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In vivo bulblet multiplication in LA lilium hybrids through scaling technique

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ABSTRACT

Lilium is a high value flower but difficult to multiply through conventional propagation methods. Therefore, an attempt has been made with an objective to multiply maximum bulblets through scaling under different storage durations and temperature conditions. The experiment was conducted in a completely randomized design (factorial) consisting of different storage durations (0, 3, 6, 9 and 12 weeks), type of scales (outer and inner) and temperature regime (4° and 2°C) on three lilium cultivars, namely/ Brindisi, Ercolana and Pavia. The results showed that bulblet production decreased from outer to inner scales and showed a positive correlation with the scale width. Nine week storage resulted in early bud sprouting and root initiation due to breaking of dormancy. However, size of bulblets were recorded more in the scales planted just after harvesting. Total weight of bulblets per scale was directly related to the number of bulblets per scale produced. Maximum number of bulblets (3.28) per scale was produced in the cv. Pavia followed by Ercolana (3.10) and Brindisi (2.18). Six weeks storage duration at 4°C was recorded best for cv. Brindisi. However, 9 week storage (4°C) was best for cvs Ercolana and Pavia with regard to maximum bulblet production. While, maximum rooting and root length were recorded in the cv. Brindisi followed by Ercolana and Pavia.

Key words: *In vivo* bulblet, lilium, multiplication, scaling, storage.

INTRODUCTION

Lilium is one of the leading cut flowers in the world market and amongst the most beautiful ornamental bulbous cut flowers. This high value crop is very popular pot plant as well due to different colours like white, pink, yellow *etc*. The genus *Lilium* belongs to the family Liliaceae and is native to Northern hemisphere in Asia, Europe and North America. In India, lilium is found growing naturally in Nilgiri Hills and Himalayan regions (Bose and Yadav, 2). Its bulbs are non-tunicated, consisting of fleshy scales joined at the basal plate. About 76% of the total world lily bulb production takes place in the Netherlands (Qu *et al.*, 13). In India suitable climatic conditions for lilium cultivation have been identified in different regions. Although, vernalized lilium bulbs could be made to flower anywhere in the country by providing optimum growing conditions, yet commercial cultivation of lilium is restricted to Jammu & Kashmir, Himachal Pradesh, Uttarakhand, Tamil Nadu (Ooty and adjoining region) and North Eastern states without much environmental manipulation.

Lilium bulbs exhibit a distinct period of dormancy, which can be broken by regulating the storage duration and temperature to enable root and shoot development (De-Hertogh and Le Nard, 4). It triggers the developmental processes leading to dormancy breaking (De-Hertogh *et al*., 3; Bewley, 1). Earlier Dhiman (5) and Park (12) have studied the effect of temperature and scale position and storage in Asiatic lily. Sharma *et al.* (15) have standardized propagation medium and growth regulators for scaling in Oriental lily. Cultivation of liliums under north Indian plain conditions is a new intervention. However, availability of quality planting material to a common grower at an affordable price has always remained the bottleneck. Multiplication through bulbs, bulblets and bulbils is slow, whereas; micropropagation need controlled environment conditions and trained technician. Hence, multiplication through scaling holds an effective way for commercial multiplication of bulblets of lilium hybrids at comparatively cheaper and faster rates, hence the present study were carried out.

MATERIALS AND METHODS

The present experiment was conducted at the Experimental Farm of the Division of Floriculture and Landscaping, ICAR-IARI, New Delhi, India during 2016-17. The experiment was laid out in a completely randomized design(factorial) (Gomez and Gomez, 7) consisting of different storage durations, *viz*., 0, 3, 6, 9 and 12 weeks and storage temperature regimes, *viz.* 4 and 2°C on three LA lily cultivars, namely, Brindisi, Ercolana and Pavia. The bulbs were lifted from the experimental field during last week of April, 2016 and after removal of the soil particles adhered on the surface were dipped into the fungicidal solution of carbendazim (0.2%) for 1 h before storage. Cold

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storage with automatically controlled parameters like temperature and humidity were used for the storage of bulbs at desirable temperatures (4 and 2°C) and durations (3, 6, 9 and 12 weeks) except for the control lots maintained at room temperature. The relative humidity of the storage chambers was adjusted at 70-80% throughout the storage period. The bulbs were stored according to the storage durations and taken out at subsequent intervals for planting of the outer and inner scales under *in vivo* conditions in moist coco-peat as growing medium.

