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Improved Micro Propagation Protocol in *Lilium hybrida* var. Pollyanna

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Abstract: *In an experiment conducted on* invitro *propagation of Asiatic* Lilium hybrida *cv. Pollyanna. Bulb scales were used as the explants. In vitro culture media for efficient regeneration of Lilium bulb scales were standardized. Pre-treatment with carbendazim (0.2%) + 8-HQC (200 ppm) + GA_3 (100 ppm) for 2 hours followed by surface sterilization with 0.1% mercuric chloride for 7 min. resulted highest healthy cultures (80%). MS medium supplemented with 0.2 mgl^{-1} NAA and 1.0 mgl^{-1}BAP was the most effective combination for inducing and shoots proliferation. Rooting was best achieved on half-strength MS medium supplemented with 1.0 mgl^{-1} IBA, where the rooting was 100%.*

Key words*: Bulb scales, Asiatic hybrid lily,* In vitro *culture, MS medium*

Lilies (*Lilium* sp.) is one of the most fascinating bulbous flower crops used as cut flower and potted plant all over the world. Among various types of lilies, hybrids of Asiatic and oriental lilies and *L. longiflorum* seems to be the most promising in florist trade. Asiatic lilies are quite popular mainly because of its large flower size, an array of petal colors, forms, long keeping quality and capacity to rehydrate after continuous transportation. Lilies are usually propagated by scaling, a technique which produces 3-5 bulbs from each bulb scale, depending on species, cultivar and scale size. Scale propagation makes it difficult to obtain large numbers of bulbs from disease-free stock or new cultivars in a short period of time (Stimart and Ascher, 1978). Bulbous plant, like lily, has proved to be ideal for tissue culture, as it's regeneration potential is usually high (Chu and Kurtz, 1990). Therefore, *in vitro* propagation is essential to produce large numbers of bulblets in short period to meet the demand of the industry. Though bulb scales are most commonly used as explants for regeneration, being under-ground parts there is a high contamination risk (Pelkonen, 2005). This can be overcome by careful pretreatment and sterilization procedures. Although the different culture media have not been compared in terms of *in vitro* growth of lilies, the MS medium is presently the most used one. Culture initiation and differentiation can be promoted by adjusting phytohormone concentrations. Keeping in view the potentialities of tissue culture, the present studies were conducted to describe a simple method for mass multiplication of Asiatic hybrid lily (*L. hybrida* L) cv. Pollyanna through tissue culture at IARI, New Delhi during 2006-07.

MATERIALS AND METHODS

Preparation of material

Bulbs of Asiatic lily cv. Pollyanna (10-12 cm size) previously kept in cold storage at 4 ^{0}C for 6 weeks were used for culturing the scales in MS medium. All outer damaged or diseased scales were removed and discarded. Bulb scales were rinsed with running tap water for 20-30 min. until all attached soil particles were removed. Three procedures of pre-treatments with carbendazim (0.2%), mancozeb (0.2%), 8-HQC (200 ppm) and GA_3 (100 ppm) were employed. The pretreated bulb scales were swirled with 0.1% $HgCl_2$ for 0, 5, 7 and 10 min. durations to

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find out the best duration for surface sterilization. Full strength MS basal and half strength MS media supplemented with 3% sucrose and agar-agar @ 0.8% w/v along with one conc. of auxin, NAA (0.2 mgl^{-1}) and three conc. of cytokinin, BAP (0.5, 1.0 and 2.0 mgl^{-1}) was tested for shoot initiation and multiplication. Cultures were incubated at 25°C under white fluorescent light and light/dark cycle of 16/8 hours. For rooting, micro-shoots were transferred on to half strength MS medium supplemented with two concentrations of auxins, namely IBA (0.5 and 1.0 mgl^{-1}) and NAA (0.5 and 1.0 mgl^{-1}). All cultures were sub-cultured on fresh medium in every four weeks.

