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Effect of plant growth substances and bio-enhancer on the regeneration of bulblet from scale of *Lilium* under *in vivo* condition

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Abstract
Liliums are widely praised for their use as cut flowers and pot plants. *Lilium* (*Lilium* spp) belongs to the family Liliaceae. In general, lilies reproduce sexually through seeds and asexually through the development of daughter bulbs and axillary bulblets. Daughter bulbs develop in the axils of leaves and axillary bulblets that develop underground or above ground, and scales (Hartmann *et al.,* 1997) [5]. Scale propagation is widely used for Lilium multiplication in various conventional methods. Scaling is a quick and cost-effective way of producing bulbs from vegetative growth (Tang *et al*., 2020) [15]. From the point of view experiment was carried out in the Department of Horticulture, Sikkim University to examine the effect of growth substances and bio enhancers on the regeneration of microbulblet from the scale. The experiment was laid out in Completely Randomized Design (CRD) and replicated thrice. For the regeneration of microbulblet from scales, the scales were treated with Kinetin (growth substances) and bio enhancers namely Coconut water, Vermiwash and Cow urine. The performance of bio enhancers was found better in comparison to kinetin and control. Non-significant variations was recorded in days taken for sprouting, production of microbulblet/scale and size of microbulblet. Among these different treatments, the minimum days taken for sprouting was recorded when treated with cow urine @ 10% but production of more number of microbulblet/scale was observed when treated with coconut water @ 50%. The maximum weight of microbulblet was obtained when treated with vermiwash @ 50%. However, the maximum length and breadth of microbulblet were recorded when kinetin was used @ 75 ppm. Number of roots and shoots per microbulblet and shoot length showed non-significant variation among all treatments. Maximum length of the root was recorded when treated with cow urine and maximum number of scales/microbulblet was produced with Kinetin @ 50 ppm.
Keeping in view the results obtained in the study for the regeneration of microbulblet from scale and development of bulbs from scale, bio enhancers like coconut water, vermiwash and cow urine can be used as substitutes inspite of chemical growth substances in organic farming and can be recommended to the farmers for quality production of quality planting materials of lilium in the Eastern Himalayan Region of India.

Keywords: *Lilium* spp. scales, growth substances, bio-enhancers, microbulblet

Introduction
Lilium (*Lilium* spp) is one of many flowers planted for commercial purposes, and in addition to being a significant garden plant, it also holds a key position in the cut flower trade. According to Panda and Mohanty (2016) [11], compared to any other cut flower or plantation crop in India, these flowers are now the country's biggest source of income. Due to their abundance and variety of colours, lilies are one of the world's most widely sold cut flowers (Bala *et al*., 2019) [2]. Liliums are widely praised for their use as a cut flower and pot plant. Lilium belongs to the family Liliaceae
In general, lilies reproduce sexually through seeds and asexually through the development of daughter bulbs and axillary bulblets. Daughter bulbs develop in the axils of leaves and axillary bulblets that develop underground or above ground, and scales (Hartmann *et al.*, 1997) [5]. Scale propagation is widely used for Lilium multiplication in various conventional methods. Scaling is a quick and cost-effective way of producing bulbs from vegetative growth (Tang *et al*., 2020) [15]. However, according to Park (1996) [13], commercial-size bulbs are obtained only after 3-4 years. Scale propagation is a quick method of multiplication, especially in cultivars that don't generate stem bulbils (Bose and Yadav, 1998) [3].
One of the critical challenges in the growth of horticultural crops, particularly in the floriculture industry, is the need for high-quality planting materials. Additionally, imported bulbs, which are quite expensive, make up the majority of the bulbs utilised in Lilium's

commercial manufacturing. Currently, India imports Lilium bulbs from other nations to use in the manufacturing of flowers. The Netherlands is the top exporter and supplier, according to Bala *et al*. (2019) [2]. For commercial production, there has been considerable interest in Lilium bulb production *in vivo* (Panda & Mohanty, 2016) [11]. Various studies have been conducted on different aspects of scaling. However, adequate information about growth substances, bio-enhancers, and *in vivo* conditions is still being determined. Therefore, to reduce the bulb cost and to meet the increasing demand for flowers throughout the year by providing sufficient planting material, there is an urgent need to develop cheaper multiplication techniques indigenously to increase bulblet production.

