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Bulblet production from node explant grown *in vitro* in hybrid lilies

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Abstract

An attempt was made to regenerate bulblets from *ex vitro* node explants of hybrid lilies in the year 2007. Node sections (3-4 mm) isolated from the middle part of the stem in hybrid lilies were cultured on Murashige and Skoog (MS) medium supplemented with several combinations of NAA and BA. Growth regulator-free medium was not effective in inducing bulblet regeneration. A significant increase in the percent of explant producing bulblets and number of bulblets per explant was observed when 2 mg/l NAA was used in combination with 1.5 or 2 mg/l BA. The heaviest bulblets were obtained with 2 mg/l NAA in combination with 1.5 mg/l BA after 90 days of culture. The cultivar Apeldoorn produced greater number of bulblets whereas more weighty bulblets were produced in the cultivar Beartix. 1 or 2 mg/l IBA was most effective in producing roots. The rooted bulblets were hardened with 80-82% survival success after 30 days of transfer in the pots.

Keywords: Asiatic hybrids; Bulblet; Growth regulators; Micropropagation; Node explant; Oriental hybrids

Introduction

Lilium belongs to the family Liliaceae and is characterized by an annual thermoperiodism and a number of striking features such as scaly bulbs, short lanceolate leaves and beautiful flowers. Over the past few years, the importance of lily has increased enormously, especially in The Netherlands. The area under cultivation has increased from 102 ha in 1960 to 2419 ha in 1990, representing a 24-fold increase (Betties and White, 1993, Kumar et al., 2006). Lily is a low volume, high value crop and ranks fourth in the international flower trade (Anonymous, 1996). It has a wide applicability in the floral industry as cut flower and potted plants (Jana and Roy Choudhary, 1989). Lilies can be propagated by both sexual and asexual reproduction. Most of the commercial grown cultivars are propagated through vegetative means by way of above ground bulbils instead of underground bulbscales (Maesato et al., 1991; Dilta et al., 2000; Lian et al., 2003; Kumar et al., 2001; Kumar et al., 2006).

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, bulbscales have remained the prime choice of explants to regenerate bulblets in *Lilium* because bulblets seem to be the most productive (Lian et al., 2003; Kumar et al., 2006). Niimi (1984) reported *in vitro* propagation of *Lilium rubellum* from the explants of leaves, scales, stem, tepals and other organs, some of these cultures had almost the same bulblet productivity as the explants of scale. Reports are also available on bulblet regeneration from stem explants in *Lilium* (Sheridan, 1968; Nhut et al., 2001; Nhut et al., 2002a; Nhut, 2003). Bacchetta et al., (2003) developed a protocol for the development of pseudobulblets from *in vitro* shoot tip derived stem nodes in *Lilium*. Keeping in view, the above, in the present investigation an attempt was made to regenerate bulblets from *ex vitro* node explants of hybrid lilies.

Materials and Methods

Stem segments (4-5 cm) from middle of the stem with leaves of asiatic hybrids (Alaska, Apeldoorn and Beartix) and oriental hybrids (Siberia and Marco Polo) were procured from the plants growing in the fields of the Department of Floriculture and Landscaping, University of Horticulture and Forestry, Solan (H.P.), India during the year 2007. After removing the leaves, the explants were washed with distilled water supplemented with Tween-20. The explants were surface sterilized with 5% sodium hypochlorite for 8-9 min and washed 3-4 times with sterilized distilled water before culturing. Nodal sections (3-4 mm) were cultured on MS medium (Murashige and Skoog, 1962) supplemented with 3% (w/v) sucrose, 100 mg/l meso-inositol and in all possible combinations of 0, 1 and 2 mg/l NAA and 0, 1.5 and 2 mg/l BA. The medium was adjusted to pH 5.8, followed by addition of 0.8% (w/v) Agar. The cultures without growth regulators served as control. Three replications with 10 explants in each were maintained for each treatment. The explants were cultured in Erlenmeyer flask (100 ml) containing 30 ml of medium and closed by non-absorbent cotton plugs. The cultures were maintained under 16/8 h light/dark photoperiod provided with cool-white fluorescent lamps (50 μmol m^{-2} s^{-1}) at a temperature of 25±2 ^{o}C. The cultures were transferred to fresh media every 30 days. Percent regeneration, average number of bulblets and fresh weight recorded after 90 days of culturing were analyzed statistically using factorial completely randomized design consisting of two factors each with three replications. The first factor (A) was cultivars (five levels) and the second factor (B) was MS medium with growth regulators (nine levels).

