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Bulblet Regeneration from Leaf and Internode Explants of Oriental Hybrid Lilies

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ABSTRACT

Bulblets were regenerated in two oriental lily hybrid cultivars 'Siberia' and 'Marco Polo' from leaf and internode explants cultured on Murashige and Skoog (MS) medium supplemented with α-naphthalene acetic acid (NAA) and 6-benzyladenine (BA) singly, or in combination. Bulblet regeneration and average fresh weight per bulblet was highest with 1 mg/l NAA and 0.5 mg/l BA in both cultivars, whereas the number of bulblets per explant was greater with 0.2 mg/l NAA and 0.5 mg/l BA. Bulblet regeneration, the number of bulblets per explant and fresh weight was greatest in leaf than internode explants. The bulblets attaining 14 to 16 cm in size flowered in the second year of growth period without any phenotypic variations.

Keywords: growth regulators, *in vitro*, *Lilium*, regeneration
Abbreviations: BA, 6-benzyladenine; **NAA**, α-naphthalene acetic acid

INTRODUCTION

Lilium is one of the world's leading cut flower crops. It has a wide applicability in the floral industry as a cut flower and as potted plants (Jana and Roy Choudhury 1989). Among the various types of lilies, asiatic, oriental and *Lilium longiflorum* hybrids have premium potential in the floral trade. Oriental lilies are the most expensive among various lily forms, as their bulbs are most expensive and require special technology for bulb production program. Several organs of lilies have regenerative capability viz., stem nodes (Nhut 2003), flower buds (Corti *et al*. 1988), stem apices (Park *et al*. 1996), peduncle and petals (Takayama and Misawa 1979), bulb scale (Kumar *et al*. 2006) and root (Kapoor *et al*. 2008). Although many explants have commonly been used, bulb scales have remained the prime choice of explant to regenerate bulblets in *Lilium* because scales seem to be the most productive (Stimart and Ascher 1978; Lian *et al*. 2003; Kumar *et al*. 2006). Niimi and Onozawa (1979) and Niimi (1984) reported that the leaf segments of several lilies freely produced bulblets and are an alternative to scales as a source of material for propagation. Takayama and Misawa (1979) reported that in *Lilium speciosum* segments of leaves excised just before anthesis were a poor source for the production of bulblets, as only 1 out of 150 segments survived, whereas segments excised from young leaves in *L. longiflorum* developed bulblets (Stenberg *et al*. 1977), thus showing inconsistent regenerative ability of leaf explants (Niimi 1984). Reports are also available on bulblet regeneration from stem explants in *Lilium* (Sheridan 1968; Niimi 1984; Nhut *et al*. 2002; Nhut 2003). Keeping the above in mind, the present investigation was undertaken to regenerate bulblets from leaf and internode explants of oriental hybrid lilies.

MATERIALS AND METHODS

Preparation of plant material

The plant material of oriental lily hybrid cvs. 'Siberia' and 'Marco Polo' growing in the fields of the Department of Floriculture and Landscaping, University of Horticulture and Forestry, Solan, India was procured during the month of March after the emergence of the stem with flower buds (unopened). Intact leaves and stem segments were taken from the middle part (10-15 nodes from the terminal bud) of the plant. They were washed with tap water and surface sterilized with 5% sodium hypochlorite for 5-7 min. Following surface sterilization, the leaf and stem segments were washed 3-4 times with sterile distilled water under aseptic conditions and cut with a sterilized razor blade (Glassvan, Delhi, India) into 3-5 mm transverse sections and used as explants.

Cultural conditions

The sterilized explants were cultured with the abaxial side down on the MS (Murashige and Skoog 1962) medium supplemented with 30 g/l (w/v) sucrose, 0.2 and 1 mg/l α-naphthelene acetic acid (NAA) and 0.5 and 1 mg/l 6-benzyladenine (BA), alone or in combinations. Cultures without growth regulators served as controls. The explants were cultured in 100 ml Erlenmeyer flasks (Borosil Bombay, India)) containing 35 ml of medium. The medium was adjusted with 1 N HCl and/or 1 N NaOH to pH 5.8. Difco bacto agar (0.8%, w/v) was added to the medium before autoclaving at 121°C, at the pressure of 1.1 kg/cm^2 for 15 min. All the cultures were kept under a 16-hr photoperiod at 24 ± 2°C with a photosynthetic photon flux density (PPFD) of 50-60 µmol/m^2/s provided by white cool fluorescent lamps (40W each, Philips). The cultures were transferred to fresh medium at four weeks interval.

