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Low cost strategy for micropropagation of *Lilium* Asiatic hybrid cv. Toscana

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ABSTRACT

A low cost protocol for *in vitro* propagation of *Lilium* cv. Toscana has been developed through incorporation of cost-effective media components. MS medium supplemented with 0.75 mg l^{-1} BAP (6-benzylaminopurine) and 0.5 mg l^{-1} NAA (α-naphthalene-acetic acid) was prepared with tapioca granules, table sugar and tap water in different combinations in place of agar-agar, sucrose and distilled water, respectively. Culture medium containing all the cost effective components was found to be the best for *in vitro* establishment of cultures yielding 6.00 bulblets per explant and medium supplemented with tapioca granules as cost effective component was found to be the best for *in vitro* multiplication of bulblets giving 3.70 bulblets per *in vitro* formed bulblet five weeks from third subculture. Tapioca supplemented MS medium containing 1 mg l^{-1} NAA was significantly better than all the other modified media giving 86.62% *in vitro* rooting, 2.86 average root number and 4.60 cm average root length. For hardening of *in vitro* rooted bulblets, coco peat, peat moss and coco peat in combination with peat moss were found to be at par giving 100% survival.

Key words: *Lilium*, micropropagation, low-cost strategy

INTRODUCTION

Lilium, a monocot belonging to the family Liliaceae, is one of the leading cut flowers of the world. It has become commercially important due to its bold, beautiful and fascinating form of flowers, long vase life and capacity to rehydrate after long transportation. *Lilium* is native to the Northern hemisphere and is widely distributed over China, South Canada, Siberia and extends upto Florida in USA. In India, *Lilium* is found in Nilgiri hills and in the Himalayan region.

Lilium can be propagated by both sexual and asexual means. Most commercially grown cultivars are propagated through scaling, a technique which produces 3-4 bulbs from each bulbscale depending upon bulbscale size and variety. Though a bulb under ideal conditions may yield anywhere between 50 to 100 bulblets, this rate is far too low to meet the present day demand for planting material. Also, reduced vigour of bulbs with repeated cycles of vegetative propagation is reported which may be due to accumulation of soil borne diseases (Van Aartrijk and Blom-Barnhoorn, 1983). Thus, mass propagation through tissue culture is needed for research and development of the *Lilium* industry. Cost effective micropropagation would facilitate commercialization of the technology.

In this paper, we describe a rapid and low-cost protocol for micropropagation of *Lilium* using cheaper medium components.

MATERIAL AND METHODS

Bulbs of *Lilium* cv. Toscana were collected from a private nursery at Darang, Palampur (HP), India. Bulbscales were excised from mother bulbs of 12cm -14 cm diameter stored in saw dust at 5°C for six weeks after harvest. The scales were surface sterilized in 0.2% solution of bavistin (carbendazim) for 8-9 min., washed with sterile water and treated with 0.1% solution of $HgCl_2$ for 3 min., followed by thorough washing with sterile water. For *in vitro* establishment of cultures, basal segment each of about $1cm^2$ was excised from surface sterilized scales and inoculated onto standard MS medium (Murashige and Skoog, 1962) and MS medium modified by replacing sucrose, distilled water and agar-agar with table sugar, tap water (potable drinking water) and tapioca granules (*Manihot esculentum*), respectively (Table 1). MS medium (standard and modified) was supplemented with BAP (0.75 mg l^{-1}) and NAA (0.5 mg l^{-1}).

On formation of *in vitro* bulblets, these were separated and individually subcultured both on standard as

well as modified media (Table 1). After 3-4 subcultures each of 4-5 weeks, *in vitro* induction of rooting was attempted in *in vitro* formed bulblets on MS standard medium and MS modified media supplemented with NAA (Table 2).