The *in vitro* produced bulblets were separated and individual bulblet was transferred to MS medium supplemented with 0.5, 1, 1.5 and 2 mg/l IBA after 30 days. The data were analyzed statistically using factorial completely randomized design consisting of two factors each with three replications. The first factor (A) was cultivars (five levels) and the second factor (B) was MS medium with growth regulators (five levels). The rooted bulblets were taken out of the culture vessels, washed with water to remove the adhering agar and treated with 0.2% bavistin (Carbendazim, a fungicide) for 30 min and were transferred to the pots (4" diameter) containing cocopeat. The hardening and acclimatization procedures were followed as described by Kumar et al., (2007). When the leaves were dried, the bulblets were removed from the pots, washed thoroughly and dried at room temperature.

The bulblets were treated with 0.1% (w/v) bavistin and stored in cocopeat at 2 ^{o}C. Percent survival of bulblets was recorded 30 days after transfer to pots.

Results and Discussion

The nodal sections (3-4 mm) did not produce bulblets on growth regulator-free medium till 90 days of culturing (Table 1). Bulblets were initiated on the explants growing in media containing NAA or BA alone or in combinations (Figure 1 A-E). The percent of explants producing bulblets was greater with 2 mg/l NAA in combination with 1.5 mg/l BA, which was statistically on a par with 2 mg/l NAA in combination with 2 mg/l BA. Niimi (1984) reported that in *Lilium rubellum*, the ability to regenerate bulblets was greatest in explants of stem where 89% of the explants produced bulblets. Sheridan (1968) also reported that in *Lilium longiflorum*, the explants excised from the terminal portion of the stem can easily regenerate bulblets. An efficient system for the *in vitro* shoot regeneration of *L. longiflorum* cv. Nellie White was developed by culturing pretreated protocorm-like bodies derived from young stem traverse thin cell layers exposed to various treatments of different medium volume, pH and sucrose concentrations (Nhut et al., 2002a). The interaction between treatment and cultivar revealed that the greatest bulblet regeneration was achieved with 2 mg/l NAA in combination with 2 mg/l BA, followed by 2 mg/l NAA in combination with 1.5 mg/l BA in cultivar Alaska. Among the cultivars, the bulblet regeneration was highest in Apeldoorn followed by Beartix and Siberia. The lowest response was recorded in Marco Polo. The number of bulblets was significantly higher when used in maximum concentrations of BA and NAA in this experiment (Table 2). Nhut (1998) and Bacchetta et al., (2003) developed psuedobulblets from *in vitro* shoot-tip derived stem nodes in *Lilium*. Azadi and Khosh-Khui (2007) recorded 5.41 bulblets from explants of bulb scale on a medium containing 0.1 mg/l BA and 0.1 mg/l NAA in *Lilium ledebourii*. Similarly, Ishioka and Tanimoto (1993) reported 7.4 bulblets from the explants of leaf with 10 μM NAA and 10 μM BA in *Lilium longiflorum*. Embryo-like structures were obtained through somatic embryogenesis from pseudobulblets transverse thin cell layers and transverse young stem sections of *L. longiflorum* (Nhut et al., 2002b). The interaction between treatment and cultivar revealed that 2 mg/l NAA in combination with 2 mg/l BA and 1.5 mg/l BA, produced greatest number of bulblets in cultivars Alaska and Beartix, respectively. Among the cultivars, Marco Polo produced maximum number of bulblets per explant, which was statistically on a par with all other cultivars. A differential effect of growth regulators under similar media, was observed on the average fresh weight of regenerated bulblets. The average fresh weight differed significantly with NAA or BA (Table 3). The highest average fresh weight of 324.8 mg was observed with a combination of 2 mg/l NAA and 1.5 mg/l BA. All other combinations induced the production of lower quantities of fresh weight. Niimi (1984) reported heaviest bulblets (135 mg) from the explants of stem n *Lilium rubellum*. It was also observed that the average fresh weight was higher, when higher concentration of NAA (2 mg/l) was used in combination with BA. The interaction between treatment and cultivar revealed the maximum average fresh weight of 401.3 mg with 2 mg/l NAA and 1.5 mg/l BA in cultivar Beartix. Among the cultivars, Beartix recorded heaviest bulblets, followed by Apledoorn. The bulblets forming roots were greater with 1.5 mg/l IBA, which was statistically on a par with 2 mg/l IBA (Table 4, Figure 1. F). the results also revealed that the rooting response of individual cultivars varied with the treatment.