For *in viv*o propagation through scales, bulbs were taken from cold storage and individual bulb scales were excised from the mother bulb with hands in such a way so that a small portion of basal plate remained attached to ensure bulblet formation and rooting. Diseased, rotten, broken and ones without basal plate scales were discarded. The detached scales were then disinfected by dipping them in carbendazim (0.2%) for one hour and maintained in the shade. In the present experiment, coco-peat was used as the propagation medium for scaling. Commercial coco peat bricks were soaked overnight in tap water to make it sufficiently moist for scaling or bulblet induction on the scales. The medium was treated with a fungicidal solution (0.2% carbendazim) before use and then filled into plastic seed trays. The prepared scales were then planted in such a way so that a thin layer of 1 cm coco-peat remains above the scales. Both outer and inner scales were used for bulblet multiplication. Fifteen scales were used in each treatment with five scales per replication. Routine cultural operations like weeding, loosening of potting medium and watering *etc*. were done as per the requirement. Observations on important parameters were recorded and analyzed.

RESULTS AND DISCUSSION

Multiplication of bulblets through scales under *in vivo* conditions was found to be significantly influenced by storage duration, storage temperature and types of scale. Sprouting percentage of outer and inner scales was recorded 100% irrespective of storage duration and temperature in all the three cultivars. However, days to bulblet initiation varied significantly under different storage periods (Table 1). Scales of cv. Brindisi sprouted earlier than Ercolana and Pavia. Irrespective of cultivars, the earliest bud sprouting was recorded in 12 week storage period, which was statistically at par with 9 weeks storage period followed by 6 week storage period. Of the two scale type studied, outer scales sprouted earlier than inner scales and storage temperature of 4°C had marked affect than 2°C, which was statistically significant in all the three cultivars. Storing bulbs at cold temperature is known to trigger the increase of the growth promoters and or the decrease of inhibitors, which result in rapid bulblet induction. The time required for bulb sprouting decreased with enhancement in storage duration was earlier reported by Dhiman (5), Malik *et al*. (10) and Lee *et al*. (9).

Bulblet multiplication is a major consideration in the commercial floriculture. Maximum numbers of bulblets were produced in cv. Brindisi when stored for 6 week duration (Table 1). However, cvs Ercolana and Pavia produced the maximum bulblets when stored for 9 weeks. Irrespective of cultivars, outer scales produced more bulblets as compared to inner scales and 4°C storage temperature was found to be effective as compared to 2°C. Among all the three cultivars, maximum numbers of bulblets were produced in Pavia followed by Ercolana and Bridisi (Fig. 1). Interaction effect of the storage duration and type of scale revealed that 6 week storage duration in cv. Brindisi outer scales resulted in the highest number of bulblets per scale. However, 9 week storage period was sufficient for the highest bulblet induction from outer scales of cvs Ercolana and Pavia. Average number of bulblets per scale was more in the outer scales than inner scales, which showed that production of bulblet is directly related to the scale width. Among all the cultivar studied, cv. Pavia resulted in the highest number of bulblets per scale followed by Ercolana and Brindisi. The genotypic differences along with environmental conditions could be accounted for this variation (Table 1a). Interaction data of the storage duration and storage temperature showed that 4°C storage temperature had marked effect than 2°C on bulblet multiplication. In case of Brindisi, 4°C storage temperature combined with 6 week storage period had marked effect on bulbet multiplication. But in case of cvs Ercolana and Pavia, 9 week storage duration with 4°C storage temperature resulted in highest No. of bulblets per scale. Cultivar Pavia produced highest number of bulblets per scale followed by cvs Ercolana and Brindisi (Table 1b). The physical environment exerts a marked influence on dormancy, which is usually broken by a period of

Table 1a. Interaction of storage period × scale type on number of bulblets production per scale in LA lilium hybrids.