Statistical analysis

Three replications with 15 explants in each replications were maintained for each treatment and data were analyzed statistically using CRD/ factorial CRD. The statistical analysis based on mean values per treatment was made using the technique of analysis of variance (SAS Institute, 2002). The comparative LSD multiple range test (P= 0.05) was used to determine difference between treatments. Percentage data were transformed using Arc sin transformation before analysis.

RESULTS AND DISCUSSION

Effect of different pre treatments on in vitro culture establishment

The bulb scales of *Lilium hybrida* var. Polyanna were subjected to various pre-treatments to break the dormancy and to establish contamination-free cultures. Table 1 summarizes that the highest explant survival percentage (66.67) was recorded when the bulb scales were agitated in carbendazim (0.2%) + 8 HQC (200 ppm) + GA_3 (100 ppm) for 2 hours followed by treatment comprising carbendazim (0.2%) + 8 HQC (200 ppm) + GA_3 (100 ppm) + mancozeb (0.2%) (62.22%). Significantly highest (64.44%) shoot bud sprouting was observed in bulb scales treated with carbendazim (0.2%) + 8 HQC (200 ppm) + GA_3 (100 ppm) for 2 hours compared to control (37.78%). The bulb scale segments pre-treated with carbendazim (0.2%) + 8 HQC (200 ppm) + GA_3 (100 ppm) for 2 hours showed reduced duration (15.67 days) for bud sprouting as compared to those treated with carbendazim (0.2%) + 8 HQC (200 ppm) + GA_3 (100 ppm) + mancozeb (0.2%) (18.3 days) and control (16.33 days). Previously, Kawarabayashi and Asahira (1989) had also effectively used these two chemicals to control the microbial contaminations in bulb scale explant of *Lilium*. The existence of dormancy strongly affects the morphogenesis of differentiating shoots in lilium. Hence, GA_3 have been commonly used for overcoming the dormancy present in bulb scales (Aguettaz *et al*., 1990; De Klerk, 1992).

Effect of surface sterilization on in vitro explant survival

Surface sterilization with 0.1% $HgCl_2$ for 7 min. (Fig. 1) resulted in the highest (95.55%) explant survival. The maximum bud sprouting (88.89%) was

Table 1
Effect of different pre-treatments on culture establishment from bulb scale segments of *Lilium hybrida* cv. Polyanna

Treatment details	*Explant survival (%)*	*Bud sprouting (%)*	*Healthy cultures (%)*	*Duration required for sprouting {days)*
GA_3 (100 ppm) + Distilled water	42.22 [a]	37.78 [b]	09.89 [a]	16.33 [ab]
(Control)	(40.45)*	(37.79)	(17.85)	
Carbendazim (0.2%) + 8 HQC (200 ppm)	66.67 [a]	64.44 [a]	62.34 [b]	15.67 [b]
+ GA_3 (100 ppm)	(55.01)	(56.10)	(55.35)	
Carbendazim (0.2%) + Mancozeb (0.2%)	62.22 [a]	53.33[ab]	52.48 [b]	18.33 [a]
+ 8 HQC (200 ppm) + GA_3 (100 ppm)	(52.11)	(46.90)	(45.45)	
S.Em ±	7.73	5.33	4.56	0.72
$CD_{P=0.05)}$	26.70	18.46	15.75	2.50

Means with the same alphabetical letters are not significantly different.