Among the cultivars, Siberia recorded maximum number of bulblets forming roots, which differ significantly from all other cultivars.

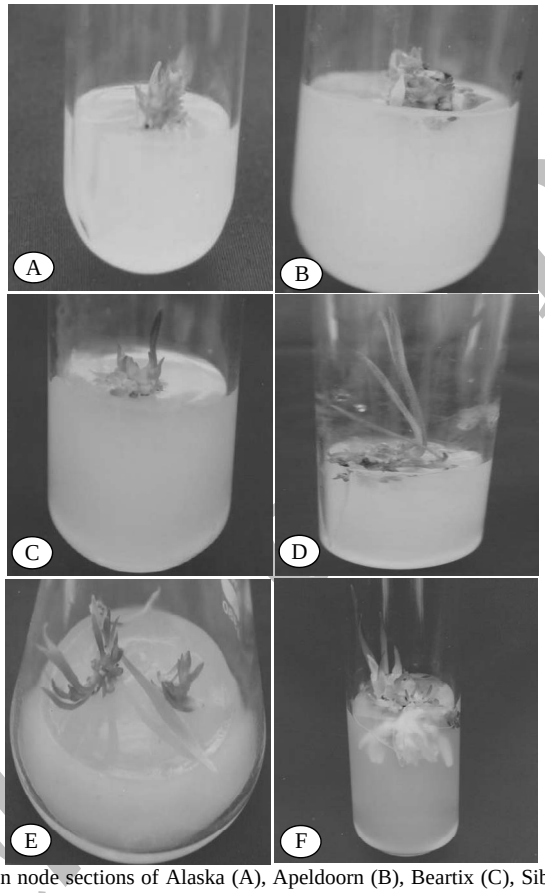

Figure 1. A-F. Bulblets on node sections of Alaska (A), Apeldoorn (B), Beartix (C), Siberia (D) and Marco Polo (E) on MS medium supplemented with 2mg/l NAA and 1.5 mg/l BA., 90 days of culturing, Rooted bulblets on MS medium supplemented with 1.5 mg/l IBA, 30 days of culturing (F).

The rooted bulblets were hardened in cocopeat in plastic pots (4" diameter). Depending upon the cultivar, about 80-82% survival of the bulblets was recorded after 30 days of transfer in pots. From the above results, it may be concluded that the sections of *ex vitro* explants of node in hybrid lilies could regenerate bulblets when cultured on nutrient medium supplemented with growth regulators. The cultivar Beartix produced heaviest bulblets, which is the basic need for vigour and growth of the plant for production of quality flowers.

Table 1. Analysis of variance (ANOVA): Effect of NAA, BA, cultivar and their interaction on percent explants regeneration in hybrid lilies.

Treatment (mg/l)		Asiatic hybrids		Oriental hybrids Mean			Mean
NAA	BA	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	
0	0	0.00	0.00	0.00	0.00	0.00	0.00
		(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
1	0	36.00	58.00	57.67	54.00	52.00	55.53
		(48.45)	(49.61)	(49.41)	(47.30)	(46.15)	(48.18)
2	0	56.00	58.00	54.33	54.00	52.67	55.00
		(48.55)	(49.61)	(47.49)	(47.30)	(47.10)	(47.99)
0	1.5	58.00	56.00	58.00	56.00	53.67	56.33
		(49.61)	(48.45)	(49.16)	(48.45)	(46.53)	(48.53)
0	2	60.00	60.33	60.67	55.33	53.00	57.87
		(50.77)	(50.97)	(51.16)	(40.07)	(46.72)	(49.54)
1	1.5	67.33	70.00	69.33	70.00	69.00	69.13
		(55.18)	(56.79)	(56.38)	(56.79)	(56.17)	(54.97)
1	2	68.00	63.00	64.67	69.33	70.00	67.00
		(55.55)	(52.54)	(53.57)	(56.38)	(56.79)	(54.97)
2	1.5	76.67	75.67	70.00	72.00	73.00	74.20
		(61.14)	(60.48)	(56.79)	(58.06)	(58.70)	(59.03)
2	2	78.00	68.67	72.33	75.67	75.00	73.93
		(62.03)	(56.00)	(58.27)	(60.45)	(60.00)	(59.35)
Mean		55.96	56.63	56.33	56.26	55.38	
		(48.13)	(48.59)	(48.53)	(48.50)	(41.05)	

