IN VITRO BULBLET PRODUCTIVITY IN DIFFERENT EXPLANTS OF HYBRID LILIES

Ranjana Kapoor[1], Surinder Kumar[1]*, Jitender Kumar Kanwar[1] and Pawan Kumar Mahajan[2]

[1]Department of Biotechnology, [2]Department of Basic Sciences, University of Horticulture & Forestry, Solan (H.P.), INDIA – 173 230
*Corresponding author: e-mail: skuhf07@yahoo.co.in

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A B S T R A C T

Lilium is one of the most important floriculture crops and is in great demand as cut flower and potted plant. Different explants have been used to regenerate lilies *in vitro*. In the preliminary experiment different levels of NAA and BA were used to examine their effect on bulblet regeneration, average number and fresh weight of bulblets. The most effective treatment; 2 mg l^{-1} NAA and 1.5 mg l^{-1} BA was selected to compare bulblet productivity in different explants in the five hybrid lily cultivars. The bulblet formation was highest (78.64%) in the bulbscale explants. Average number of 3.1 bulblets per an explant was recorded in root explants followed by node, bulbscale, leaf and internode. Bulblets formed in the bulbscale explants were the largest with an average fresh weight of 350.6 mg. The cultivar 'Beartix' produced the greatest number and the largest bulblets in the explants of node. The bulblets stored at 2°C for 30 days in cocopeat survived with 80-82% success.

Key words: *Lilium*, Asiatic hybrids, growth regulators, micropropagation, oriental hybrids, bulbscale, leaf, root, node, internode, explants, *in vitro*

INTRODUCTION

Lilium is one of the most important ornamental plants all over the world. It has a wide applicability in floral industry as cut flower and potted plant. Among various types of lilies, asiatic, oriental and *L. longiflorum* hybrids have premium potentialities in the florist trade. Numerous studies have been made on *in vitro* regeneration of bulblets in lily (Kumar et al., 2006). Different kinds of explants such as bulbscales

(Han et al., 2004; Kumar et al., 2007), leaves (Niimi, 1984; Kim et al., 2005), stem sections (Doung, 2003; Ebrahim, 2004) and roots (Kumar et al., 2008) have been used, however, bulbscales have remained the choicest. The aim of the study presented was to compare bulblet productivity in the explants of scales, leaves, nodes, roots and internodes of some asiatic and oriental hybrids.

MATERIAL AND METHODS

Pre-cooled bulbs (2°C for 6 weeks) of lily asiatic hybrids ('Alaska', 'Apeldoorn' and 'Beartix') and oriental hybrids ('Siberia' and 'Marco Polo') were obtained from the Department of Floriculture and Landscaping, University of Horticulture & Forestry, Solan (H.P.) India.

The bulbs were grown in earthenware pots (25 cm diameter) containing sand, soil and farmyard manure (FYM) mixed in the ratio 1:1:1 under glasshouse maintained at temperature 25 ±2°C and 90% relative humidity. Thirty days after sprouting, twenty unfolded, young leaves, 10 cm apical part of stem, 2.5 cm long roots and one mother bulb were separated in each cultivar, washed with tap water and then with distilled water supplemented with Tween-20. The explants, namely leaf fragments (3-4 mm), nodes (2-3 mm), internodes (3-4 mm), middle zones (4-5 mm) of the 2.5 cm long roots and the basal portion (3-4 mm) of the inner scales were isolated and surface sterilized with 5% NaOCl (v/v) for 5-6 min followed by washing 3-4 times with sterile distilled water. The explants were placed on Murashige and Skoog's (1962) medium (MS) supplemented with 30 g l^{-1} sucrose and solidified with 8 g l^{-1} agar. The following growth regulators separately or in combination were added to the medium:

- 1.0 or 2 mg l^{-1} naphthalene acetic acid (NAA);
- 1.5 or 2 mg l^{-1} 6-benzyladenine (BA).

