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In vitro propagation of *Lilium* (*Lilium longiflorum*)*

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Lilium (*Lilium longiflorum*) is a high value crop and ranks fourth in the international flower trade. Propagation rate from bulb scales and stems is very low and produces 1–2 bulblets/bulb scale in one years' time which is not sufficient for large scale cultivation of this plant. Numerous studies have been made on the *in vitro* regeneration of bulblets in lily using different explants (Kumar *et al*. 2006). Although many explants have commonly been used, bulb scales have remained the prime choice as explants to regenerate bulblets in *Lilium* because bulblets seem to be the most productive (Lian *et al*. 2003). One of the best and most prolific vegetative propagation methods for lilies is *in vitro* scale culture (Bahr and Compton, 2004). This study reports a successful and reliable protocol for mass multiplication of *Lilium* under *in vitro* conditions.

The experiment was conducted during 2008–09. Fresh bulbs of *Lilium* were collected from polyhouse of Central Institute of Temperate Horticulture, Srinagar. The middle scales of bulbs were separated and washed thoroughly under running tap water. The explants were surface sterilized with 70% ethanol for 30 sec. followed by 5% sodium hypochlorite for 10 min. and washed 5–6 times with sterilized distilled water before culturing. Basal segments of the scales measuring about 1cm × 1 cm were aseptically cut and each segment with the dorsal side in contact with the medium was placed in test tubes containing 15–20 ml of the culture medium. Murashige and Skoog (1962) medium supplemented with 3% (w/v) sucrose and 0.8% (w/v) agar was used during the investigation. The *p*H of the medium was adjusted to 5.8 using 0.1 N NaOH/0.1 N HCl. The culture vessels containing the media were autoclaved at 121^{0}C at 1.06 kg/cm^2 for 20 min. All the cultures were maintained at 25 ± 2 ^{0}C under light/dark cycle of 16/8 hr with a photosynthetic photon flux density (PPFD) of 50 µ mol/m^2/S provided by cool white fluorescent lamps at 65–70% relative humidity. For shoot induction and multiplication, scales of bulblets were inoculated on MS medium supplemented with different concentrations of BA (Benzyl adenine) 0.0–2.0 mg/leither individually or in combination with 0.0–2.0 mg/l NAA (á-naphthalene acetic acid). The shoots induced were sub cultured onto the fresh MS medium with same plant growth regulators after every four weeks. The frequency of explants producing bulblets, number of shoots/explant, shoot length and fresh weight were recorded. A set of cultures containing MS medium without growth regulators served as control.

The *in vitro* produced bulblets were separated and individual bulblet was transferred to MS media supplemented with 0.5, 1, 1.5 and mg/l IBA or NAA after 30 days of third subculture. After achievement of sufficient number of roots, the rooted plantlets were taken out of the culture vessels, washed with water to remove the adhering agar and treated with 0.2% bavistin (Carbendazim) for 10 min. and were finally transferred to the pots containing cocopeat or soilrite:soil. Potted plantlets were covered with transparent polythene bags to ensure high humidity and watered every 3–4 days with half strength MS salt solution for two weeks. Polythene bags were gradually perforated after four weeks and were removed after eight weeks in order to acclimatize plants to field conditions. After eight weeks, acclimatized plants were transferred to greenhouse, where ambient conditions of temperature and humidity were maintained. The hardening and acclimatization procedures were followed as described by Kumar *et al*. (2007).