LSD (P=0.05) Treatment (A) = (0.80); Cultivar (B) = (0.05); AxB= (1.10)
Figures in parentheses are arc-sine transformed values

Table 2. Effect of NAA, BA, cultivar and their interaction on average number of bulblets in hybrid lilies.

Treatment (mg/l)		Asiatic hybrids			Oriental hybrids		Mean
NAA	BA	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	
0	0	0	0	0	0	0	0
1	0	2.33	2.00	1.66	1.00	1.66	1.73
2	0	1.66	1.66	1.66	1.66	2.00	1.73
0	1.5	1.69	2.00	1.33	1.66	1.66	1.66
0	2	1.69	2.00	2.66	2.69	3.33	2.46
1	1.5	2.33	2.66	2.33	3.00	2.00	2.46
1	2	2.66	2.33	2.00	1.66	2.33	2.20
2	1.5	1.76	2.33	4.00	2.67	3.00	2.73
2	2	4.00	2.67	2.00	2.33	3.66	2.93
Mean		2.02	1.96	1.96	1.85	2.18	

LSD (P=0.05) Treatment (A) =0.56; Cultivar (B) =0.37; AxB =0.78

Table 3. Effect of NAA, BA, cultivar and their interaction on average fresh weight (mg) in hybrid lilies.

Treatment(mg/l)		Asiatic hybrids			Oriental hybrids		Mean
NAA	BA	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	
0	0	0	0	0	0	0	0
1	0	134.30	126.30	115.30	133.00	143.00	130.30
2	0	125.00	133.30	151.30	143.30	128.00	136.10
0	1.5	144.00	192.00	202.00	135.30	194.00	173.40
0	2	193.70	204.30	183.30	172.30	162.70	183.20
1	1.5	196.30	208.70	203.00	218.30	243.30	214.00
1	2	208.00	265.30	196.70	187.00	193.00	210.10
2	1.5	307.30	351.70	401.30	301.00	262.70	324.80
2	2	221.70	238.30	339.70	291.00	211.30	260.40
Mean		172.25	190.88	199.22	164.54	170.96	

LSD (P = 0.05) Treatment (A) = 1.30; Cultivar (B) = 0.93; A x B = 2.10

Table 4. Effect of IBA, cultivar and their interaction on percent rooting in hybrid lilies.

Treatment(mg/l)	Asiatic hybrids			Oriental hybrids		Mean
IBA	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	
0	41.67	47.67	43.00	41.67	42.00	43.20
	(40.20)	(43.60)	(40.98)	(40.20)	(40.40)	(41.09)
0.5	69.00	73.00	69.67	83.00	87.33	76.40
	(56.17)	(58.70)	(56.61)	(65.66)	(69.18)	(61.26)
1	72.67	76.00	73.00	86.67	86.00	78.87
	(58.49)	(60.67)	(58.70)	(68.63)	(68.04)	(62.90)
1.5	83.67	76.33	80.33	85.00	79.00	80.87
	(66.23)	(60.90)	(63.70)	(67.26)	(62.73)	(64.16)
2	86.00	76.33	72.67	86.33	77.00	79.67
	(68.04)	(60.90)	(48.49)	(68.36)	(61.39)	(63.43)
Mean	70.60	69.87	67.73	76.53	74.27	
	(57.83)	(56.97)	(55.69)	(62.01)	(60.35)	

LSD (P =0.05) Treatment (A) = (1.00); Cultivar (B) = (1.00); A x B = (1.30)
Figures in parentheses are arc-sine transformed values

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