In light of those mentioned above, this study was carried out with the objective to examine the impact of plant growth substances and bio-enhancers on the regeneration of micro-bulbs from scales.

Materials and Methods

From a farmer's field, suitable, healthy Lilium bulbs were gathered. The scales were taken off, washed in tap water, treated with fungicide, and dried for 24 hours under shade conditions. To regenerate bulblets from scales, an experiment was carried out under room conditions at the Post Graduate Laboratory of the Department of Horticulture at Sikkim University. After being dried, the scales (outer and middle) were put in disposable plastic cups which contained coco peat and perlite in an equal proportion used as growing media. Irrigations have been followed whenever necessary. Outer and middle scales were chosen because they have more carbohydrates, which Panda and Mohanty reported (2016) [11]. Each plastic cup contained three scales. The experiment was laid out in a Completely Randomized Block Design (CRD) with ten treatments and replicated thrice. The treatment details are as follows: T_1- Coconut water @ 100%, T_2-Coconut water @ 50%, T_3-Vermiwash @ 100%, T_4-Vermiwash @ 50%, T_5-Cow urine @ 10%, T_6-Kinetin @ 100 ppm, T_7-Kinetin @ 75 ppm, T_8-Kinetin @ 50 ppm, T_9-Kinetin @ 25 ppm and T_{10}-Control. In the treatments mentioned above, the scales were dipped for 30 minutes before planting in the media, and a second foliar spray was applied after one month. In this experiment, parameters like the number of micro-bubbles per scale, days taken for the development of the micro-bulblet, fresh weight of micro-bulblet, size of micro-bulblet, root and shoot development and the number of micro-bulblet were recorded.

Artemisia sp. spray was used as per requirement for protection from fungal infection (Hrytsyk *et al*., 2021) [6]. The data were analysed statistically using a completely randomized design (CRD). The critical difference at 5% level of significance for each character was worked out to compare the significance among the treatment mean.

Result and discussion

According to the data in Table. 1, not all scales generated bulblet. Almost in all the treatments, few scales failed to sprout. According to Niimi (1985) [10], temperature and light availability also affect sprouting. The ideal temperature is between 20 and 25 °C, and more light should be available. But the current experiment was conducted throughout winter at temperatures between 10 and 18 °C, therefore the scales may have failed to sprout. The data also revealed a non-significant effect on the days taken for sprouting of Lilium scales. The number of days taken for sprouting ranged from 42.67 days to 57.42 days observed under treatments T_5 (cow urine @ 10%) and T_9 (Kinetin @ 25%) respectively. By the effect of different treatments, the number of micro-bulblet/scales recorded non-significant variation as per the data obtained in Table 1. When treated with Coconut water @ 50% (T_2) resulted in the maximum number of microbulblet/scale, i.e., 1.22 and a minimum number of microbulblet was recorded when treated with Vermiwash @ 100% (T_3), Vermiwash @ 50% (T_4), T_5 (Cow urine @ 10%), T_8 (kinetin @ 50 ppm), T_9 (kinetin @ 25 ppm) and T_{10} (control) showed an equal number of bulblets /scale, i.e. 1. The non-significant difference among the treatment in number of microbulblets/scale may be due to temperature and light as Niimi (1985) [10] had concluded that a temperature of 25 °C favoured more microbulblet per scale, but the present experiment was carried out under temperatures 9 to 18 °C. The tendency of the outer and middle scales of lily bulbs to produce more bulblets is connected with the overall carbohydrate content of those scales (Akcal & Kahraman, 2016 [1]; Panda & Mohanty, 2016 [11]; Matsuo, 1972 [9]; Park, 1996) [13]. However, Gray (1973) [4] contradicted the above statement. In his experiment, he concluded that there is no critical temperature or day length requirement for bulbing. In *in vitro* conditions, the addition of BA into the medium increased the regeneration of bulblets to a moderate level (52.5–59.5%) as compared with control and produced approximately four bulblets per explants (Kumar *et al*., 2007) [7]. Therefore, the application of kinetin and coconut water may be used to increase the regeneration of microbulblet in scale while taking favourable temperature and light into consideration.

