Bulblet regeneration from *ex vitro* root explant in lily hybrids

R. Kapoor, S. Kumar, J. K. Kanwar

Department of Biotechnology, University of Horticulture & Forestry, Solan, India

ABSTRACT: The influence of growth regulators on *in vitro* bulblet formation from *ex vitro* roots was studied in asiatic and oriental hybrids of *Lilium*. The root segments (3–4 mm) isolated from the middle zone of 2–3 cm *ex vitro* root were cultured on Murashige and Skoog (MS) medium containing 1 or 1.5 mg/dm^3 naphthalene acetic acid (NAA) and/or benzyladenine (BA). Bulblets were not produced in the presence of NAA and BA alone. A significant increase in the per cent explants producing bulblets was observed with 1.5 mg/dm^3 NAA and 1 mg/dm^3 BA. Maximum number of bulblets and average fresh weight per bulblet was observed with 2 mg/dm^3 NAA and 1.5 mg/dm^3 BA after 90 days of culture. No differences were found among cultivars in bulblet regeneration of explant or bulblet number although more weighty bulblets occurred in cv. Apeldoorn. About 82% bulblet survival was recorded in coco peat after 30 days of transfer to pots.

Keywords: lily hybrids; cvs. Alaska; Apeldoorn; Beartix; Siberia; Marco Polo; NAA; BA; IBA; *in vitro*; bulblet regeneration; *ex vitro* root; rooting; hardening

Lilium is one of the leading cut flower crops in the world. It ranks fourth in the international flower trade (Anonymous 1996) and has a wide applicability in the floral industry as cut flowers and potted plants (Jana, Roychoudhary 1989). Over the past few years, the importance of lily has increased enormously, especially in the Netherlands. The area under lily cultivation has increased from 102 ha in 1960 to 2,419 ha in 1990, representing 24-fold increase (Betties, White 1993).

Among the various types of lilies, asiatic, oriental and longiflorum hybrids have premium potential in the floral trade. It can be propagated by both sexual and asexual reproduction. Most of the commercially grown cultivars are propagated through vegetative means, to maintain uniformity and genetic purity, by way of above ground bulbils and under ground bulbscales (Maesato et al. 1991; Dilta et al. 2000; Kumar et al. 2001). Numerous studies have been made on *in vitro* regeneration of bulblets in lily (Kumar et al. 2006). Although many explants have been commonly used, bulb scale remained the choicest explant to regenerate bulblets in *Lilium* because scales seemed to be most productive (Stimart, Ascher 1978; Takayama, Misawa 1979; Lian et al. 2003; Kumar et al. 2007).

The use of root as a source of explant for *in vitro* propagation is limited to a small number of species (Sankhola et al. 1995; Vinocur et al. 2000). Kumar et al. (2008) reported regeneration of bulblets from the middle zone of *in vitro* roots in oriental lily hybrid cv. Marco Polo. The root explant source offers obvious advantages (ease of manipulation, availability, less oxidation after excision etc.) in comparison with other organ cultures (Son, Hall 1990). The efficiency of root for the multiplication of a large number of genetically identical plants and for propagating individual genotypes as clone render root as a potential source of explant mass propagation (Ostazeki, Henson 1965). In the present work an attempt was made to develop a protocol for *in vitro* propagation of asiatic and oriental hybrid lily from *ex vitro* root explant.

MATERIALS AND METHODS

Preparation of material

Pre-cooled bulbs (2°C for 6 weeks) of asiatic (Alaska, Apeldoorn and Beartix) and oriental (Siberia and Marco Polo) lily hybrids were procured from the Department of Floriculture and Landscaping, Solan, India. The bulbs were raised in earthen-ware pots (12" diameter) filled with soil:sand:FYM mixed in the ratio 1:1:1 in glass-house maintained at 25 ± 2°C for developing roots. The roots (2–3 cm long) were

excised from the bulbs and washed thoroughly under running tap water. The root segments (3–4 mm) from the middle zone of the 2–3 cm long roots were used as explant. The explants were surface sterilized with 0.1% mercuric chloride ($HgCl_2$) for 3–4 min and rinsed three times under aseptic conditions with sterile distilled water to remove the sterilization solution.

Cultural conditions

The sterilized explants were cultured on the Murashige and Skoog (MS 1962) regeneration medium supplemented with 8 g/dm^3 (w/v) agar, 30 g/dm^3 (w/v) sucrose and 1 and 1.5 mg/dm^3 naphthalene acetic acid (NAA) and benzyladenine (BA), respectively. The cultures without growth regulators served as control. The explants were cultured in 100 ml Erlenmeyer flasks (Borosil) and culture tubes (25 × 150 mm diameter) containing 30 ml and 20 ml of medium, respectively. The flasks/culture tubes were plugged with non-absorbent cotton. The pH of the medium was adjusted to 5.8 before autoclaving at 121°C, at the pressure of 1.1 g k/cm^2 for 15 min. All the cultures were incubated in a room with controlled conditions of 24 ± 2°C under 16 h photoperiod with a photosynthetic photon flux density (PPFD) of 50 to 60 μmol/m^2/s.