The number of explants forming bulblets was recorded after four weeks and the number of bulblets and average fresh weight after 13 weeks of culture.

Statistical analysis

Three replications with 10 explants in each replication (30 explants) were maintained for each treatment and the data were analyzed statistically using factorial completely randomized design (Gomez and Gomez 1984). The statistical analysis based on mean value per treatment was made using ANOVA. The comparative LSD multiple range test (P = 0.05) was used to determine differences between treatments.

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Short Communication

Table 1 Effect of growth regulators, cultivars and explants on bulblet regeneration in oriental lily hybrids.

Treatment (mg/l)		Leaf			Internode			Overall
NAA	BA	Siberia	Marco Polo	Mean	Siberia	Marco Polo	Mean	mean
0	0	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
0	0.5	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
0	1	66.33 (54.54)	63.33 (52.74)	64.83 (53.64)	0 (0.00)	0 (0.00)	0 (0.00)	32.41 (26.82)
0.2	0	74.00 (59.35)	73.00 (58.70)	73.50 (59.02)	0 (0.00)	0 (0.00)	0 (0.00)	36.75 (29.51)
1	0	74.33 (59.56)	76.33 (60.89)	75.33 (60.23)	0 (0.00)	0 (0.00)	0 (0.00)	37.47 (30.11)
0.2	0.5	79.00 (62.73)	78.00 (62.03)	78.50 (62.30)	66.00 (54.34)	65.33 (53.93)	65.67 (54.14)	72.08 (58.26)
0.2	1	73.00 (58.71)	78.67 (62.50)	75.83 (60.60)	65.00 (53.73)	66.67 (54.74)	65.83 (54.24)	70.83 (57.42)
1	0.5	78.00 (62.04)	75.67 (60.45)	76.83 (61.25)	76.00 (60.68)	76.67 (61.12)	76.33 (60.90)	76.58 (61.07)
1	1	78.00 (62.03)	75.33 (60.22)	76.67 (71.13)	75.00 (60.00)	75.67 (60.45)	75.33 (60.23)	76.00 (60.68)
Mean		58.07 (46.55)	57.81 (46.39)	57.94 (46.47)	31.33 (28.80)	31.59 (25.58)	31.43 (25.50)	

$LSD_{0.05}$ Treatment (A) = (0.53); Explant (B) = (0.25); Cultivar (C) = (0.25); A x B = (0.75); A x C = (0.75); B x C = (0.35); A x B x C = (1.06)
Figures in parentheses are arc-sine transformed values

Table 2 Effect of growth regulators, cultivars and explants on number of bulblets in oriental lily hybrids.

Treatment (mg/l)		Leaf			Internode			Overall
NAA	BA	Siberia	Macro Polo	Mean	Siberia	Macro Polo	Mean	mean
0	0	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
0	0.5	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)	0 (1.00)
0	1	1.25 (1.50)	1.08 (1.44)	1.16 (1.47)	0 (1.00)	0 (1.61)	0 (1.71)	0.58 (0.73)
0.2	0	1.33 (1.52)	1.58 (1.60)	1.45 (1.56)	0 (1.00)	0 (1.00)	0 (1.00)	0.73 (1.28)
1	0	1.57 (1.60)	2.33 (1.82)	1.95 (1.71)	0 (1.00)	0 (1.00)	0 (1.00)	0.97 (1.35)
0.2	0.5	3.41 (2.10)	2.16 (1.78)	2.78 (1.94)	2.16 (1.78)	2.50 (1.87)	2.33 (1.82)	2.55 (1.88)
0.2	1	2.30 (1.81)	1.58 (1.60)	1.94 (1.71)	1.58 (1.60)	1.45 (1.56)	1.51 (1.58)	1.72 (1.65)
1	0.5	2.08 (1.75)	1.91 (1.70)	1.99 (1.73)	1.91 (1.70)	1.25 (1.50)	1.58 (1.60)	1.78 (1.67)
1	1	2.75 (1.93)	2.66 (1.91)	2.70 (1.92)	2.56 (1.88)	2.16 (1.78)	2.36 (1.83)	2.53 (1.88)
Mean		1.63 (1.58)	1.47 (1.54)	1.55 (1.56)	0.91 (0.77)	0.81 (0.74)	0.86 (0.75)	