Each treatment was applied in three replications of ten explants each. The explants were cultured in a growth room at 25°C and a 16 hour photoperiod under artificial light at the intensity of 100 µmol m^{-2} s^{-1}. The explants cultured without growth regulators served as control. The cultures were transferred to fresh medium at 30 days intervals.

After ninety days, the following data were recorded: percentage of explants producing bulblets, average number of bulblets per an explant and fresh weight of a bulblet.

The individual bulblets were separated and transferred to MS medium without growth regulators. When the leaves have dried, the bulblets were removed, washed thoroughly and dried at room temperature. Then the bulblets were treated with 0.2% bavistin (Carbendazim, a fungicide) and stored in cocopeat at 2°C. The survival percentage of the bulblets was recorded after 30 days of storage.

All data were analyzed using completely randomized design (Gomez and Gomez, 1984). The statistical analysis based on mean values per an

explant was made using analysis of variance. The comparative LSD multiple range test (P = 0.05) was used to determine differences between treatments.

In the preliminary experiment different levels of NAA and BA alone or in all possible combinations were used to examine their effect on bulblet regeneration, average number and fresh weight of bulblets in the explants of bulbscale, leaf, node, internode and root of *Lilium*. For this purpose, explant-wise analysis of variance (one-way classification) technique was applied in which treatment means of five cultivars were taken into consideration.

RESULTS AND DISCUSSION

After thirty days of inoculation, about 70% to 80% uncontaminated cultures were obtained when the initial explants were surface sterilized with 5% NaOCl. The analysis of the data revealed that the treatment with 2 mg l^{-1} NAA and 1.5 mg l^{-1} BA was the most effective in explant regeneration and stimulation of bulblet formation with heaviest fresh weight in all the cultivars tested and was selected for comparing bulblet productivity subsequently in different explants in *Lilium* (Tab. 1).

Of the cultured explants, about 78% developed bulblets in bulblscale explant, which differed significantly from other explants (Fig. 1, Tab. 2). These results are contrary to Niimi (1984) who reported that the ability to regenerate bulblets was greatest in explants of stem in *Lilium rubellum* but support others, who reported that *in vitro* scale culture was the best and most prolific method (Kumar et al., 2006). No significant differences were observed in the explants producing bulblets among the cultivars.

The greatest average number of bulblets per an explant was observed in the explants of root (Tab. 3). Kumar et al. (2008) reported greatest number of bulblets with 2 mg l^{-1} NAA and 2 mg l^{-1} BA from *in vitro* explant of root in oriental hybrid lily. Cavallini and Natali (1989) obtained somatic embryos and regenerated plants from root explant in *Brimeura amethystina*. The efficiency of root for multiplication of a large number of genetically identical plants makes it a potential source of explant for mass propagation (Ostazaki and Henson, 1965). The least number of bulblets was recorded in the explants of internode. Among the cultivars, 'Beartix' formed greatest number of bulblets, which was statistically on a par with 'Apeldoorn', 'Siberia' and 'Marco Polo'. The interaction between cultivars and explants revealed that the cultivar 'Beartix' produced greater number of bulblets (4.0) in the explants of node. A different effect of explant under similar environmental conditions was observed in case of fresh weight of bulblets (Tab. 4). The explants of bulbscale produced the largest bulblets, followed by node and leaf explants. Niimi (1984) reported heaviest bulblets in the explant of stem in *L. rubellum*. The differences in the results may be due to differences in cultural conditions and genotypes used. Among the cultivars, 'Alaska' recorded the highest

Table 1. Effect of NAA and BA on explant regeneration, mean number of bulblets and mean fresh weight in different explants of five hybrid cultivars of *Lilium*

