Bionature, 34 (1), 2014 : 15-20

IN VITRO REGENERATION AND BULBLET PRODUCTION IN ASIATIC *LILIUM*

MANISH KAPOOR* AND AMARJEET SINGH

Department of Botany, Punjabi University, Patiala-147001 Punjab INDIA
Email: jdmanishkapoor@yahoo.com

(Accepted: April, 1st 2014).

ABSTRACT

Bulb scale explants of two Asiatic hybrids of Lilium, viz. Bengalope and Vivaldi cultured on MS medium supplemented with NAA (0.1 mg/L) exhibited early bud initiation, higher number of shoot buds and more shoot length than other media combinations. The initiation and elongation of buds was delayed in the explant cultured on the media supplemented with NAA (0.5 mg/L) or BAP (0.01, 0.05 and 0.1 mg/L), whereas, the buds failed to elongate in the media containing both NAA and BAP. The root formation was obtained only in media containing NAA (0.1 and 0.5 mg/L) and BAP (0.01 mg/ 1), however, the number and length of roots were more on medium containing NAA (0.1mg/L). The explants cultured on MS media containing NAA or BAP alone exhibited bulblet formation, however, the number and size of bulblets was maximum on the medium containing NAA (0.1 mg/L). The maximum bulblet size was in the half strength MS medium containing NAA (0.1 mg/ L) and elevated level of sucrose (9%).

Keywords : *Lilium, in vitro* regeneration.

Introduction

Lilium is a top ranking cut flower in the global flower trade ln India, the production of Asiatic hybrid lilies has increased considerably for cut flower production in the recent years. Lilies are propagated vegetatively through bulbs, stem bulblets, aerial stem bulbils and adventitious bulblets. However, it is susceptible to many viral as well as fungal diseases. The systemic built up of the pathogens due to vegetative propagation adversely deteriorates the crop performance, generation after generation (Anonymous 1983). The tissue culture technique, therefore, offers an alternative method for the mass production of bulblets and maintenance of disease free germplasm. Aartrijk *et al* (1990) reported the *in vitro* bulblet production through adventitious bud formation from the bulb scale leaves, cotyledons, stem segment, petals, sepals, peduncles, pedicels, stamens and ovary explants. However, *in vitro* bulblet production through bulb scales has been reported to be the most suitable method for commercial propagation in *Lilium* (Kawarabayashi and Asahira 1989, Lesham et al 1982, Park el al 1996). Since Asiatic *Lilium* hybrids have gained significant importance in Northwestern hills and plains as commercial crop, the present studies were conducted to work out the protocol for *in vitro* regeneration and bulblet production in two Asiatic *Lilium* cvs., viz. Bengalope and Vivaldi

Material and Methods

Healthy bulbs of Asiatic *Lilium* hybrid cvs., Bengalope and Vivaldi were selected and washed thoroughly in tap water containing teepol (0.1 percent). The outermost scale layer of bulbs was removed and the scales from the next two layers were used as

explant. The scales were rinsed in 70% ethyl alcohol and surface sterilized with 0.1% mercuric chloride for two minutes. Subsequently, the scales were cut vertically into two pieces and then cultured aseptically in the Murashige and Skoog (MS) (1962) basal medium containing sucrose (3%) and gelled with agar (7g/l). The medium was further supplemented with different concentrations of NAA and BAP, as mentioned in Tables 1 to 3. The explants were placed aseptically with abaxial surface on the medium and incubated at 23±2°C under 16 hour light (3500lux intensity) and 8 hours dark condition.

Observation were recorded for days to bud initiation, number of shoot buds per bulb scale, shoot length (cm), days to root formation, number of roots per bulblet, average root length (cm), number of bulblets per explant and average diameter of bulblets (cm) after 8 weeks of incubation.

In order to study the effects of sucrose concentration in the medium on the growth of the bulblet, the bulb scale explants were incubated on half strength MS medium containing NAA (0.1mg/L and 0.5mg/L) and 3, 6 or 9% sucrose. The explants were sub cultured on the same media after 8 weeks. The observations were recorded after 16 weeks for the diameter of the bulblets. The data presented are a mean of five replications each representing five cultures.

Results and Discussion

Bud initiation:

Among the various media evaluated the earliest bud initiation (25.53 and 26.67 days) took place in MS medium containing NAA (0.1mg/L), closely followed by the MS medium containing NAA 0.5mg/L (27.00 and 27.60 days) in both the cultivars, Bengalope and Vivaldi, respectively (Table 1). Aartrijk and Blom-Bamhoorn (1981) have also reported that NAA (0.1mg/L) in the medium increased number of plantlets/ explant in *Lilium*. Medium containing BAP (0.05, 0.01 and 0.1mg/L) alone or in combination with NAA delayed the initiation of buds on the explants in both the cultivars. The maximum number of days taken for bud initiation was however, 43.24 and 43.96 days in basal medium in both the cultivars, Bengalope and Vivaldi, respectively.