Storage period (wk)	Brindisi		Ercolana		Pavia	
	Outer scale	Inner scale	Outer scale	Inner scale	Outer scale	Inner scale
0	1.80	1.73	2.73	2.00	2.20	2.20
3	2.13	2.00	2.23	2.33	2.30	2.37
6	2.33	2.03	2.27	2.20	2.97	2.17
9	2.07	2.00	3.23	2.97	3.43	3.13
12	2.30	2.07	3.07	2.70	3.27	3.07
CD at 5%	NS		0.33		0.23	

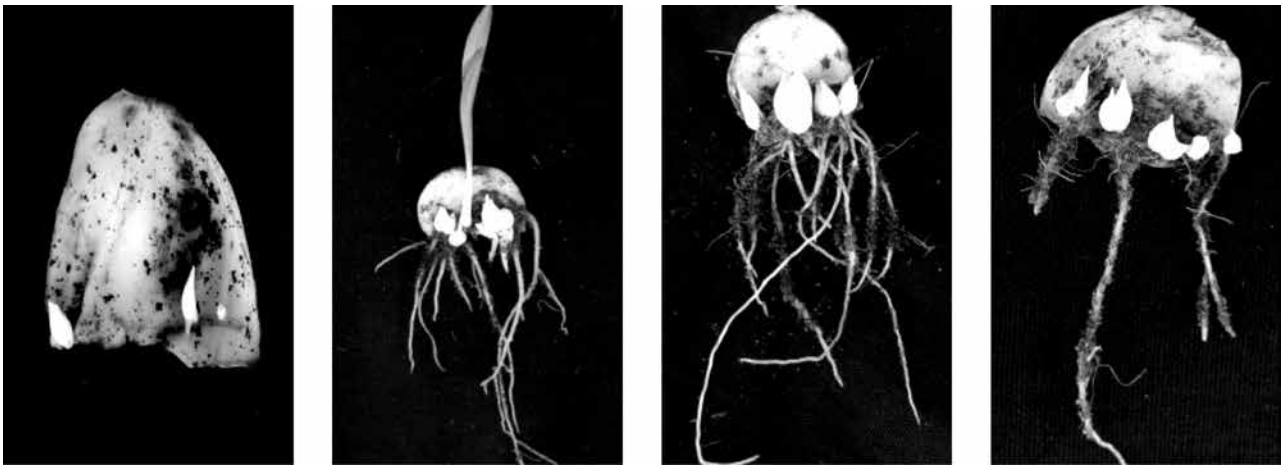

Fig. 1. Stages of vegetative propagation in LA lilium hybrids through scaling technique. (a) Bulblet induction from scales, (b) Bulblets produced from scales in cv. Brindisi, (c) Bulblets produced from scales in cv. Ercolana, (d) Bulblets produced from scales in cv. Pavia.

cold treatment. Not only storage at low temperature but also long storage durations at low temperatures lead to breaking of dormancy, thus resulting in early sprouting and maximum bulblet production. Abscisic acid concentration in the scales of Lilium bulbs decreased as storage duration extended, and it declined to a constant low level after bulbs had been stored for 6-9 weeks at 4°C. This result indicates that the decrease in the endogenous ABA concentration during bulb storage is related to dormancy release in lilium bulbs (Rong-Yan, 14). These findings have also been supported earlier findings of Moshrefi *et al*. (11) and Singh (16) in Asiatic lilies. Scale segments obtained from the outer scales tended to induction of higher number of bulblets, which can be correlated with the total carbohydrates content in the scales (Park, 12).

Significantly higher bulblet size was observed in the 6 week storage duration followed by control (scale planted just after harvest) and 3 week storage duration in the cv. Brindisi. However, in case of cvs Ercolana and Pavia, the maximum bulblet size was attained in the scales planted just after harvesting. This may be due to the fact that scales planted immediately after harvest remained in the coco-peat for a longer duration as compared to other treatments. Thus, one can recommend to multiply bulblets from the scales just after harvesting of bulbs if have enough scales because it will take shorter period to achieve commercial size of bulbs. As the number of bulblet per scale increased, individual size of bulblets is decreased. Hence, it will take comparatively longer period to get commercial size of bulbs. When compared to the types of scale, outer scales attained bigger bulblet size as compared to the inner scales in all the three cultivars studied. Similarly, 4°C storage temperature stored bulb's scales attained bigger bulblet size among all the three cultivar. Interaction of storage duration and types of scales influenced bulblet size in cv. Brindisi. However, it was statistically non-significant in cvs Ercolana and Pavia (Table

Table 1b. Interaction of storage period × storage temperature on number of bulblets per scale in LA lilium hybrids.