Phytohormone concentration [mg l^{-1}]		Percentage of explants producing bulblets*					Average number of bulblets per an explant*					Average fresh weight of a bulblet [mg]*				
NAA	BA	BS	L	N	IN	R	BS	L	N	IN	R	BS	L	N	IN	R
0	0	75.3	0	0	0	0	1.3	0	0	0	0	117.0	0	0	0	o
1	0	76.5	73.5	51.5	31.9	0	1.9	1.3	1.7	0.8	0	197.5	199.4	130.4	86.8	0
2	0	77.1	74.9	55.0	32.2	0	2.1	1.9	1.7	0.7	0	203.0	193.8	136.2	97.6	0
0	1.5	76.8	0	56.3	0	0	1.7	0	1.7	0	0	233.8	0	173.5	0	0
0	2	78.7	68.2	57.9	52.2	0	2.2	1.2	2.5	1.9	0	206.8	1209.1	183.3	140.3	0
1	1.5	76.7	74.8	69.1	64.6	63.4	2.7	2.0	2.5	1.8	2.1	326.2	199.2	213.9	190.1	128.8
1	2	77.5	57.0	67.0	65.9	63.2	3.3	1.9	2.2	1.9	2.1	350.6	191.2	210.0	203.3	127.8
2	1.5	80.5	76.1	73.9	75.1	71.4	3.9	2.6	2.9	2.8	3.2	364.2	263.1	324.8	210.3	150.3
2	2	78.7	75.5	73.5	75.0	74.5	2.6	1.8	2.8	2.5	2.4	198.0	229.4	260.4	203.1	138.5
$LSD_{0.05}$**		2.8	0.8	1.4	1.2	1.5	0.3	0.4	0.6	1.0	0.4	4.1	2.5	1.3	0.7	0.3

*Data in table are means for five hybrid lily cultivars 'Alaska', 'Apeldoorn', 'Beartix', 'Siberia' and 'Marco Polo'
**Least significant difference at 5% level of significance
BS = bulbscale; L = leaf; N = node; IN = internode; R = root