• Values in parenthesis indicate arc sin transformation

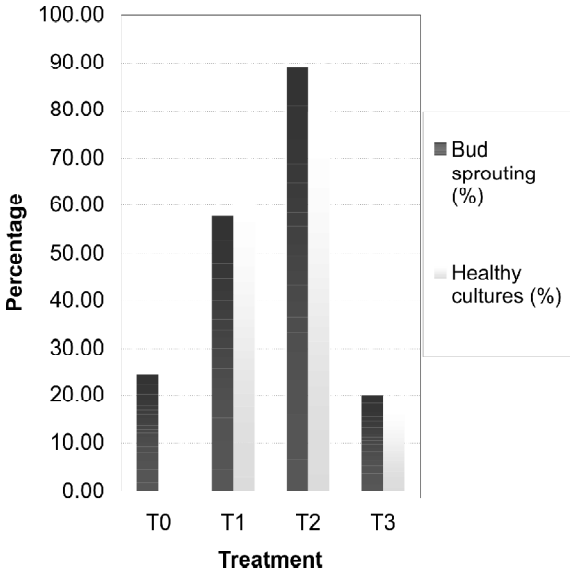

Figure 1: Effect of mercuric chloride (0.1%) on surface sterilization of bulb scale segments of *Lilium*

observed when bulb scales were agitated in 0.1% $HgCl_{2\ for}$ 7 min., which was significantly higher from all other treatments. The healthiest cultures (80.00%) were achieved when the explants were treated with 0.1% $HgCl_2$ for 7 min. Shorter exposure time (5 min.) in 0.1% $HgCl_2$ resulted in quicker sprouting (15.67 days) followed by treatments comprising 7 and 10 min. with 16.33 and 18.0 days for bud sprouting respectively. The highest number of shoots (5.66) per explant was achieved when exposed for 7 min.

Effect of media modifications on in vitro culture establishment

Significantly higher bud sprouting percentage was observed on full-strength MS medium (92.25%) as compared to half-strengh MS medium (65%) (Table 2). The explant cultured on full-strength MS medium sprouted earlier (16.92 days) than those cultured on half-strength MS medium, which took 20.08 days. The highest number of shoots (4.42) was recorded on full-strength MS medium as compared to the half-strength MS medium (3.75). The highest number of leaves (4.13) was observed in full-strength MS medium compared to half- strength MS medium (3.5). Though there was no significant difference between the two media for average length of leaves, the highest average value (5.82 cm) was observed on full-strength MS medium. MS medium containing higher concentrations of macro- and micro-elements may have induced early growth on bulb scales (Niimi, 1995; Pinsan *et al*., 2000; Chunlin *et al*., 2004; Sevimay *et al*., 2005).

Effect of NAA and BAP on culture establishment and shoot regeneration

The cultures were incubated on MS medium supplemented with 0.2 mgl^{-1} NAA + 1.0 mgl^{-1} BAP exhibited the maximum bud sprouting (98.78%) (Table 3).Significantly lowest duration required for bud sprouting (14.66 days) was observed on MS medium supplemented with 0.2 mgl^{-1}NAA + 1.0 mgl^{-1} BAP over the other treatments. Significantly higher number of shoots per explant was observed in MS medium supplemented with 0.2 mgl^{-1} NAA + 1 mgl^{-1} BAP (6.33). Control treatment resulted in least number of shoots, which were 3.33 shoots per explant. Maximum number of leaves (6.0) was recorded on MS medium supplemented with 0.2 mgl^{-1} NAA + 1.0 mgl^{-1} BAP . Longest length of leaves

Table 2
Effect of MS medium modifications on *in vitro* culture establishment on bulb scale segments of *Lilium hybrida* cv. Polyanna

Medium	*Sprouting (%)*	*Duration for sprouting (days)*	*Number of shoots per explant*	*Number of leaves per shoot*	*Av. length of leaves (cm)*
Full-strengh MS	92.25 [a] (75.56)*	16.92 [a]	4.42 [a]	4.13 [a]	5.82 [a]
Half-strengh MS	65.00 [b] (53.85)	20.08 [b]	3.75 [a]	3.50 [a]	4.76 [a]
S.Em ±	3.22	0.45	0.23	0.25	0.38
$CD_{(P=0.05)}$	12.22	3.41	0.88	0.83	1.26

Means with the same alphabetical letters are not significantly different.