The observation in Table 1. Towards the average weight of microbulblet showed a highly significant influence of different treatments. The maximum weight of the microbulblet was recorded under treatment T_4 (Vermiwash @ 50%), i.e., 0.13 g. At par, results were found in T_1 (coconut water @ 100%) and T_8 (Kinetin- 50 ppm), i.e., 12 g, T_5 (Cow urine @ 10%), i.e., 0.11 g and T_6 (Kinetin @ 100 ppm) and T_9 (kinetin @ 25 ppm), i.e., 0.10 g. The minimum weight of microbulblet was recorded under T_{10} (control), i.e., 0.05 g, which was found at par with T_2 (Coconut water @ 50%) and T_3 (vermiwash @ 100%), i.e., 0.08 gm. This may be due to the presence of kinetin and auxin in vermiwash, and the scale position tends to produce microbulblet having more weight, as reported by Akal and Kahraman, 2016 [1]; Panda and Mohanty, 2016 [11]. Outer and middle scales produce microbulblet having more weight than inner scales because, in outer and middle scales, there is more carbohydrate accumulation, which is used by microbulblet to derive more food.

The experimental finding of the maximum length of microbulblet (10.60 mm) was reported in Kinetin @ 50 ppm (T_8) which was found to be at par with treatment T_7(Kinetin @ 75 ppm) having a value of 10.20mm with highly significant variation from other treatments. The minimum length of microbulblet (6.96 mm) was recorded by control (T_{10}) which was found to be at par with treatment T_2 (coconut water @ 50%), i.e., 8.10 mm. However, a non-significant difference was obtained in the breadth of the microbulblet. The range of the breadth of the microbulblet recorded was from 6.30 mm (T_7) to 4.33mm (T_{10}). A study of data

presented by Panda and Mohanty (2016) [11] revealed that the maximum circumference found in the medium scale differed significantly from other scales. It was followed by the outer scale, while the minimum was recorded with the inner scale. Moreover, the same result has been recorded by Akcal and Kahraman (2016) [1]. Marinangeli *et al*., (2003) [8] also observed that inner scales produced bulblets with a significantly smaller diameter than those measured in bulblets from middle and external scales. The use of Kinetin may also have helped in the increase of diameter as Uranbey (2010) [16] recorded that Kinetin was suitable for the induction of bulblet production on 2-4 bulb scales of *Muscari azureum*.

Data regarding the total number of roots per microbulblet and root length (cm), as presented in Table 2, showed non-significant and highly significant results respectively. The use of different treatments could have affected the root length. As per Table 2, the maximum average root number (2.83) recorded was in control (T_{10}) while the minimum average root number (1.33), was recorded when treated with coconut water @ 50% (T_2).

Concerning root length, cow urine @ 10% (T_5) showed the maximum result (4.00 cm), which was at par with coconut water @ 100% (T_1) (3.73 cm), Kinetin @ 100 ppm (T_6) (3.38 cm) and Kinetin @ 75 ppm (T_7) (3.36 cm) and Vermiwash @ 100% (T_3) (1.33 cm). T_3 and T_{10} i.e., vermiwash @ 100% and control, respectively, showed the minimum result (1.33 cm). A higher amount of Kinetin suppressed rooting, and a lower amount of Kinetin is favourable for root formation. Similar results have been advocated by Skoric *et al*. (2012) [14] in *Lilium martagon*. The scale position also affected the root length. According to Panda and Mohanty (2016), the middle scale had maximum and longer root length, followed by the outer and inner scales, as there is more carbohydrate accumulation in the middle and outer scales. As per Akcal and Kahraman (2016) [1] the outer scale gave well results in root number and root length per microbulblet.

