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 I\(^{-1}\) BAP (6-benzylaminopurine) and 0.5 mg I\(^{-1}\) 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 I\(^{-1}\) 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 Aartijk 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 I\(^{-1}\)) and NAA (0.5 mg I\(^{-1}\)).

On formation of *in vitro* bulblets, these were separated and individually subcultured both on standard as
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 \textit{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 \(M_s\) medium (Table 1) having all the cost effective components such as tapioca granules, table sugar and tap water. The least effective modified medium was \(M_t\) having tapioca alone as the cost effective component, which produced 1.24 bulblets per explant. In vitro induced bulblets when multiplied on modified \(M_s\) 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 \(M_t\) 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, \(R_t\) 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 \(R_s\) 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 \(R_t\).
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having tapioca as the cost effective component, yielding 59.58% rooting average root number 1.65 and average root length 0.45 cm. R2 medium was significantly better than the other modified media. R1 and R3 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 P1, P2, and P3 and only 61.88% of in vitro rooted bulblets survived on P4 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 8.5 min. and 0.1% solution of HgCl2 for 3 min. Priyadarshi and Sen (1992) achieved a high rate of sterilization using Bavistin (0.2%). Novak and Petra (1981) Rybczynski and Gomolinska (1989) and Dabrowski et al (1992) recommended the use of sodium hypochlorite for successful sterilization of bulbscales of Lilium for in vitro culture.

M4 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 (1996) in banana and Okuno et al (1996) in Brassica campestris, tried to bring down the cost of in vitro multiplication 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.


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.

REFERENCES


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