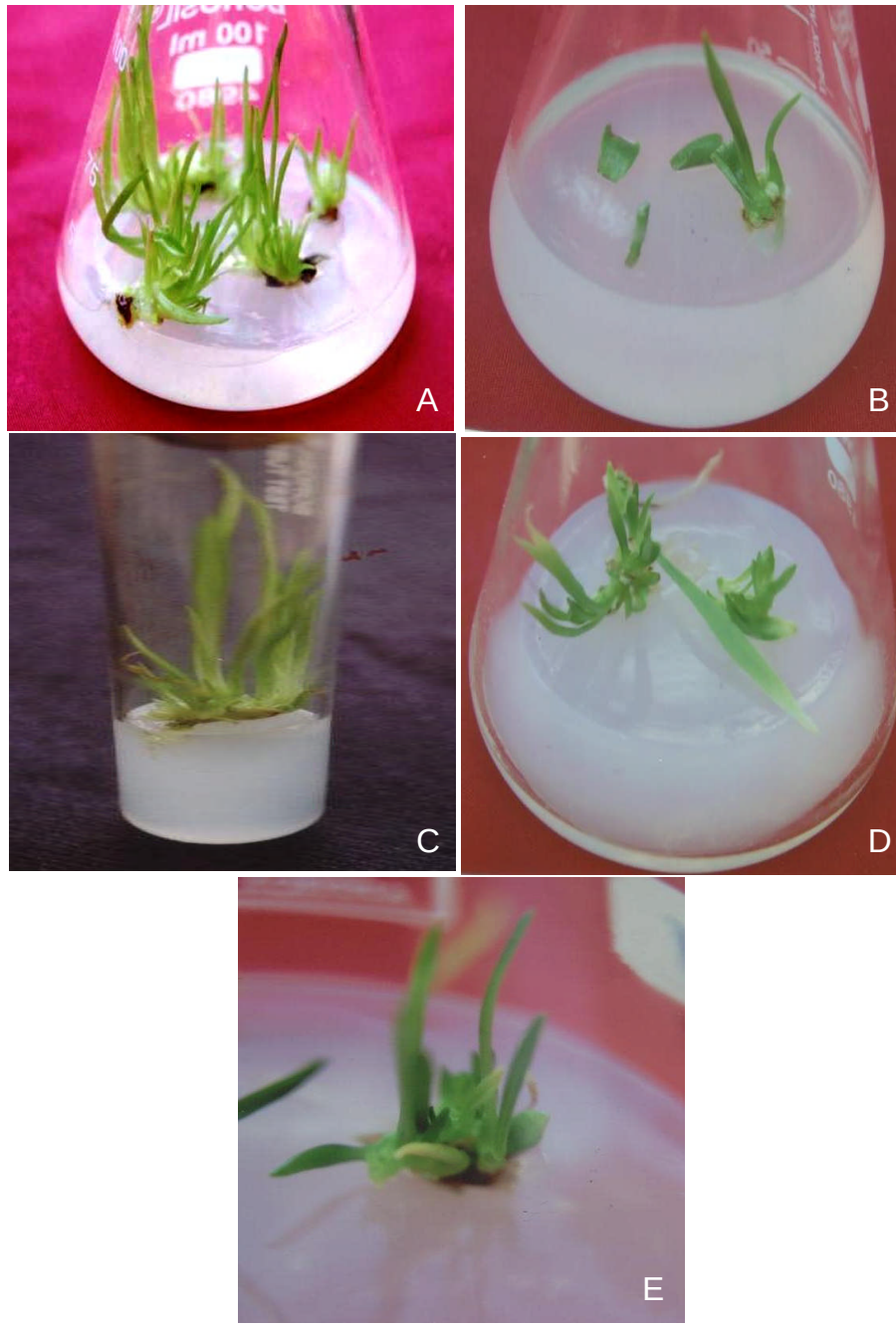

Figure 1. Bulblet regeneration in lily cv. 'Marco Polo', 'Alaska', `Apeldoorn', 'Siberia', and 'Beartix' in the explants of **A.** bulbscale, **B.** leaves, **C.** roots, **D.** nodes and **E.** internodes after 90 days of culture on MS medium supplemented with 2 mg l^{-1} NAA and 1.5 mg l^{-1} BA

Table 2. Effect of an explant, cultivar and their interaction on bulblet regeneration in hybrid lilies

Explant	Bulblet regeneration rate [%]					Explant mean
	Asiatic hybrids			oriental hybrids		
	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	
Bulbscale	75.00±1.15*	79.22±1.18	78.00±0.87	80.00±0.58	81.00±0.58	78.64
Leaf	76.11±1.16	76.11±1.74	74.22±0.62	78.00±1.73	75.44±0.87	75.98
Node	76.56±0.87	75.56±0.87	70.00±0.58	72.00±1.15	73.00±0.58	73.42
Internode	73.00±0.58	74.00±0.58	75.56±0.87	76.00±0.58	76.22±0.62	74.96
Root	77.00±0.72	75.33±0.91	77.33±0.71	74.00±1.05	78.00±0.73	76.33
Mean	75.17	76.22	74.44	76.50	76.42	
$LSD_{0.05}$**	Explant A = 1.22; Cultivar B = 1.36; A x B = 2.72					

Data were analyzed using factorial completely randomized design
*Mean of 30 explants ± standard error
**Least significant difference at 5% level of significance

Table 3. Effect of explant, cultivar and their interaction on mean number of bulblets in hybrid lilies

Explant	Mean number of bulblets per an explant					Explant mean
	Asiatic hybrids			oriental hybrids		
	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	
Bulbscale	2.75±0.14	3.00±0.12	2.00±0.06	3.08±0.05	2.80±0.06	2.73
Leaf	2.16±0.03	2.66±0.03	2.66±0.01	2.75±0.03	2.66±0.02	2.58
Node	1.76±0.02	2.33±0.02	4.00±0.06	2.67±0.02	3.00±0.06	2.75
Internode	1.50±0.06	2.08±0.05	2.40±0.06	1.91±0.03	1.25±0.03	1.83
Root	3.30±0.10	3.33±0.07	2.66±0.05	3.00±0.04	3.60±0.05	3.17
Mean	2.29	2.68	2.74	2.68	2.66	
$LSD_{0.05}$**	Explant A = 0.07; Cultivar B = 0.08; A x B = 0.16					

