly more in IBA treatment as compared with the NAA. Among the different lisianthus genotypes tested, Echo Double Blue exhibited the maximum number of roots per cutting (11.6) followed by Echo Double Yellow (11.0) and Echo Double Pink Picotee (10.2). The events leading to adventitious rooting strongly depend on the mother plant nutritional status, both in terms of minerals and carbohydrates, as well as on sink establishment at cutting bases (da Costa *et al.*, 3). The auxins have been reported to enhance rooting through the translocation of carbohydrates and other nutrients to the rooting zone. The increased number of roots may be due to increase in the internal free NAA that enhanced the root formation and may also be due to translocation of carbohydrates from the leaves which play an important role in development of roots. Also, the enhanced hydrolysis activity in the presence of exogenously applied growth hormones would be the main reason for the increased rooting in auxin treated cuttings. The more number of roots obtained

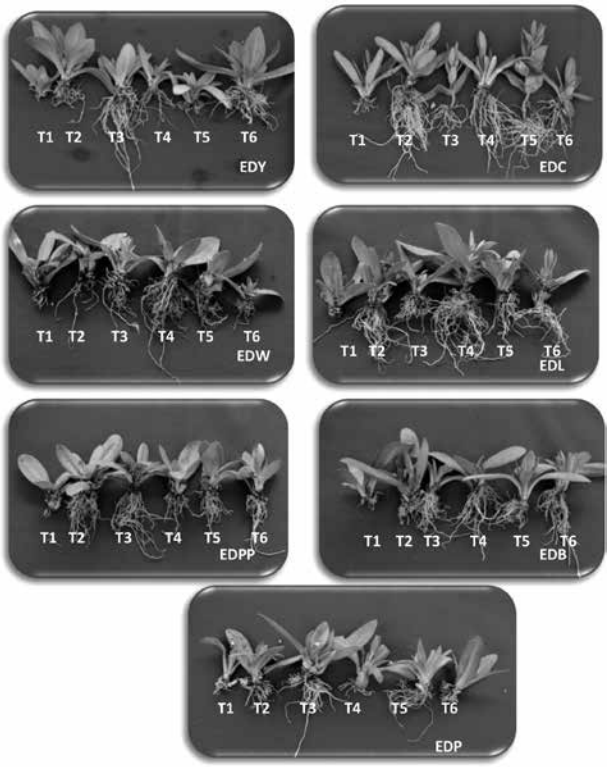

Fig. 1. Effect of growth regulators on rooting of different lisianthus genotyps.

Table 2: Effect of growth regulators on number of roots per cutting in different lisianthus genotypes.

Treatments/ Variety	Number of roots per cutting							
	EDY	EDC	EDW	EDL	EDPP	EDB	EDP	Mean
T1:(Control: (Distilled Water)	2.5±0.5	2.0±0.6	2.2±0.4	2.7±0.5	3.5±0.8	2.3±0.8	2.5±0.5	2.5±0.7c
T2: (IBA: 250ppm)	11.0±1.4	12.0±2.0	8.2±2.6	6.8±2.9	15.0±2.7	13.8±1.9	9.0±2.1	11.3±3.7a
T3: (IBA:500ppm)	13.8±2.2	9.5±2.8	9.0±1.4	6.3±1.9	12.3±2.2	15.8±1.2	8.5±2.2	10.6±3.5a
T4: (NAA:250ppm)	10.5±1.2	10.0±2.2	10.7±2.8	7.5±2.1	10.0±3.0	13.0±2.6	6.5±1.4	9.7±2.9 a
T5: (NAA:500ppm)	12.3±2.7	7.2±1.7	8.8±1.7	5.8±1.9	8.3±1.8	12.8±3.1	6.3±1.2	8.8±3.3b
T6: (IBA:250ppm +NAA:250ppm)	14.3±1.5	8.8±1.7	9.0±1.8	7.5±1.0	11.8±2.6	11.2±2.5	7.7±2.2	10.0±3.1a
Mean	11.0±3.3ab	8.3±3.7c	8.0±3.3c	6.1±2.4d	10.2±3.2ab	11.6±3.9a	6.8±2.7d	

with the application of growth chemicals clearly reflects that they not only initiate rooting but also help in subsequent rapid growth of roots in numerical strength. Further, the role of auxins in inducing more number of roots have been demonstrated by Prince *et al.* (11) in carnation and Bhatia *et al.* (2) in gerbera.