$LSD_{0.05}$ Treatment (A) = (0.12); Explant (B) = (0.006); Cultivar (C) = (0.006); A xB = (0.018); A x C = (0.018); B x C = (0.08); A x B x C = (0.025)
Figures in parentheses are arc-sine transformed values

Bulblet storage

The procedure for bulblet storage is the same as described by Kumar *et al*. (2007). The bulblets, following storage at 2°C for 8 weeks, were transferred to earthenware pots (25 cm diameter) containing soil: sand: FYM (farm yard manure) mixed in a 1: 1: 1 ratio. The process was repeated until the size (14 to 16 cm) of bulblets required for flowering was achieved. Repeated storage and transfer to soil was carried out for breaking dormancy and growth of bulblets to attain standard size (14 to 16 cm) for getting quality flowers.

RESULTS AND DISCUSSION

In both cultivars, the explants failed to regenerate bulblets in growth regulator-free medium or when the medium was supplemented with 0.5 mg/l BA (**Table 1**). About 76% of the explants regenerated bulblets when 1 mg/l NAA was used in combination with 0.5 mg/l BA, which was statistically equal to the combination 1 mg/l NAA and 1 mg/l BA (**Fig. 1**). Niimi (1984) reported that about 72 and 89% of leaf and stem explants, respectively regenerate bulblets in *Lilium rubellum*. Bulblet regeneration was significantly higher in leaf explants (57.94%) than internode explants (36.85%). NAA at both levels was more effective in regenerating bulblets than BA. NAA is essential for the formation and growth of bulblets in scale culture (Stimart and Ascher 1978). Van Aatrijk *et al*. (1985) also noticed that auxins and their distribution within the tissue are important factors in the process of bud regeneration in lily scale culture. The interaction effect of treatment × explant revealed that 0.5 mg/l BA in combination with 0.2 and 1 mg/l NAA resulted in highest explant regeneration in leaves and internodes. Bulblet regeneration differed significantly between both cultivars in both explants.

The highest average number (2.55 bulblets/explant) was achieved with a combination of 0.2 mg/l NAA and 0.5 mg/l BA, which was statistically equal to the combination of 1 mg/l NAA and 1 mg/l BA, where 2.53 bulblets/explant were produced (**Table 2**). Similar results were reported by other researchers in different lily species/cultivars (Niimi and Onozawa 1979; Kumar 2002; Becchetta *et al*. 2003). Hussey (1975) reported that leaf propagation of monocotyledonous plants is generally regarded as difficult in several species of Liliaceae, Iridaceae and Amryllidaceae. The highest number of bulblets was recorded in cv. 'Siberia' in leaf explants whereas the lowest response was observed in cv. 'Marco Polo' in internode explants. The treatment × explant interaction revealed the best response with either concentration of NAA when used in combination with 0.5 or 1 mg/l BA in both explants. Similarly, the treatment × explant × cultivar interaction showed that the response of the treatments varied depending on the type of explant and cultivar.

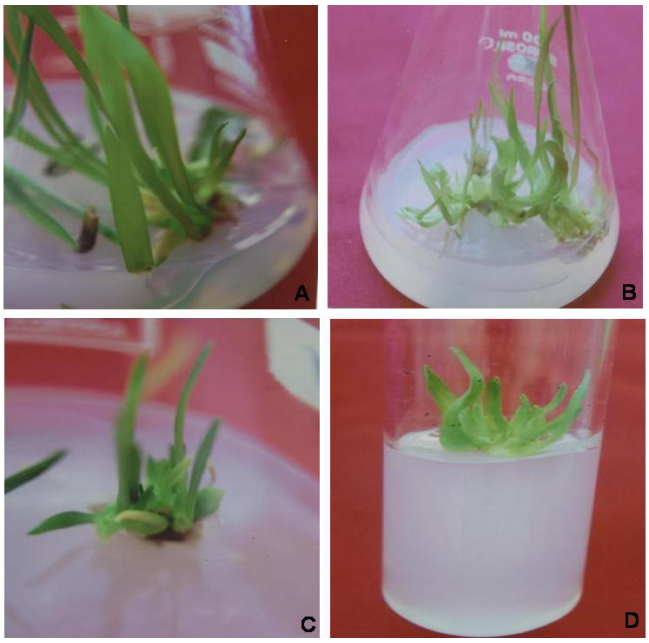

Fig. 1 Bulblet regeneration from leaf and internode explants. Leaf explants of (**A**) 'Siberia' (**B**) 'Marco Polo'. Internode explants of (**C**) 'Siberia' and (**D**) 'Marco Polo' on MS medium supplemented with 0.5 mg/l NAA and 1 mg/l BA after four weeks of culture.