Each treatment with ten cultures/treatment was replicated three times and observations in stages of development were recorded periodically. The data were analyzed by comparing means using one way ANOVA and the significance was determined by Duncan's Multiple Range Test using SPSS for windows (v. 15). Data given in percentages were subjected to arcsine (vX) transformation (Snedecor and Cochran 1980) before statistical analysis. The excised scales did not produce

*Short note

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bulblets on growth regulator free medium (Table 1). Bulblet formation initiated on the explants grown on media containing NAA or BA alone or in combinations (Fig 1a). The optimum response in terms of percentage of explants producing bulblets was recorded on MS medium supplemented with BA 2.0 mg/l + NAA 0.5 mg/l where 94.0% of the explants regenerated bulblets and the bulblets were regenerated without any visible callus formation as an intermediate step. Kumar *et al*. (2008) observed that 45% of the explants regenerated bulblets from the middle zone of *in vitro* root in oriental hybrid cv. Marco Polo. Similarly, LingFei *et al.* (2009) also observed the highest frequency of regeneration (93.3%) and the largest number of shoots/leaf (3.83) were obtained on NN basal medium supplemented with 0.5 mg/l TDZ and 1.0 mg/l NAA. The number of shoots/explant was significantly higher 22.0 with BA 2.0 mg/l + NAA 0.5 mg/l as compared to other treatments (Table 1, Fig 1b). Azadi and Khosh-Khui (2007) recorded 5.41 bulblets from explants of bulb scale on a medium containing 0.1 mg/l 1 BA and 0.1 mg/l NAA in *L. ledebourii*. A different effect of growth regulators, under similar environmental conditions was observed on length of shoots and fresh weight of regenerated bulblets. The highest shoot length (12 cm) and highest mean fresh weight of 365 mg was observed on MS medium supplemented with 2.0 mg/l BA + 0.5 mg/l NAA (Table 1). Kumar *et al*. (2008) recorded highest fresh weight of 171.7 mg in *in vitro* root with 1.5 mg/l NAA and 2 mg/l BA in oriental hybrid cv. Marco Polo. Among different rooting treatments, MS medium supplemented with 2 mg/l NAA gave best results and explants cultured on this medium recorded highest rooting (93%). Also the number of roots (19) and length of roots (5.2 cm) was higher than other treatments (Fig 1c). The rooted bulblets were hardened in coco peat and soilrite: sand (1:1 w/v) in plastic pots (Fig 1d) and the survival of bulblets was significantly higher in coco-peat (95%) as compared to soilrite: sand after eight weeks of transfer. Saifullah *et al.* (2010) observed greatest number andlength of shoots were produced in a medium containing BAP (2.0 mg/l), IBA (1.0 mg/l), IAA (1.0 mg/l). Thakur *et al*. (2002) reported that for hardening *in vitro* rooted bulblets of *Lilium*, coco-peat, peat moss and coco-peat + peat moss (1:1) gave 100% survival whereas sand:soil:FYM (1:1:1) was the least effective with only 62% surviving plantlets. This study on *in vitro* mass multiplication of *Lilium* spp revealed that large number of high quality planting material of *Lilium* can be achieved in a very short period of time (six months).

Table 1 Effect of BA and NAA on shoot regeneration and multiplication of *Lilium*