Table 1: Effect of different culture media on days to bud initiation and shoot growth in Asiatic *Lilium* cvs., Bengalope and Vivaldi, 8 weeks after incubation.

Culture medium (mg/L)	Days to bud initiation			Number of shoot buds/bulbscale			Shoot length (cm)		
	Bengalope	Vivaldi	Mean	Bengalope	Vivaldi	Mean	Bengalope	Vivaldi	Mean
MS + NAA 0.1	25.53	26.67	26.10	3.72	3.28	3.50	11.26	10.72	10.99
MS + NAA 0.5	27.00	27.60	27.30	2.64	2.55	2.60	8.54	7.78	8.16
MS + BAP 0.01	36.72	37.68	37.20	2.66	2.80	2.73	4.72	4.36	4.54
MS + BAP 0.05	37.70	38.30	38.00	2.20	2.00	2.10	4.08	3.64	3.86
MS + BAP 0.1	36.60	37.30	36.95	2.20	2.20	2.20	4.80	4.20	4.50
MS + NAA 0.1 + BAP 0.05	38.23	39.27	38.75	1.79	1.21	1.50	-	-	-
MS + NAA 0.2 + BAP 0.05	36.00	38.00	37.00	1.50	1.10	1.30	-	-	-
MS + NAA 0.3 + BAP 0.05	38.21	43.99	41.10	1.30	1.00	1.15	-	-	-
MS basal	43.24	43.96	43.60	0.87	0.64	0.76	3.80	3.80	3.80
MEAN	35.40	37.40	-	1.58	1.50	-	4.13	3.53	-
LSD (p=0.05)									
A (Treatment)		0.88			0.4			0.74	
B (Variety)		0.41			NS			0.34	
A×B		1.24			NS			NS	

Table 2: Effect of different culture media on *in vitro* root formation in Asiatic *Lilium* cvs., Bengalope and Vivaldi, after 8 weeks of incubation.

Culture medium (mg/L)	Days to root formation			Number of roots/bulblet			Root length (cm)		
	Bengalope	Vivaldi	Mean	Bengalope	Vivaldi	Mean	Bengalope	Vivaldi	Mean
MS + NAA 0.1	28.75	29.15	28.95	3.44	2.82	3.13	0.27	0.22	0.25
MS + NAA 0.5	29.10	30.10	29.60	3.16	2.58	2.87	0.23	0.21	0.22
MS + BAP 0.01	34.00	34.00	34.00	2.39	2.40	2.40	0.20	0.20	0.20
MS + BAP 0.05	-	-	-	-	-	-	-	-	-
MS + BAP 0.1	-	-	-	-	-	-	-	-	-
MS + NAA 0.1 + BAP 0.05	-	-	-	-	-	-	-	-	-
MS + NAA 0.2 + BAP 0.05	-	-	-	-	-	-	-	-	-
MS + NAA 0.3 + BAP 0.05	-	-	-	-	-	-	-	-	-
MS basal	-	-	-	-	-	-	-	-	-
MEAN	1.90	1.94		0.87	0.86		0.077	0.069	
LSD (p=0.05)									
A (Treatment)		0.40		0.30				0.03	
B (Variety)		NS		NS				NS	
A×B		NS		NS				NS	

Table 3: Effect of different culture media on *in vitro* bulblet production in Asiatic *Lilium* cvs., Bengalope and Vivaldi, after 8 weeks of incubation.

Culture medium (mg/L)	Number of bulblets/explant			Average diameter of bulblets (cm)		
	Bengalope	Vivaldi	Mean	Bengalope	Vivaldi	Mean
MS + NAA 0.1	7.08	5.22	6.15	0.34	0.24	0.29
MS + NAA 0.5	3.72	2.38	3.05	0.16	0.16	0.16
MS + BAP 0.01	3.24	2.08	2.66	0.20	0.20	0.20
MS + BAP 0.05	1.36	1.12	1.24	0.12	0.16	0.14
MS + BAP 0.1	1.20	1.00	1.10	0.12	0.12	0.12
MS + NAA 0.1 + BAP 0.05	-	-	-	-	-	-
MS + NAA 0.2 + BAP 0.05	-	-	-	-	-	-
MS + NAA 0.3 + BAP 0.05	-	-	-	-	-	-
MS basal	-	-	-	-	-	-
MEAN	1.84	1.31		0.10	0.09	
LSD (p=0.05)						
A (Treatment)		0.45			0.04	
B (Variety)		0.21			NS	
A×B		0.64			NS	

Table 4: Effect of different concentrations of sucrose on diameter of bulblets in Asiatic *Lilium* cvs., Bengalope and Vivaldi, after 16 weeks of incubation.