Table 3 Effect of growth regulators, cultivars and explants on mean fresh weight per bulblet in oriental lily hybrids.

Treatment (mg/l)		Leaf			Internode			Overall
NAA	BA	Siberia	Macro Polo	Mean	Siberia	Macro Polo	Mean	mean
0	0	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
0	0.5	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
0	1	124.6 (2.09)	130.4 (2.12)	127.5 (2.10)	0 (0.00)	0 (0.00)	0 (0.00)	131.1 (2.12)
0.2	0	159.7 (2.20)	233.7 (2.37)	196.7 (2.28)	0 (0.00)	0 (0.00)	0 (0.00)	98.3 (1.14)
1	0	191.3 (2.28)	206.3 (2.31)	198.8 (2.30)	0 (0.00)	0 (0.00)	0 (0.00)	99.4 (1.35)
0.2	0.5	159.3 (2.20)	232.7 (2.37)	196.0 (2.28)	184.3 (2.26)	203.3 (2.31)	193.8 (2.29)	194.9 (2.28)
0.2	1	141.3 (2.15)	186.2 (2.27)	163.7 (2.21)	212.7 (2.33)	196.0 (2.29)	204.3 (2.31)	184.0 (2.26)
1	0.5	194.7 (2.29)	255.7 (2.41)	225.2 (2.35)	237.7 (2.37)	205.0 (2.31)	221.3 (2.34)	223.2 (2.34)
1	1	199.7 (2.30)	289.7 (2.46)	244.7 (2.38)	191.0 (2.28)	165.3 (2.82)	178.2 (2.25)	211.4 (2.34)
Mean		130.1 (1.72)	170.5 (1.81)	150.3 (1.77)	113.8 (1.28)	85.5 (1.01)	88.6 (1.02)	

$LSD_{0.05}$ Treatment (A) = (0.032); Explant (B) = (0.0015); Cultivar (C) = (0.0015); A xB = (0.0045); A x C = (0.0045); B x C = (0.002); A x B x C = (0.0063)
Figures in parentheses are arc-sine transformed values

Fig. 2 Flowering plants of (A) 'Marco Polo' (B) 'Siberia' in pots containing soil: sand: FYM mixed in a 1: 1: 1 ratio.

The failure of the explants to regenerate bulblets with NAA or BA singly in internode explants may be due to the fact that a proper auxin-cytokinin balance is required. Maesato *et al*. (1991) also reported that a critical auxin-cytokinin balance is required for organ regeneration from bulb scales in *Lilium japonicum*.

The maximum average fresh weight of bulblets was achieved with 1 mg/l NAA and 0.5 mg/l BA (**Table 3**). The fresh weight was significantly higher in leaf explants than in internode explants. In contrast, stem explants produced heaviest bulblets in *L. rubellum* (Niimi 1984). The difference in the results may be due to the difference in the genotype and cultural conditions. Niimi and Onozawa (1979) observed a relatively higher fresh weight with 1 mg/l NAA in combination with 0.1 mg/l BA in *L. rubellum*. The production of heavier bulbs from leaf explants may be due to that reserve material is being manufactured in leaves and transported to the new bulblets (Niimi and Onozawa 1979; Langens-Gerrits *et al*. 1997). The treatment × explant interaction revealed the highest response with 1 mg/l NAA and 1 mg/l BA in leaf explants, whereas the best interaction was achieved with 1 mg/l NAA and 0.5 mg/l BA in internode explants of both cultivars. The treatment × explant × cultivar interaction showed that the combination of 1 mg/l NAA and 1 mg/l BA produced heaviest bulblets in leaf explants, whereas the combination of 1 mg/l NAA and 0.5 mg/l BA produced heaviest bulblets in internode explants of both cultivars. The heaviest bulblets were produced in leaf explants of cv. 'Marco Polo'.

Bulblets attaining 14 to 16 cm size flowered (**Fig. 2**) in the second year of growth period in pots without any phenotypic variations.

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