As per the data presented in Table 2, the effect of different treatments on the number of shoots per microbulblet and shoot length was found non-significant. The maximum number and length of the shoot were found in T_1 (coconut water 100%).

The number of scales per microbulblet differed significantly among the treatment interactions as presented in Table 2. Then treated with Kinetin @ 50 ppm (T_8) recorded the maximum average scale per microbulblet (4.78) as Kinetin helps in cell division which may have led to more scales per microbulblet. T_7 (4.73), i.e., Kinetin @ 75 ppm, T_6 (4.67), i.e., Kinetin @ 100 ppm, T_1 (4.50) under coconut water @ 100%, T_2 (4.34) under coconut water @ 50%, T_5 (Cow urine @ 10%) 4.33, T_9 (4.17) under Kinetin @ 25 ppm and T_4 (4.00) under Vermiwash @ 50% had at par results with kinetin @ 50 ppm. The minimum average number of scales per microbulblet was recorded when treated with vermiwash @ 100% (T_3) i.e., 2.33.

Table 1: Effect of different treatments on the average days taken for sprouting (Days), average number of microbulblet/scale, total weight (gm), length (mm) and breadth (mm) of the microbulblet

Treatment	Days Taken for Sprouting (Days)	Number of Microbulblet /Scale	Microbulblet Weight (gm)	Microbulblet Length (mm)	Microbulblet Breadth (mm)
T_1	49.92	1.08	0.12	9.52	6.28
T_2	51.94	1.22	0.08	8.1	5.27
T_3	48.5	1	0.08	8.69	5.54
T_4	42.78	1	**0.13**	9.17	5.95
T_5	48.67	1	0.11	8.97	5.92
T_6	43.57	1.17	0.1	9.17	5.9
T_7	54.33	1.13	0.1	10.2	6.3
T_8	53.44	1	0.12	**10.6**	5.73
T_9	57.42	1	0.1	7.99	5.56
T_{10}	56.67	1	0.05	6.96	4.33
SE(m) ±	6.731	1.18	0.12	0.46	0.4
CD @ 5%	NS	NS	**0.04	**1.37	NS

NS – Non-significant, * - Significant and ** - Highly significant

Table 2: Effect of different treatments on the average root number, root length (cm), shoot number, shoot length (cm) and average scale number per microbulblet

Treatment	Total Number of Root / Microbulblet	Root Length (cm)	Total Number of Shoots/Microbulblet	Shoot Length (cm)	Number of Scale / Microbulblet
T_1	2.39	3.73	2.5	15.16	4.5
T_2	1.33	2.61	1	7.19	4.34
T_3	1.83	1.33	1.33	7.78	2.33
T_4	1.55	1.35	1.33	9.83	4
T_5	2.5	4	1.33	10.12	4.33
T_6	2.39	3.38	1.78	10.81	4.67
T_7	2.2	3.36	1.13	1.28	4.73
T_8	1.72	2.17	1	4.95	4.78
T_9	1.83	2.22	0.67	7.52	4.17
T_{10}	2.83	1.33	1.33	14.77	3.67
SE(m) ±	0.49	0.41	0.44	3.07	0.29
CD @ 5%	NS	**1.21	NS	NS	**0.86