All the media compositions shown in Tables 1 & 2 were supplemented with 3 mg/l thiamine-HCl, 0.1g/l inositol, 3% sucrose; pH was adjusted to 5.8 before autoclaving at 121 °C and 15 psi for 15 min. Cultures were maintained at 25±2°C under 16 h photoperiod provided by cool, white, fluorescent lamps (40 µmole $m^{-2} s^{-1}$). Survival and establishment of *in vitro* rooted bulblets was studied after transplanting the bulblets into pots containing different soil mixtures (Table 3). These were kept at 25 °C under 16 h photoperiod of 3000 lux intensity and 75% relative humidity. All experiments were repeated thrice with 72 replicates. Data recorded for different parameters were subjected to completely randomized design (CRD) (Gomez and Gomez, 1984). Statistical analysis based on mean values per treatment was made using analysis of variance (ANOVA) technique of CRD.

RESULTS AND DISCUSSION

Pre-treatment of bulbscale segments with 0.2 % carbendazim solution for 8-9 min. followed by 0.1 % solution of $HgCl_2$ for 3 min. yielded 94% cultures free from contamination.

In vitro induction of bulblets and *in vitro* multiplication of bulblets on standard MS medium supplemented with 0.75 mg l^{-1} BAP and 0.5 mg l^{-1} NAA was carried out to standardize a general protocol for micropropagation of *Lilium* cv. Toscana. The same protocol was modified by adding different but cheaper components into the medium. There were significant differences among different media in terms of number of bulblets per explant as well as the rate of multiplication of bulblets. Among these media, the maximum number of bulblets per explant (8.0) was produced on M_5 medium (Table 1) having all the cost effective components such as tapioca granules, table sugar and tap water. The least effective modified medium was M_4 having tapioca alone as the cost effective component,which produced 1.24 bulblets per explant. . *In vitro* induced bulblets when multiplied on modifified M_3 medium gave the maximum multiplication rate of 3.70 bulblets per *in vitro* bulblet. The lowest multiplication rate of 1.46 bulblets per bulblet was obtained on M_5 medium (Table 1).

For induction of rooting, *in vitro* formed bulblets were separated and cultured singly on various rooting media (Table 2). Out of four modified media, R_3 having tap water as the cost effective component was the best, yielding 86.62% rooting, 2.86 average root number and 4.6 cm average root length. It was followed by R_2 with 74.25 % rooting, average root number 2.19 and average root length 1.35 cm. Out of all the cost effective media, the least effective medium for *in vitro* induction of rooting was R_4

Table 1. *In vitro* establishment of scale segments and *in vitro* bulblet multiplication of *Lilium* cv. Toscana on standard MS medium (1962) and modified MS medium

MS basal medium +BAP (0.75 mgl^{-1}) + NAA (0.5 mgl^{-1})	Type of gelling agent	Type of sugar used (30 gl^{-1})	Type of water used	No. of bulblets per basal segment (at seven weeks from inoculation)	Rate of multiplication of bulblets (at six weeks from subculture)
M_1 (standard medium)	Agar-agar (8g/l)	Sucrose	Distilled water	11.20	5.75
M_2 (modified medium)	Agar-agar (8g/l)	Table sugar	Distilled water	3.20	3.20
M_3 (modified medium	Agar-agar (8g/l)	Sucrose	Tap water	4.40	3.70
M_4 (modified medium)	Tapioca granules (125/l)	Sucrose	Distilled water	1.24	1.95
M_5 (modified medium)	Tapioca granules (125/l)	Table Sugar	Tap water	8.00	1.46

Table 2. *In vitro* induction of rooting in *in vitro* bulblets of *Lilium* cv. Toscana on standard MS medium (1962) and modified MS at six weeks from culture