There was significant improvement in the root length in all the lisianthus genotypes with the treatment of cuttings with different doses of growth regulators (Table 3). Treatment of lisianthus cuttings with 250 ppm IBA induced the longest roots (7.7 cm). However, no significant differences for root length were observed among T_2 (IBA:250ppm), T_3 (IBA:500ppm), T_4 (NAA:250ppm) and T_6 (IBA+NAA:250ppm each). Among the different auxins, IBA treatment induced longer roots compared with the NAA treatment. The IBA induced roots were longer with well distributed root hairs whereas, NAA induced smaller and stumpy roots with less root hairs. No synergistic effect for root length was observed. The longest roots were recorded in Echo Double Blue (7.9cm), whereas, the smallest roots were produced in Echo Double Pink (5.0 cm). The genotypes x growth regulators interaction were found significant for root length. The maximum root length (11.5 cm) was observed in Echo Double Blue when treated with IBA 250 ppm. Earlier studies by Bhatia *et al.* (2) have also revealed the efficacy of IBA over NAA in inducing good quality roots in *in vitro* propagated gerbera. The root length varied significantly among the different genotypes. Similar results have been obtained in carnation by Kumar *et al.* (5).

Data presented in Table 4&5 revealed that fresh and dry weight of the roots varied significantly among the various auxin treatments and genotypes. The highest fresh and dry weight of the roots per cutting was recorded with 250 ppm IBA (2.257g; 0.670g). The lowest fresh and dry weight was recorded in the control (0.350g; 0.072g). When two auxin sources were compared, IBA induced roots exhibited more fresh weight than the roots induced by NAA. Among the different lisianthus genotypes the maximum fresh weight was recorded in Echo Double Pink Picotee (2.113g; 0.660g) followed by Echo Double Blue (2.09g; 0.644), respectively. The significantly higher fresh and dry root weight may be due to the enhanced number of roots as well as longer roots in auxin treated cuttings. Earlier, Kumar *et al.* (5) also

Table 3: Effect of growth regulators on root length in different lisianthus genotypes.

Treatments/ Variety	Root Length (cm)							
	EDY	EDC	EDW	EDL	EDPP	EDB	EDP	Mean
T1:(Control: (Distilled Water)	3.1±0.8	2.4±0.6	3.0±0.9	2.7±0.5	1.9±0.6	2.7±0.6	2.5±0.8	2.6± 0.7d
T2: (IBA: 250ppm)	8.5±1.1	7.8±2.8	7.5±1.2	5.1±0.6	7.9±1.0	11.5±0.8	5.3±0.7	7.7± 2.4a
T3: (IBA:500ppm)	10.5±0.7	7.2±0.9	6.2±0.9	5.3±1.0	5.7±0.9	8.3±1.0	5.8±1.1	7.0±1.9 b
T4: (NAA:250ppm)	6.5±2.4	6.4±0.8	6.3±0.8	6.7±1.3	5.3±0.4	8.0±1.1	4.5±0.6	6.2± 1.5c
T5: (NAA:500ppm)	6.5±1.0	6.9±0.6	5.5±0.9	5.2±0.6	4.9±0.7	7.6±0.6	4.2±0.7	5.8± 1.3c
T6: (IBA:250ppm +NAA:250ppm)	8.9±1.5	6.0±0.5	6.8±1.9	5.5±0.8	7.2±0.6	9.5±0.7	7.5±1.1	7.3± 1.7ab
Mean	7.3±2.7b	6.1±2.2c	5.9±1.8c	5.1±1.4d	5.5±2.1cd	7.9±2.8a	5.0±1.7d	

Table 4: Effect of growth regulators on fresh weight of the roots in different lisianthus genotypes.