For explanations see Table 2

Table 4. Effect of explant, cultivar and their interaction on mean bulblet weight (mg) in hybrid lilies

Explant	Mean bulblet weight (mg)					Explant mean
	Asiatic hybrids			oriental hybrids		
	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	
Bulbscale	385.8±2.89	335.0±1.73	264.6±2.31	379.5±2.31	388.2±1.73	350.6
Leaf	306.4±2.31	202.7±1.15	317.0±1.15	199.7±2.31	289.9±1.53	263.1
Node	305.3±1.15	350.7±0.58	401.3±0.58	301.0±1.73	262.7±1.15	324.2
Internode	213.0±1.73	174.7±1.15	185.0±2.89	238.3±1.73	205.0±1.15	203.2
Root	164.7±1.14	162.0±1.23	147.3±1.79	143.7±1.64	134.0±1.07	150.0
Mean	275.0	245.0	263.0	252.4	255.9	
$LSD_{0.05}$**	Explant A = 9.24; Cultivar B = 10.32; A x B = 20.65					

For explanations see Table 2

average fresh weight followed by 'Beartix' and 'Marco Polo'. Other cultivars produced smaller bulblets with the smallest in 'Apeldoorn'. The interaction between cultivars and explants revealed that the largest bulblets were produced in the 'Beartix' in the explants of node followed by 'Marco Polo' in the explants of bulbscale. The bulblets stored at 2°C in cocopeat for 30 days survived with 80-82% success depending upon the type of explant and cultivar.

The study presented revealed that the root explants produced higher number of bulblets but the largest bulblets were produced in the explants of bulbscale. The formation of largest bulblets in the explants of bulbscale may be due to the fact that the available sugars, which are normally utilized to sustain the growth, have formed storage sugars (Stoddart, 1964), thereby increasing mass of the bulblets. Hence the bulblets produced had higher fresh weight.

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WYDAJNOŚĆ TWORZENIA CEBUL *IN VITRO* Z RÓŻNYCH EKSPLANTATÓW LILII HYBR.

Ranjana Kapoor, Surinder Kumar,
Jitender Kumar Kanwar i Pawan Kumar Mahajan

S T R E S Z C Z E N I E

We wstępnych badaniach zastosowano stężenia NAA i BA celem określenia ich wpływu na regenerację cebul, liczbę tworzonych cebul i ich świeżą masę z różnych eksplantatów (łuski, liście, korzenie, międzywęźla, węzły). Najlepszą kombinacją okazała się pożywka zawierająca NAA w stężeniu 2,0 mg 1^{-1} i BA w stężeniu 1,5 mg 1^{-1} dla wszystkich badanych odmian lilii hybr. (‘Marco Polo’, ‘Alaska’, ‘Apeldoorn’, ‘Siberia’, ‘Beatrix’) i wszystkich rodzajów eksplantatów. Najwięcej i o największej świeżej masie tworzyło się cebul na eksplantatach łusek, z wyjątkiem odmiany ‘Beatrix’; eksplantaty z węzłów wytwarzały najwięcej cebul i o najwyższej masie. Wytworzone cebule przetrzymywane w temperaturze 20°C przez 30 dni przeżywały w 80-82 procentach.

Słowa kluczowe: *Lilium*, hybrydy azjatyckie, regulatory wzrostu, mikrorozmnażanie, orientalne hybrydy, łuski, liście, korzenie, węzły, międzywęźla, eksplantaty, *in vitro*