Data on explants forming bulblets were recorded after 30 days, and on number of bulblets and average fresh weight after 90 days of culture.

In vitro rooting and hardening

Bulblets were separated and individual bulblet was transferred to MS rooting medium supplemented

Table 1. Effect of NAA and BA on regeneration (%) of explant after 30 days of culture

Treatment (mg/dm^3)	Asiatic hybrids			Oriental hybrids		
	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	Mean
Control	0.00	0.00	0.00	0.00	0.00	0.00
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
NAA (1)	0.00	0.00	0.00	0.00	0.00	0.00
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
NAA (1.5)	0.00	0.00	0.00	0.00	0.00	0.00
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
BA (1)	0.00	0.00	0.00	0.00	0.00	0.00
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
BA (1.5)	0.00	0.00	0.00	0.00	0.00	0.00
	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)	(0.00)
NAA + BA (1 + 1.5)	61.43	63.00	64.00	63.33	65.33	63.41
	(51.55)	(52.54)	(53.13)	(53.93)	(59.93)	(53.02)
NAA + BA (1.5 + 1.5)	63.33	63.33	63.33	61.67	64.33	63.19
	(52.74)	(52.74)	(52.74)	(52.74)	(53.34)	(52.86)
NAA + BA (2 + 1.5)	68.00	69.00	69.00	78.00	73.00	71.40
	(55.56)	(56.18)	(56.18)	(57.21)	(58.76)	(56.77)
NAA + BA (1.5 + 1)	77.00	75.33	77.33	74.00	78.00	76.33
	(61.37)	(60.00)	(61.39)	(62.03)	(62.03)	(61,41)
NAA + BA (1.5 + 2)	77.67	75.00	74.67	78.33	73.67	74.47
	(57.21)	(60.26)	(59.19)	(59.35)	(59.13)	(59.15)
NAA + BA (1.5 + 2.5)	72.02	72.67	72.06	74.06	70.67	72.28
	(51.06)	(58.49)	(51.06)	(60.26)	(57.21)	(58.41)
Mean	37.49	38.03	38.21	39.03	38.63	
	(27.88)	(28.53)	(29.61)	(30.44)	(29.13)	

LSD (P = 0.05); treatment (A) = 0.90; cultivar (B) = 0.60; A × B = 1.30
Figures within parenthesis are arc sine transformed values