NS – Non-significant, * - Significant and ** - Highly significant

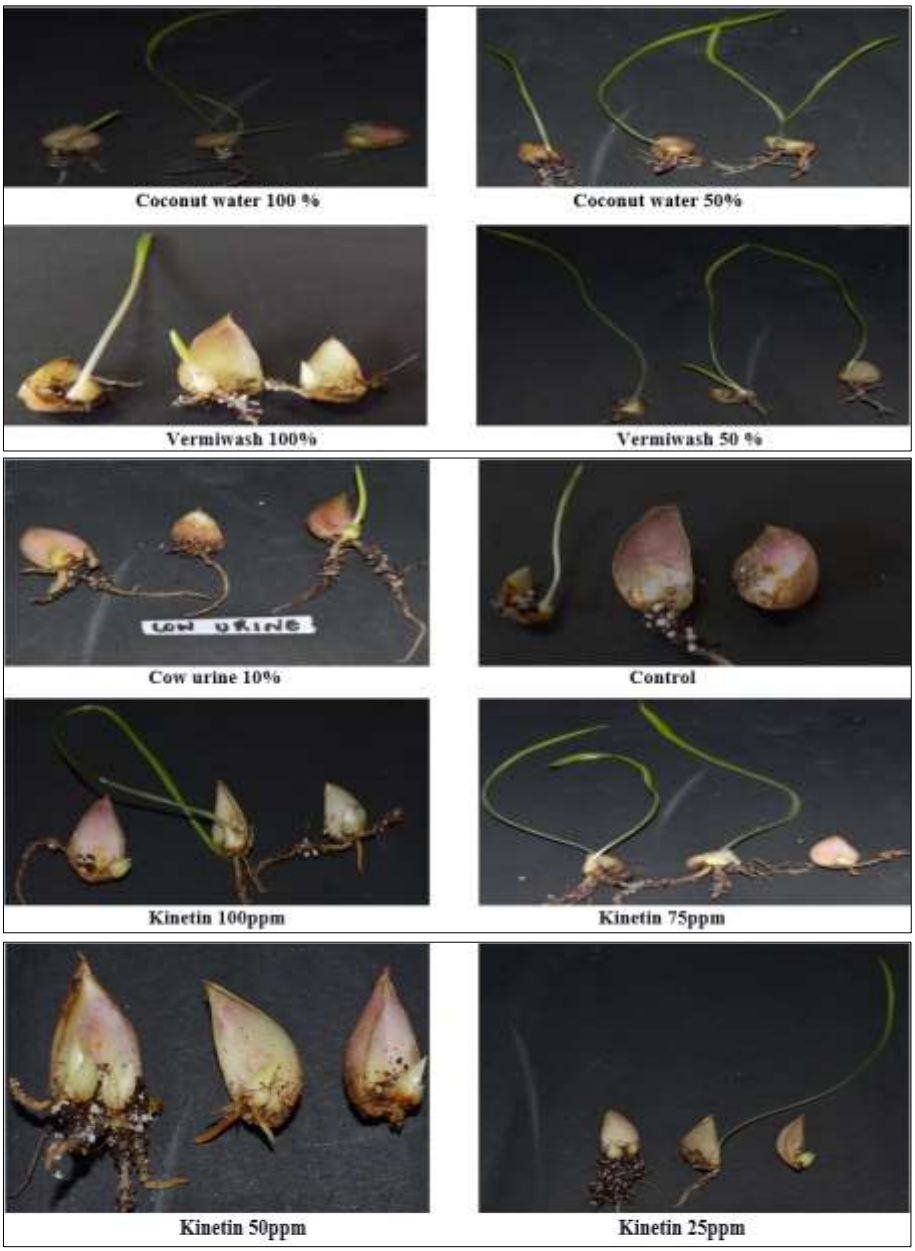

Fig 1: Regeneration of bulblets from scales with the effect of different treatments

Conclusion

The growth and development of microbulblet from scale by the use of different growth substances and bio enhancer was examined under *in vivo* condition. Application of vermiwash 50% and coconut water 50% gave the best result for the growth and development of microbulblet in terms of early sprout, number of microbulblet and size of microbulblet. From the study we can conclude that the organic growth substances and bio-enhancers can substitute for chemical growth substances in the production of bulbs in lilium *in vivo* condition while taking temperature and availability of light into consideration.

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