Media	Sucrose concentration (%)	Diameter of bulblet (cm)		
		Bengalope	Vivaldi	Mean
½ MS + NAA 0.1 mg/L	3	0.30	0.21	0.26
	6	0.44	0.34	0.39
	9	0.97	0.85	0.91
½ MS + NAA 0.1 mg/L	3	0.11	0.10	0.11
	6	0.20	0.18	0.19
	9	0.54	0.48	0.51
MEAN		0.43	0.36	-

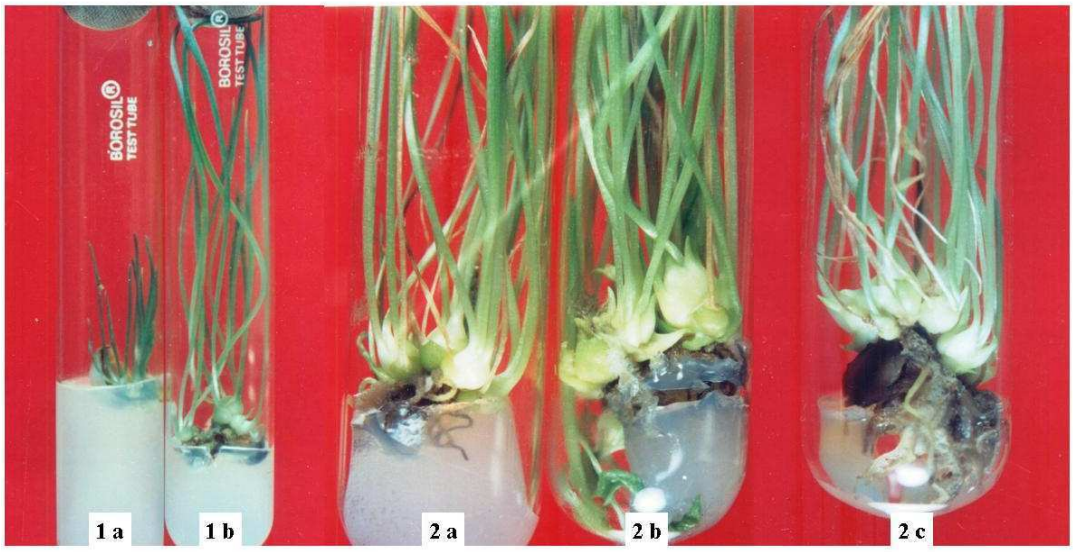

Figure1.: Showing comparative bulb formation on bulb scale explant, after 8 weeks of inoculation.
1a. MS + BAP 0.1mg/L
1b. MS + NAA 0.1 mg/L
Figure2.: Showing *in vitro* bulblet formation in bulb scale explant, after 16 weeks of inoculation.
2a. MS + NAA 0.1 mg/L + Sucrose 6%
2b. MS + NAA 0.1 mg/L + Sucrose 9%
2c. MS + NAA 0.5 mg/L + Sucrose 6%

In vitro shoot formation:

The data presented in Table 1 reveal that MS basal medium supplemented with NAA (0.1mg/L) significantly increased the number of shoot formed 8 weeks after incubation in both the cvs., Bengalope and Vivaldi, respectively. Increase in concentration of NAA to 0.5mg/L in the culture medium decreased the number of shoots. The explants cultured on medium containing BAP also showed less number of shoot buds/bulb scale as compared to NAA (0.1mg/L) supplemented medium in both the cvs., Bengalope and Vivaldi, respectively. Increase in concentration of BAP further decreased the number of shoot buds/bulb scale. The BAP induced decrease in the number of shoot buds has also been recorded earlier (Ivanova *et al*., 1981). The shoot buds showed the maximum shoot length (11.26 and 10.72cm) on medium containing NAA (0.1mg/L), in both the cvs., Bengalope and Vivaldi, respectively but, the length decreased with the increase in level of NAA to 0.5mg/L as well as addition of BAP in the medium. The combination of NAA and BAP in the medium completely inhibited the shoot growth.