Plant growth regulators (mg/l)		Bulb let formation	No. of shoots	Length of	Fresh weight/bulb
BA	NAA	(%)		shoots(cm)	let (mg)
0	0	0(0) ^{a}	0 ^{a}	0 ^{a}	0 ^{a}
0.5	0	23 (32.37) ^{cd}	1.8 ^{bc}	1.4 ^{b}	125 ^{bcd}
1	0	29 (34.81) ^{e}	2.2 ^{cd}	1.9b ^{cde}	142 ^{de}
1.5	0	39 (38.44) ^{fg}	2.4 ^{cd}	2.2 ^{def}	151 ^{ef}
2	0	56 (44.11) ^{ij}	3.7 ^{fg}	3.2 ^{ij}	172 ^{g}
0	0.5	14 (27.95) ^{b}	1.2 ^{b}	1.6 ^{bc}	107 ^{b}
0	1	21 (31.48) ^{c}	1.7 ^{bc}	1.7b ^{cd}	119 ^{bc}
0	1.5	27 (34.02) ^{de}	1.8 ^{bc}	1.8 ^{bcd}	126 ^{bcd}
0	2	38 (38.09) ^{f}	2.2 ^{cd}	2.1 ^{cdef}	135 ^{cde}
0.5	0.5	39 (38.44) ^{fg}	2.2 ^{cd}	2.2 ^{def}	143 ^{de}
0.5	1	44 (40.14) ^{gh}	2.3 ^{cd}	2.4 ^{efg}	168 ^{fg}
0.5	1.5	47 (41.14) ^{h}	2.7 ^{de}	2.6 ^{fgh}	188 ^{gh}
0.5	2	52 (42.79) ^{i}	3.2 ^{ef}	2.8 ^{ghi}	197 ^{h}
1	0.5	54 (43.45) ^{ij}	3.4 ^{ef}	2.9 ^{ghij}	217 ^{i}
1	1	59 (45.11) ^{jk}	4.9 ^{hi}	4.2 ^{k}	238 ^{ij}
1	1.5	57 (44.44) ^{ij}	4.2 ^{gh}	3.4 ^{j}	231 ^{ij}
1	2	55 (43.78) ^{ij}	3.7 ^{fg}	3.1 ^{hij}	219 ^{i}
1.5	0.5	58 (44.77) ^{j}	4.9 ^{hi}	4. ^{1k}	251 ^{j}
1.5	1	64 (46.79) ^{k}	7 ^{j}	7.1 ^{n}	283 ^{k}
1.5	1.5	59 (45.11) ^{jk}	5 ^{i}	6.2 ^{m}	275 ^{k}
1.5	2	57 (44.44) ^{ij}	4.6 ^{hi}	5.1 ^{l}	247 ^{j}
2	0.5	94 (60.55) ^{m}	22 ^{n}	12 ^{q}	365 ^{m}
2	1	85 (55.07) ^{l}	16 ^{m}	10 ^{p}	328 ^{l}
2	1.5	81(53.22) ^{kl}	11 ^{l}	9 ^{o}	312 ^{l}