* Values in parenthesis indicate arc sin transformation

(8 cm) was achieved with 0.2 mgl^{-1}NAA + 1.0 mgl^{-1} BAP whereas it was shortest in control (3.7 cm). Mean number of 7.4 shoots per subculture was achieved (in avg. of 5 sub-cultures) with 90.5% bud sprouting on MS medium supplemented with 0.2 mgl^{-1} NAA and 1.0 mgl^{-1}BAP (Fig. 2)

Table 3
Effect of NAA and BAP supplemented in MS basal medium on *in vitro* culture establishment and shoot regeneration of *Lilium hybrida* cv. Polyanna.

Treatment Number	*Growth regulators (mg/L)*	*Bud Sprouting (%)*	*Duration required for bud sprouting (days)*	*Number of shoots per explant*	*Number of leaves per shoot*	*Average length of leaves (cm)*
1	No hormone (Control)	84.11 [c] (66.55)*	19.33 [a]	3.33 [b]	2.8 [b]	3.73 [b]
2	NAA (0.2) + BAP (0.5)	92.99 [b] (74.63)	17.33 [b]	4.0 [b]	3.9 [b]	4.13 [b]
3	NAA (0.2) + BAP (1.0)	98.78 [a] (86.28)	14.66 [c]	6.33 [a]	6.0 [a]	7.40 [a]
4	NAA (0.2) + BAP (2.0)	93.11 [b] (74.77)	16.33 [b]	4.0 [b]	3.7 [b]	8.00 [a]
S.Em ±		1.18	0.34	0.47	0.56	0.58
$CD_{(P=0.05)}$		3.86	1.09	1.54	1.94	1.88

Means with the same alphabetical letter are not significantly different.

* Values in parenthesis indicate arc sin transformation

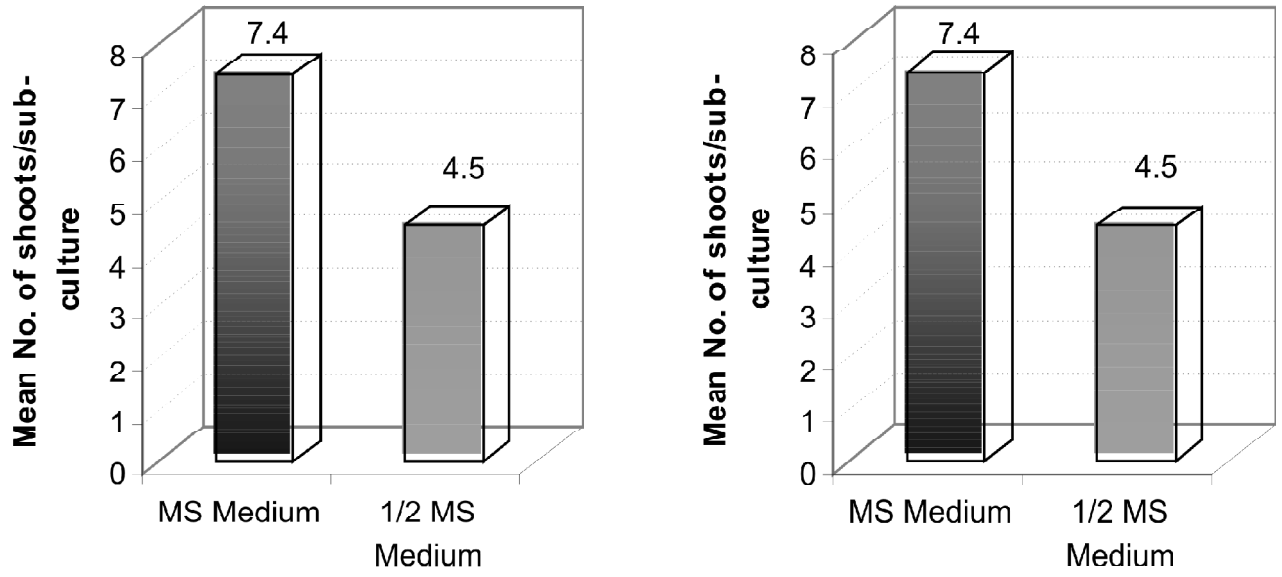

Figure 2: Mean number of shoots and bud sprouting (%) per subculture in *in vitro* multiplication of *Lilium*

