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In vitro propagation of *Lilium*

R.K. Pandey, A.K. Singh and Mamta Sharma

Assoc. Prof.(Flori) SKVAST-Jammu Division of Veg. Sci. and Flori, FOA, SKVAST Chatha Jammu INDIA

ABSTRACT : An experiment on *in vitro* propagation of *lilium* was carried out at Biotechnology Centre, Udheywalla, SKUAST- Jammu. The explants (bulb scales) were surface sterilized with 0.1% $HgCl_2$ and 2% Bavistin for 7.5 minutes and inoculated on MS medium supplemented with Kin (0.75mg/l) and NAA (0.5mg/l) which resulted in maximum proliferation. These were subcultured for further multiplication on MS medium fortified with BAP (0.75mg/l) and NAA (0.5mg/l) which resulted in maximum number of 7.2 bulblets per scale explant. The individual bulblet was separated out transferred for rooting in MS medium supplemented with NAA (1.0mg/l). This gave 97.32 per cent rooted cultures and maximum root length of 1.66cm. The plantlets with well developed roots were transferred to potting mixture of cocopeat before transplanting to green house.

Keywords : *Lilium, in vitro*, micro propagation, bulb scales

INTRODUCTION

Lilium, an important genetic resource as an ornamental plant belongs to family *Liliaceae*. It is one of the leading cut flower crops in the world because of beautiful and fascinating form of flowers, long vase life and capacity to rehydrate after long transportation.

Lilium propagation is usually done by vegetative means which produces 3-4 bulbs per bulb scale depending on size and variety. The multiplication efficacy by bulb is low and the plantlets are more susceptible to diseases. Therefore, there is a need to develop a protocol for its mass propagation. With the advent of tissue culture technique, a new era has dawned the research and development of floriculture. Through tissue culture there is not only a continuous supply of bulblets but true-to-type and disease free plants can be obtained.

MATERIAL AND METHODS

Bulbs of *lilium* were collected from Division of Vegetable Science and Floriculture, SKUAST-J, Chatha. The bulbs were thoroughly washed in running tap water for half an hour to remove traces of mud and dirt. The scales were gently teased off from the points of attachment using sterile scalpel blade. The scales were sterilized with Tween-20 for 20 minutes followed by rinsing under tap water to remove traces of detergent. Then further aseptic surface sterilization was carried out with 0.1% $HgCl_2$ and 2% Bavistin under the Laminar Flow Chamber followed by three times washing with sterile distilled water. After surface sterilization the explants were cultured on modified MS medium (Murashige and Skoog, 1962) supplemented with various combinations of different growth regulators viz. NAA, BAP, and Kin.

After establishment, the *in vitro* raised bulblets were separated and cultured individually on multiplication medium. Once suitable multiplication has been achieved, individual bulblets were transferred to rooting medium supplemented with different concentrations of NAA.

All media contained 30g/l sucrose, 0.1 g/l inositol and 8g/l agar. The pH of the media was adjusted to 5.6-5.8 with 0.1N NaOH and 0.1 HCl before autoclaving at 121°C and 15 psi for 15 minutes. All the cultures were incubated in the culture room at 25$\pm$2°C and 70 per cent relative humidity under 16 hours photoperiod and 1.5 kilolux light intensity provided by cool, white, fluorescent lamps. The rooted shoots were carefully removed, washed gently under running tap water to remove adhering agar and kept in 0.5% Bavistin for about half an hour. These were then transferred to pots with different potting mixtures.

All the trials of the experiments were repeated thrice. The data recorded for different parameters were subjected to completely randomized design (Gomez & Gomez, 1984). The statistical analysis based on mean values per treatment was made using analysis of variance (ANOVA) technique of CRD.

RESULTS AND DISCUSSION

An attempt has been made to develop a protocol for the rapid multiplication of *lilium*. The surface sterilization of scale explants with 0.1%$HgCl_2$ and 2% Bavistin for 7.5 minutes resulted in 90.62 per cent of aseptic cultures and 97.32 per cent survival of explants (Table1). Further increase in exposure time to sterilants led to killing of explants which may be due to heavy metal contamination of mercury in $HgCl_2$ proving phytotoxic for the survival of explant. This is in confirmity with those reported by Singh and Tiwari (1998) in jackfruit.

Table 1 : Effect of surface sterilant (0.1% $HgCl_2$ in combination with 2% Bavistin) on survival of explants of *Lilium*.

S. No.	Duration (min)	% of aseptic cultures after 1 week	% survival after 4 weeks
1.	2.5	29.28 (32.71)	25.30 (30.08)
2.	5.0	57.30 (49.20)	49.28 (44.56)
3.	7.5	90.62 (72.38)	97.32 (83.98)
4.	10.0	95.98 (80.98)	81.30 (64.53)
	CD (*P*=0.05)	(6.68)	(6.83)

*Values in the parenthesis are transformed values

The standard MS medium supplemented with different growth regulators was used for the *in vitro* induction and multiplication of bulblets (Table 2). The highest of bulblet proliferation per cent was obtained in MS medium fortified with Kin (0.75 mg/l) and NAA (0.5 mg/l). Similar results were also obtained by Nhut *et.al.* (2006). They observed that MS media fortified with Kin was found to be more effective in producing the complete plants compared with other media.