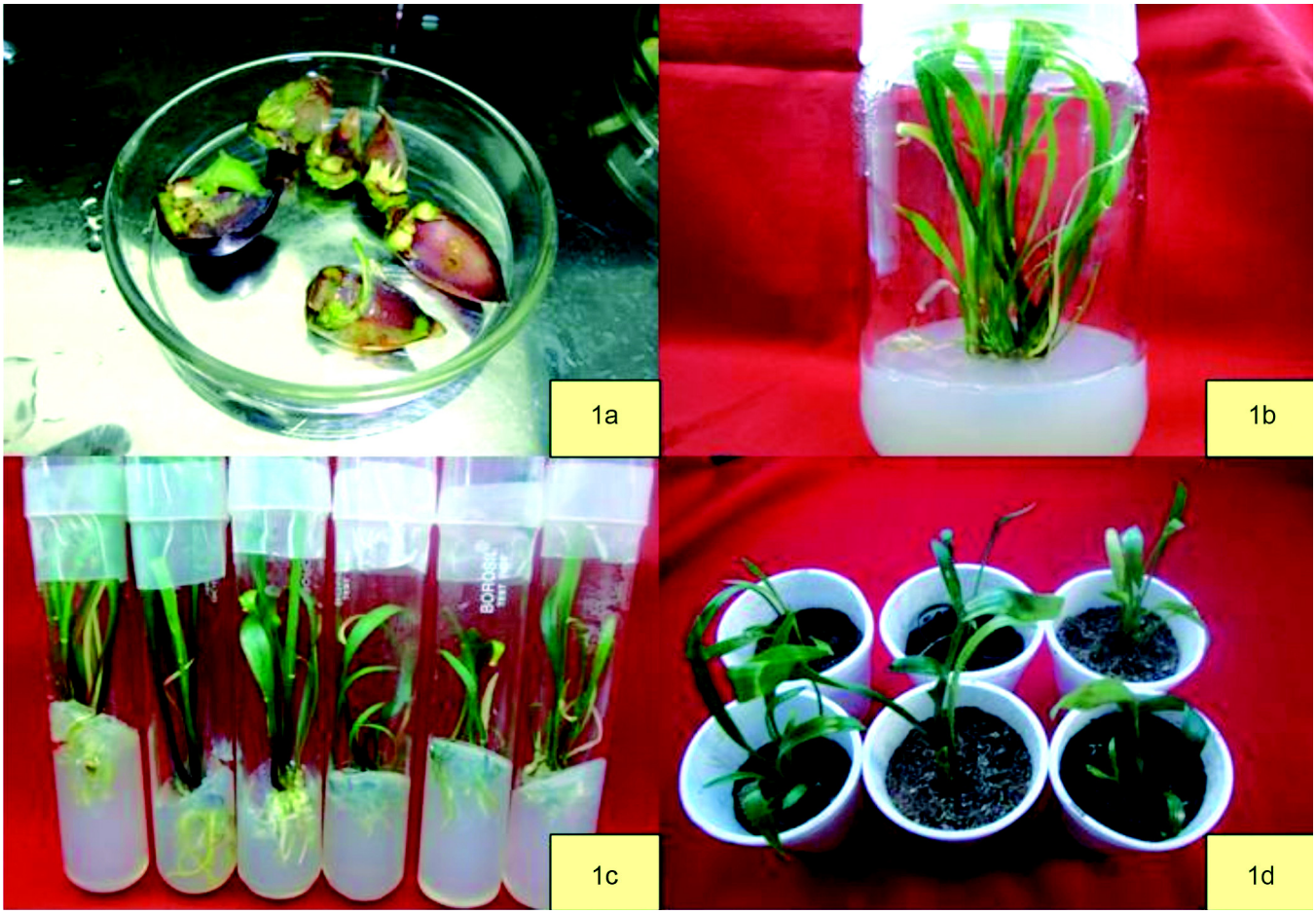

Fig 1 *In vitro* propagation of *Lilium longiflorum*

Table 2 Effect of NAA and IBA on rooting parameters of *Lilium*

Plant growth regulators (mg/l)		Rooting (%)	No. of roots	Length of roots (cm)
NAA	IBA			
0	0	0 (0)* [a]	0 [a]	0 [a]
0.5	0	57 (44.44) [c]	7 [c]	2.9 [c]
1	0	71 (49.27) [d]	12 [f]	3.3 [d]
1.5	0	84 (54.59) [e]	16 [h]	3.8 [e]
2	0	93 (59.77) [f]	19 [i]	5.2 [g]
0	0.5	41 (39.12) [b]	4 [b]	2.6 [b]
0	1	56 (44.11) [c]	8 [d]	3.1 [cd]
0	1.5	69 (48.54) [d]	11 [e]	3.7 [e]
0	2	81 (53.22) [e]	15 [g]	4.5 [f]

Figures given in parentheses are angular transformed values.

Means followed by the same letter within the columns are not significantly different (P = 0.05) using Duncan's multiple range test.

SUMMARY

Lilium longiflorum has a wide applicability in the floral industry as cut flower and potted plants. Micropropagation in bulb plants is an alternative to the conventional methods for vegetative propagation which attracts much attention because of its advantages such as many fold multiplication rate. In present study an efficient micropropagation protocol in *L. longiflorum* has been developed. The highest bulblet regeneration (94%), maximum number of shoots (22), maximum shoot length (12 cm) and heaviest bulblets with average fresh weight (365 mg) were obtained in MS medium supplemented with BA 2.0 mg/l + NAA 0.5 mg/l. The treatment of MS medium with NAA 2.0 mg/l was most effective for rooting and gave maximum rooting (93%), maximum number of roots (19) and highest root length of 5.2 cm as compared to other treatments. The micropropagation from inoculation of scales to hardening was completed only in six months.

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