MS basal medium +NAA (1mg l^{-1})	Type of gelling agent	Type of sugar used (20 gl^{-1})	Type of water used	Rooting percentage	No. of roots per bulblet	Root length (cm)
R_1 (standard medium)	Agar-agar (8g/l)	Sucrose	Distilled water	100.00	3.42	4.42
R_2 (modified medium	Agar-agar (8g/l)	Table Sugar	Distilled water	74.25	2.19	1.35
R_3 (modified medium	Agar-agar (8g/l)	Sucrose	Tap water	86.62	2.86	4.60
R_4 (modified medium	Tapioca granules (125/l)	Sucrose	Distilled water	59.58	1.65	0.45
R_5 (modified medium)	Tapioca granules (125/l)	Table Sugar	Tap water	72.41	2.02	0.86
CD at 5%				4.94	0.62	-

having tapioca as the cost effective component, yielding 59.58% rooting, average root number 1.65 and average root length 0.45 cm. R_3 medium was significantly better than the other modified media. R_5 and R_2 were statistically at par with respect to rooting percentage and number of roots per bulblet.

In vitro rooted plantlets, generated on different modified media, were transferred to different potting mixtures (Table 3). A total of 600 plantlets were transferred. 100% survival rate was achieved on P_1, P_2 and P_4 and only 61.88% of *in vitro* rooted bulblets survived on P_3 at one month from transplantation.

Tissue culture has a number of advantages in *Lilium* propagation. Though the advantages of micropropagation are tremendous, cost is a limiting factor. Attempts have been made in the present investigation to explore the possibility of cost reduction in micropropagation of *Lilium*. In the present study 94% cultures free from contamination were obtained by treating the explants with 0.2% carbendazim solution for 8-9 min. and 0.1% solution of $HgCl_2$ for 3 min. Priyadarshi and Sen (1992) achieved a high rate of sterilization using Bavistin (0.2%). Novak and Petru (1981) Rybczynski and Gomolinska (1989) and Dabrowaski *et al* (1992) recommended the use of sodium hypochlorite for successful sterilization of bulbscales of *Lilium* for *in vitro* culture.

M_5 medium consisting of table sugar, tapioca and tap water gave reasonably high number of *in vitro* bulblets per explant (Table 1). Earlier attempts by Sharma *et al* (1992) in 'Colt'- a rootstock of cherry, Ganapathi *et al* (1992) in banana and Okuno *et al* (1996) in *Brassica campestris,* tried to bring down the cost of *in vitro* muliplication on MS medium containing tap water and table sugar as cost effective components of culture medium. The present results are also supported by some earlier findings in *Lilium* cultivars by Jeong *et al* (1996).

In the present investigation, out of four modified media, R3 medium containing tap water was more effective in *in vitro* induction of rooting. Sharma and Singh (1995) in ginger and Kaul (1998) in kiwifruit suggested the use of commercial grade sugar and tap water over sucrose and distilled water, respectively, for *in vitro* shoot multiplication

Table 3. effect of various potting mixtures used for hardening of *in vitro* rooted bulblets of *Lilium* cv. Toscana

Medium	Potting mixture		% Survival at one month from transplantation to field
P_1	Coco peat		100.00
P_2	Peat moss		100.00
P_3	Sand : Soil : FYM	1:1:1	61.86
P_4	Coco peat : Peat moss	1:1	100.00

Chandra *et al* (1990) used table sugar for microtuberization and micropropagation of potato, and, glucose and fructose were found to be better than sucrose in stimulating the formation of embryos in anther culture of many genotypes of *Triticum aestivum* (Chu *et al*, 1990). Last and Brettell (1990) Orshinky *et al* (1990). Nene *et al* (1996) suggested that agar agar can be successfully replaced by tapioca in chickpea micropropagation and tobacco regeneration. Sorvari *et al* (1997) used starch as the gelling agent in *in vitro* germination of encapsulated somatic embryos of carrot (*Daucus carota*).

From the above studies, it may be concluded that table sugar, tapioca and tap water in culture medium can be effectively used at different stages of micropropagation of *Lilium* Asiatic hybrid cv. Toscana. The use of commercial grade sugar in place of the more expensive sucrose, tapioca granules in place of agar-agar and tap water instead of distilled water, could reduce the cost of *in vitro* raised plants thus making micropropagation in this variety a viable proposition for commercialization. Further, this can be also tried for other commercially important cultivars of *Lilium* and ornamental crops.

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