However, the maximum number of bulblets/explant (7.2) were produced in MS medium supplemented with BAP (0.75mg/l) in place of Kin. This may be due to the fact that BA promotes formation of adventitious buds in excised organs and *in vitro* cultured tissues. (Bhojwani and Johri, 1971). Kaur *et al* (2006) also obtained maximum number of bulblets/explant on MS medium augmented with BAP. It was also observed that with the increase in cytokinin concentration the number of bulblets/explants decreased which is also supported by the observations recorded by Hu and Wang (1983) who reported that high concentrations of cytokinin reduced the number of micropropagated shoots.

The *in vitro* induced bulblets were subcultured on MS medium fortified with BAP (0.75mg/l) and NAA (0.5 mg/l) for further multiplication. The rate of multiplication was maximum after first subculture which gradually declined with increase in subculture.

Auxin promotes adventitious root development in both intact and excised stems. For induction of rooting each bulblet was separated and cultured on MS (half-strength and full-strength) supplemented with different concentrations

Table 2 : Effect of hormonal concentrations and combinations in MS medium on the culture establishment.

S.No	Growth regulators			% proliferation	No. of bulblet/scale
	BAP	Kn	NAA		
1.	0.5	-	0.5	67.96 (55.56)	3.8
2.	0.75	-	0.5	82.62 (65.55)	7.2
3.	1.0	-	0.5	75.98 (60.68)	4.2
4.	-	0.5	0.5	65.30 (53.94)	3.0
5.	-	0.75	0.5	95.98 (80.98)	5.2
6.	-	1.0	0.5	83.96 (66.46)	4.8
	CD (*P*=0.05)			(5.96)	

* Values in the parenthesis are transformed values

Table 3 : Effect of medium and hormonal concentrations on rooting of bulblets.

S.No.	Media	NAA	% rooting after 4 weeks	Root length (cm)
1.	1/2 MS	0.5	35.98 (36.82)	0.38
2.	1/2 MS	1.0	38.64 (38.38)	0.50
3.	1/2 MS	1.5	34.64 (36.02)	0.38
4.	MS	0.5	61.30 (51.54)	0.88
5.	MS	1.0	97.32 (83.98)	1.66
6.	MS	1.5	90.62 (72.38)	1.06
	CD (*P*=0.05)		(5.614)	

of NAA. The best rooting of 100% was obtained on MS (full-strength) medium fortified with NAA (1.0 mg/l) with an average root length of 1.6cm (Table 3). These results are in concurrence with the observations made by Kwarabayahi and Asahira (1989) , Maesatok *et. al.* (1991) in *Lilium japonicum*.

The rooted shoots were successfully transplanted in cocopeat. After complete plantlet formation with well developed roots and leaves, plantlets were shifted to greenhouse.

REFERENCES

Bhojwani, S.S. and Johri, B. M. 1971. Morphogenetic studies on cultured mature endosperm of *Croton bonplandianum. New Phytal*. **70:** 761-766.

Gomez, K.A. and Gomez, A.A. 1984. Statistical procedures for agricultural research . p680. 3rd edn, John Wiley & Sons, Singapore.

Hu, Cy. and Wang, P.J. 1983. Meristem shoot tip and bud culture. In Evans DA, Sharp WR, Ammirato PV and Yamada Y (eds) . Handbook of Plant Cell Culture **Vol I** : 177-227.

Kaur, R., Thakur, N. and Sharma, D.R. 2006. Low cost strategy for micropropagation of *Lilium* Asiatic hybrid cv. Toscana. *J. Hort. Sci*. **1**(1)**:** 24-27.

Kawarabayahi, W. and Asahira, T. 1989. *In vitro* multiplication of virus-free bulbs of lilies *J. Japanese Soc. Hort. Sci.* **58:** 195-209.

Maesatok, Sarma, K. S., Fukui, H. and Hara, Z. 1991. *In vitro* bulblet induction from shoot apices of *Lilium japonicum. Hort. Sci*, **26:** 211.

Murashige, T. and Skoog, F. 1962. A revised medium for rapid growth and bioassays with tobacco tissue culture. *Physiol .Pl*. **15:** 473-497.

Nhut, D.T., Tam, N.T.D., Luan, V.Q., Thein, N.Q., Minh, N.T., Du T.X. and Le, B.V. (2006). Standardization of *in vitro* Lily (Lilium spp.) plantlet for propagation and bulb formation. Proceedings of International Workshop on Biotechnology in Agriculture.

Singh, R. and Tiwari, J.P. 1998. *In vitro* clonal propagation of jackfruit (Ar*tocarpus heterophyllus* lamk.). *Indian J. Hort.* **55**(3)**:** 213-217.