Treatments/ Variety	Root fresh weight (g)							
	EDY	EDC	EDW	EDL	EDPP	EDB	EDP	Mean
T1:(Control: (Distilled Water)	0.331	0.349	0.391	0.312	0.393	0.339	0.339	0.350±0.07e
T2: (IBA: 250ppm)	2.448	2.802	1.846	1.112	2.953	2.887	1.754	2.257±0.66a
T3: (IBA:500ppm)	2.970	1.966	1.722	1.068	2.636	3.037	1.524	2.132±0.72b
T4: (NAA:250ppm)	1.994	1.808	1.670	1.800	2.264	1.704	0.746	1.712±0.45c
T5: (NAA:500ppm)	1.846	1.321	1.018	0.701	1.553	2.000	0.774	1.316±0.49d
T6: (IBA:250ppm +NAA:250ppm)	2.860	2.350	1.839	1.362	2.880	2.629	1.819	2.248±0.56a
Mean	2.075± 0.89a	1.766± 0.80bc	1.414± 0.55c	1.059± 0.49e	2.113± 0.92a	2.099± 0.93a	1.159± 0.58d	

Table 5: Effect of growth regulators on dry weight of the roots in different lisianthus genotypes.

Treatments/ Variety	Root dry weight (g)							
	EDY	EDC	EDW	EDL	EDPP	EDB	EDP	Mean
T1:(Control: (Distilled Water)	0.065	0.070	0.064	0.058	0.100	0.069	0.081	0.072±0.027d
T2: (IBA: 250ppm)	0.709	0.799	0.565	0.300	0.924	0.865	0.526	0.670±0.211a
T3: (IBA:500ppm)	0.880	0.676	0.496	0.264	0.808	0.949	0.476	0.650±0.238a
T4: (NAA:250ppm)	0.604	0.603	0.520	0.491	0.740	0.488	0.281	0.532±0.143b
T5: (NAA:500ppm)	0.593	0.401	0.262	0.192	0.542	0.689	0.256	0.419±0.186c
T6: (IBA:250ppm +NAA:250ppm)	0.814	0.715	0.546	0.380	0.848	0.805	0.551	0.666±0.170a
Mean	0.611± 0.272b	0.544± 0.253c	0.409± 0.190d	0.281± 0.144f	0.660± 0.286a	0.644± 0.302a	0.362± 0.178e	

reported that higher number of roots, in addition to longer roots may have resulted in higher fresh and dry weight in carnation cuttings. The application of growth regulators significantly improved the root growth, and fresh and dry weight per rooted cutting in carnation (Panahi and Morteza, 8).

Using the stem cutting method, the rooted cuttings were ready for transplanting within two months, whereas, the time gap of 3-4 months was observed from seed sowing to transplanting. When the performance of different lisianthus genotypes raised through seeds and rooted cuttings was assessed, it was observed that the plants raised through stem cuttings showed better crop stand after transplanting. The seedlings showed high mortality on transplanting as compared to the rooted cuttings. Further, the genotypes raised through stem cuttings exhibited better vegetative growth, uniform and significantly early flowering than the seedlings. The stem cuttings raised crops also exhibited early flowering, better stem length, and number of flowers per stem. This may be due to the reason that the rooted cuttings had more number of adventitious roots with well-developed and dense root system, whereas, the seedlings had few elongated roots and weak root system, hence showed poor initial establishment and poor growth. It is generally assumed that dense root system has a greater absorbing power than an elongated one. This further signifies increased root growth rate and dry matter accumulation due to enhanced photosynthetic efficiency on treatment of cuttings with rooting hormones. Root architecture and morphology provide a lot of useful information about plant species and their ability to take up nutrients from the soil. These results are contradictory to earlier findings by Roh and Lawson (13), where they reported delayed flowering, poor vegetative growth and fewer flowers in rooted cuttings as compared to the seedling. These differences may be due to the genotypic differences.

This is the first report on successful vegetative propagation of different lisianthus genotypes using stem cuttings. Application of auxins significantly improved the rooting in lisianthus. The genotypes Echo Double Pink Picotee, Echo Double Blue and Echo Double White exhibited more than 70 percent rooting, indicating the scope for vegetative propagation of these genotypes. The treatment of the cuttings with 250 ppm IBA for five minutes was found suitable for induction of quality roots in lisianthus. The rooted cuttings exhibited better crop stand and uniform flowering after transplanting and were found superior for various vegetative and flowering traits than the seedling raised crop. This study has proved that the stem cuttings can be used as a viable alternative for multiplication of lisianthus crop.

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