In vitro root formation:

The shoots obtained from bulb scale explants exhibited *in vitro* root formation only on medium supplemented with NAA (0.) and 0.5mg/L) and BAP (0.01mg/L) (Table 2). The days taken for the roots initiation (1.90 and 1.94 days) as well as the number of roots (0.87 and 0.86) did not differ in both the cultivars, irrespective of the culture medium (Table 2). Length attained by the roots also did not differ much with the treatment as well as in both the cultivars (Table 2). Interestingly, though

BAP in the medium completely inhibited rooting, the addition of NAA failed to antagonize the effect of BAP. Takayama and Misawa (1980) has also reported that increase in the concentration of BAP (0.01 and 0.05mg/L) in the medium inhibited root formation in *Lilium*.

In vitro bulblet production:

In vitro bulblet formation occurred in medium supplemented with NAA or BAP alone (Table 3). Bulblet formation did not occur in the media containing combination of NAA and BAP as the buds in these mediums failed to elongate. Cultivars significantly differed in response to the effect of media on bulblet formation. In cv. Bengalope, maximum number of bulblet/explant (7.08) was produced in medium containing NAA (0.1mg/L) followed by media containing NAA (0.5mg/L) (3.72) and BAP (0.0lmg/L) (3.24) (Table 3). Minimum number of bulblets per explant (1.20) was produced in medium containing BAP (0.1mg/L). In cv. Vivaldi similar trend was observed but the number of bulblets produced was significantly lower than cv. Bengalope. These findings are in conformity with those of Rybezynski and Gomolinska (1989) who also reported the increase in bulblet production on NAA (0.1mg/L) supplemented medium from bulb scale segments of *Lilium martagon.* The medium supplemented with NAA (0.1mg/L) also produced the largest bulblet (0.34 and 0. 24cm diameter) in cvs., Bengalope and Vivaldi, respectively. However, the average size of the bulblets produced was more or less same (0.10 and 0.09cm) in both the cultivars (Table 3).

Effect of sucrose concentration on size of bulblets:

Results presented in Table 4 show that half strength MS medium containing NAA (0.1 mg/L) and elevated levels of sucrose (9%) led to maximum increase in the size of bulblet, 16 weeks after culturing, irrespective of cultivar (Table 4). The bulblets formed in cv. Bengalope were larger in size (0.43cm) than in cv. Vivaldi (0.36cm), irrespective of the culture medium. The size of the bulblets continued to decrease with the decrease of sucrose in the medium. The medium containing high concentration of NAA (0.5mg/L) produced smaller bulblets which showed further decrease in size with decrease in sucrose concentration. It can thus be inferred that half strength MS medium containing NAA (0.1mg/l) and sucrose (9%) is the most ideal medium for *in vitro* bulblet production.

REFERENCES

Aartrijk, J. Van and Blom-Barnhoorn, G.J. (1981). Growth regulator requirements for adventitious regeneration from *Lilium* bulb scale tissue *in vitro* in relation to duration of bulb storage and cultivar Scientia Horticulture. **14**: 261-268.

Aartrijk, J.Van, Blom-Barnhoorn, G.J. and Van der Linde, P.C.G. (1990). Lilies. Handbook of Plant Cell Culture. **5**: 535-575.

Anonymous. (1983). Ziekten en afwijkingen bij bolgewassen, Lisse, Bulb Research Center.

Ivanova, N.V. (1983). Effect of the date of bulb scale detachment on the propagation coefficient in Lilies. *Byulletin Glavnogo Botanicheskago Sada*. **129**: 72-76.

Kawarabayashi, W. and Asahira, T. (1989). *In vitro* multiplication of virus free bulbs of lilies. Journal of Japanese Society of Horticultural Science. **58**: 195-209.

Leshem, B., Lilien-Kipris, H. and Steinitz, B. (1982). The effect of light and of explant orientation and subsequent growth of bulblet on *Lilium longiflorum* Thumb bulb scale sections cultured *in vitro*. Scientia Horticulture. **17**: 129-136.

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

Park, S.Y., Kim, S.D., Cho, J.T., Kim, T.T. and Paek, K.Y. (1996). Effect of growth regulator on *in vitro* propagation through shoot tips, bulb scale and

bulblet culture of regenerated bulblets in *Lilium concolor* var. Parthneion. RDAJ Agriculture Science Biotechnology **38**: 302-306.

Rybezynski, J.J. and Gomolinska, M. (1989). 6-Benzyladenine control of the initial bulblet formation of wild Lily, *Lilium martagon*. Acta Horticulture **251**: 183-189.

Takayama, S. and Misawa, M. (1980). Differentiation in *Lilium* bulb scales grown *in vitro*. Effect of activated charcoal, physiological age of bulbs and sucrose concentration on differentiation and scale leaf formation *in vitro*. Physiologia Plantarum **48**: 121-125.