Growth regulators have a very important role in both induction of differentiation and growth. Regeneration of bulblets on growth regulator-free media indicates that endogenous hormones involved in such morphogenic responses were already present in the explants. This can also be explained with the fact that bulb scales form new bulblets in *in vivo* scale propagation easily without exogenous growth regulators. Hence, addition of growth regulators to the culture media is not necessary to regenerate bulblets from bulb scale explants, as reported for other *Lilium* species (Novak

and Petru, 1981 and Niimi, 1995). The use of cytokinins in combination with auxins on the morphogenesis of differentiating shoots from lilium bulb scales have earlier been studied by Niimi (1995) and Sevimay *et al.*, (2005).

In vitro rooting of bulblets

Pronounced effects of NAA and BAP were observed on in vitro rooting (Table 4). Highest rooting (100%) in shortest duration (15.6 days) with maximum number of primary roots per shoot (8.66) were obtained in ½ MS medium supplemented with 1.0 mgl^{-1} IBA (100%), followed by 0.5 mgl^{-1} IBA (97.77%). Synthetic auxin NAA at 0.5 and 1.0 mgl^{-1} did not show any significant differences for rooting (82.22 and 84.44%, respectively) but values are lower than the IBA treatments. Least rooting (66.66%) was observed in control (without hormones). Micro-shoots cultured on hormone-free medium (control) took longest duration for root initiation (23 days). The highest mean root length was also recorded with IBA 1.0 mgl^{-1} (4.96 cm), followed by 0.5 mgl^{-1} IBA (3.66 cm). These results are in conformity with those reported by Maesato *et al.*, (1991) in *L. japonocum* and Kawarabayashi and Asahira (1989) in *L. speciosum* cv. 'Uchida.

Plate: 1. Different stages of micro propagation

However, according to the results of the present study, supplementation of NAA, 0.5 and 1.0 mgl^{-1} to the half-strength MS medium were also quite good as compared to control. These results are in close conformity with reports of Sevimay *et al.* (2005) and Chauvin *et al.* (2005), who obtained better rooting of micro-shoots on half-strength MS

Table 4
Effect of NAA and IBA on *in vitro* rooting of *Lilium hybrida* cv. Pollyanna.

Treatment Number	*Growth regulators (mg/L)*	*Rooting (%)*	*Duration for root initiation (days)*	*Number of primary roots per shoot*	*Mean length of roots after 4 wks (cm)*
1	½ MS + No hormone (Control)	66.66 [b] (59.00)*	23.00 [a]	3.66 [b]	1.76 [b]
2	½ MS + NAA (0.5)	82.22 [ab] (78.32)	18.00 [ab]	6.00 [ab]	2.60 [ab]
3	½ MS + NAA (1.0)	84.44 [ab] (80.54)	17.33 [b]	8.00 [ab]	2.83 [ab]
4	½ MS + IBA (0.5)	97.77 [a] (87.87)	16.33 [b]	7.66 [a]	3.66 [ab]
5	½ MS + IBA (1.0)	100.00 [a] (89.96)	15.66 [b]	8.66 [a]	4.96 [a]
S.Em ±		7.65	1.63	1.3	0.76
$CD_{(P=0.05)}$		24.03	5.23	4.16	2.45

Means with the same letter are not significantly different.

* Values in parenthesis indicate arc sin transformed

medium fortified with 0.3 mgl^{-1} NAA. Both endogenous and synthetic auxins act synergistically and induce root induction.

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