Storage period (wk)	Brindisi		Ercolana		Pavia	
	4°C	2°C	4°C	2°C	4°C	2°C
0	1.77	1.77	2.37	2.37	2.20	2.20
3	2.20	1.93	2.47	2.10	2.30	2.37
6	2.27	2.10	2.30	2.17	2.70	2.43
9	2.07	2.00	3.13	3.07	3.33	3.23
12	2.20	2.17	3.07	2.70	3.20	3.13
CD at 5%	NS		NS		NS	

Table 1c. Interaction of storage period × scale type on the size of bulblets (mm) in LA lilium hybrids.

Storage period (wk)	Brindisi		Ercolana		Pavia	
	Outer scale	Inner scale	Outer scale	Inner scale	Outer scale	Inner scale
0	11.43	11.54	10.85	10.67	10.77	10.43
3	11.38	10.78	10.31	10.47	9.94	9.81
6	11.97	11.49	10.27	10.31	10.19	9.99
9	10.22	10.39	9.65	9.67	9.85	9.85
12	10.06	9.88	10.41	10.31	8.73	8.78
CD at 5%	0.40		NS		NS	

1c). Interaction data of storage duration and storage temperature showed that 4°C storage temperature had significant effect on the size of bulblet in cvs Brindisi and Pavia (Table 1d). This may be due the fact that low temperature below 4°C may suspend metabolic activity in the bulbs, so slow rate of size enlargement. In case of scales planted just after harvesting, no cold treatment was given, hence once the scales sprouted, it attained the maximum growth. Longer duration in the cocopeat media is also the contributing factor affecting size enlargement. Among all the three cultivars, maximum bulblet size was observed in the cv. Brindisi followed by cvs Ercolana and Pavia. A significant difference in the bulblet size was also reported by Kapoor *et al*. (8) but on different growing media.

Table 1a revealed that there was a significant effect of the storage duration on weight of bulblets per scale produced in all the three cultivars. Maximum weight of bulblets was recorded in cv. Pavia followed by cvs Ercolana and Brindisi. Total weight of bulblets per scale was directly related to the number of bulblets per scale produced. Cultivar Brindisi produced maximum weight of bulblets at storage duration of 6 weeks, whereas, cvs Ercolana and Pavia produced the maximum weight at 9 week storage duration. Dhiman and Sindhu (6) and Sharma *et al.* (15) also worked on lilium and found significant result. Weight of the outer scales was more than the inner scales and 4°C storage was found to be significant among all the cultivars.

Table 1d. Interaction of storage period × storage temperature on the size of bulblets (mm) in LA lilium hybrids.

Storage period (wk)	Brindisi		Ercolana		Pavia	
	4°C	2°C	4°C	2°C	4°C	2°C
0	11.49	11.49	10.76	10.76	10.60	10.60
3	11.48	10.68	10.56	10.22	10.09	9.65
6	11.70	11.76	10.22	10.36	10.07	10.11
9	10.56	10.05	9.85	9.48	10.02	9.68
12	9.99	9.94	10.41	10.31	9.19	8.31
CD at 5%	0.40		NS		NS	

When root system was compared in the three cultivars, statistically significant variation was observed in the days to root initiation under different storage periods (Table 2). Earliest root initiation was observed in the 12 week storage duration and was statistically at par with the 9 week storage period in all the cultivars. When compared with the types of scales, outer scales of cvs Brindisi and Pavia were earlier to root than inner scales. 4°C storage temperature had pronounced effect on early root initiation in cvs Ercolana and Pavia whereas, 2°C stored scales rooted early in cv. Brindisi. Number of primary roots (Roots arising directly from the bulblets) was recorded maximum under 9 week storage duration and outer scales produced more number of primary roots than inner scales in all the 3 cultivars

Table 1e. Effect of different storage duration, scale type and temperature on bulblet attributes in LA lilium hybrids.

Treatment	Days to bulblet initiation			No. of bulblets			Size of bulblets (mm)			Weight of bulblets per scale (g)		
	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia
Storage duration (week)												
0	36.57	36.00	45.30	1.77	2.37	2.20	11.49	10.76	10.60	1.93	2.57	2.40
3	21.00	25.53	26.72	2.07	2.28	2.33	11.08	10.39	9.87	2.25	2.48	2.53
6	19.03	20.33	22.18	2.18	2.23	2.57	11.73	10.29	10.09	2.38	2.47	2.60
9	18.05	19.42	21.18	2.03	3.10	3.28	10.30	9.66	9.85	2.25	3.30	3.47
12	17.42	18.93	20.57	2.18	2.88	3.17	9.97	10.36	8.75	2.38	3.08	3.37
CD at 5%	0.73	1.30	1.09	0.18	0.24	0.17	0.28	0.43	0.28	0.19	0.23	0.27
Scale type												
Outer	21.61	24.31	26.33	2.13	2.71	2.83	11.01	10.30	9.90	2.31	2.91	3.02
Inner	23.22	23.78	28.05	1.97	2.44	2.59	10.81	10.29	9.77	2.17	2.65	2.73
CD at 5%	0.46	NS	0.69	0.11	0.15	0.10	0.18	NS	NS	0.12	0.15	0.17
Storage temp. (°C)												
4	22.85	22.97	26.19	2.10	2.67	2.75	11.04	10.36	10.00	2.28	2.87	2.95
2	21.98	25.11	28.19	1.99	2.48	2.67	10.78	10.22	9.67	2.20	2.69	2.79
CD at 5%	0.46	0.82	0.69	NS	0.15	NS	0.18	NS	0.18	NS	0.15	NS

Table 2. Effect of different storage duration, scale and temperature on rooting parameters in LA lilium hybrids.

Treatment	Days of to root initiation			No. of primary roots			Length of primary roots (cm)			Dia. of primary roots (mm)			No. of secondary roots		
	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia	Brindisi	Ercolana	Pavia
Storage duration (week)															
0	38.57	38.00	47.30	6.03	6.13	4.60	11.70	11.29	9.08	1.17	1.02	0.98	4.59	4.99	2.94
3	23.02	27.57	28.70	6.22	6.02	3.46	13.55	9.80	5.89	1.27	1.04	1.04	7.01	3.84	3.04
6	21.23	22.38	24.22	6.92	5.68	5.25	18.69	8.84	11.93	1.31	1.00	1.13	12.98	6.12	5.05
9	20.10	21.40	23.22	7.60	7.59	6.69	12.12	13.19	13.10	1.11	1.18	1.17	10.41	9.87	10.15
12	19.53	20.77	22.58	5.37	6.95	5.01	13.76	16.29	11.02	1.18	1.72	1.26	4.17	6.42	6.51
CD at 5%	0.71	1.28	1.07	0.89	1.00	0.88	2.72	1.78	1.73	0.12	0.16	0.13	1.91	1.59	0.99
Scale type															
Outer	23.67	26.24	28.41	6.40	6.91	5.00	12.82	12.48	10.16	1.23	1.23	1.14	8.03	6.88	5.86
Inner	25.31	25.81	30.00	6.46	6.04	5.00	15.11	11.28	10.25	1.19	1.15	1.09	7.64	5.62	5.22
CD at 5%	0.45	NS	0.68	NS	0.63	NS	1.72	1.13	NS	NS	NS	NS	NS	1.00	0.62
Temperature (°C)															
4	24.87	24.96	28.22	6.82	6.39	5.09	14.55	12.14	8.89	1.28	1.21	1.14	6.86	6.07	5.00
2	24.11	27.09	30.19	6.04	6.56	4.91	13.37	11.62	11.52	1.14	1.17	1.09	1.21	6.43	6.07
CD at 5%	0.45	0.81	0.68	0.57	NS	NS	NS	NS	1.09	0.08	NS	NS	1.21	NS	0.62

(Table 2). Among the cultivars, Brindisi produced the maximum number of roots followed by Ercolana and Pavia. Along with the number of roots, root length is also an important parameter because it can make uptake nutrient and water from the deeper region of growing medium. Maximum length of primary roots as observed under 6 week storage duration followed by 12 and 3 weeks in cv. Brindisi. The non-significant differences in the length of roots were observed in under different storage temperature in cvs Brindisi and Ercolana, however, differences were significant in cv. Pavia. More the branching of primary roots or number of secondary roots, higher is the rate of field survival, which was found to be maximum in cv. Brindisi among all the cultivars. Highest numbers of secondary roots were observed under 6 week storage duration followed by 9 week in cv. Brindisi, whereas, highest number of secondary roots was observed in cvs Ercolana and Pavia under 9 week storage. Higher level of endogenous auxin in the scales may be responsible for easy rooting and more branching.

Results of these investigations revealed that outer scales were more effective than inner scales for bulblet multiplication through scaling. Six week storage duration at 4°C was found to be best for bulblet multiplication in cv. Brindisi however, 9 week storage at 4°C was best for cvs Ercolana and Pavia.

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