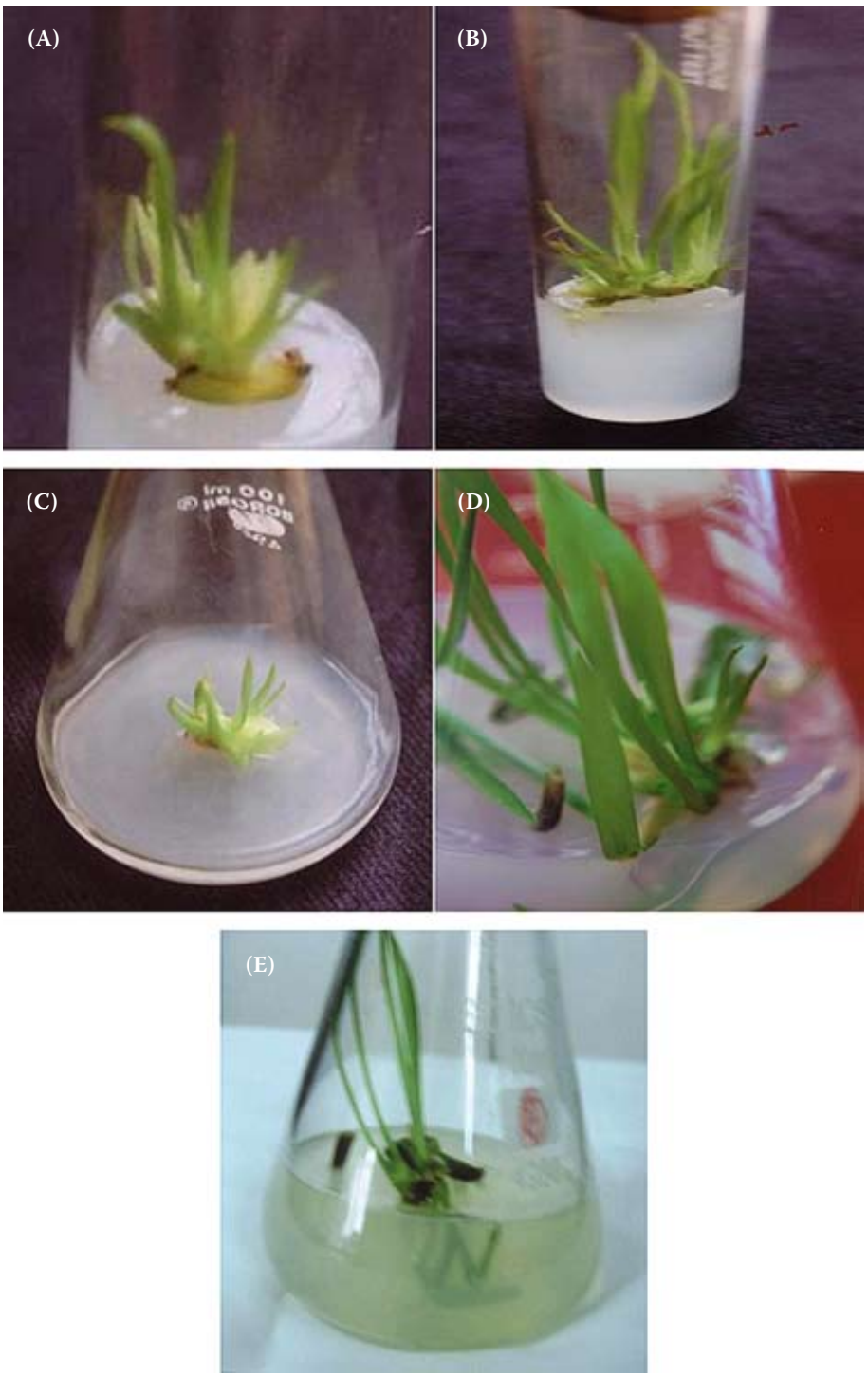

Fig. 1. Bulblet regeneration on root explant in MS medium supplemented with 1.5 mg/dm^3 NAA and 1 mg/dm^3 BA in Alaska (A), Apeldoorn (B), Beartix (C), Siberia (D) and Marco Polo (E) after 30 days of culture

with 1 mg/dm^3 indolebutyric acid (IBA). The rooted bulblets were removed from the culture tubes, washed thoroughly and transferred to pots filled with coco peat and soil:FYM mixed in the ratio 1:1. The pots were kept in glass-house maintained at 25 ± 2°C and 90% relative humidity. The per cent survival of the bulblets was recorded after 30 days of transfer to pots.

Statistical analysis

Three replications with 10 explants in each replication (30 explants) were maintained for each treatment and the data were analyzed statistically using factorial completely randomized design (Gomez, Gomez 1984). The statistical analysis based on mean value per treatment was made using the technique of

Table 2. Effect of NAA and BA on average number of bulblets after 90 days of culture

Treatment (mg/dm^3)	Asiatic hybrids			Oriental hybrids		
	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	Mean
Control	0.00	0.00	0.00	0.00	0.00	0.00
NAA (1)	0.00	0.00	0.00	0.00	0.00	0.00
NAA (1.5)	0.00	0.00	0.00	0.00	0.00	0.00
BA (1)	0.00	0.00	0.00	0.00	0.00	0.00
BA (1.5)	0.00	0.00	0.00	0.00	0.00	0.00
NAA + BA (1 +1.5)	2.02	1.69	2.06	2.33	2.33	2.08
NAA + BA (1.5 + 1.5)	2.04	1.08	2.56	2.03	2.00	2.06
NAA + BA (2 +1.5)	3.30	3.33	2.66	3.00	3.60	3.17
NAA + BA (1.5 +1)	2.66	2.00	1.66	2.00	1.66	1.99
NAA + BA (1.5 +2)	2.00	1.66	2.33	2.30	3.56	2.37
NAA + BA (1.5 +2.5)	3.00	2.66	2.00	2.10	2.66	2.48
Mean	1.36	1.18	1.20	1.24	1.43	

LSD (P = 0.05); treatment (A) = 0.50; cultivar (B) = 0.38; A × B = 0.88

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

RESULTS AND DISCUSSION

Table 1 summarizes the range of responses after 30 days of culture. The isolated root segments did not produce bulblets in the presence or absence of NAA and BA alone. The bulblet formation was initiated with NAA and BA in combination in all the cultivars (Figs. 1A,B,C,D,E). 1.5 mg/dm^3 NAA in the presence of 1 mg/dm^3 BA was most effective where 76.33% of the explants regenerated bulblets. Kumar et al. (2008) also observed that 45% of the explants regenerated bulblets from the middle zone of *in vitro* root in Marco Polo. Carmi et al. (1997) observed higher number of buds from middle zone of 2.5 cm long *in vitro* roots in *Populus tremula*. The formation of adventitious buds from root segments generally occurs after callus formation, especially at the cut end (Son, Hall 1990). In the present study,

Table 3 . Effect of NAA and BA on average fresh weight (mg) per bulblet after 30 days of culture

Treatment (mg/dm^3)	Asiatic hybrids			Oriental hybrids		
	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	Mean
Control	0.00	0.00	0.00	0.00	0.00	0.00
NAA (1)	0.00	0.00	0.00	0.00	0.00	0.00
NAA (1.5)	0.00	0.00	0.00	0.00	0.00	0.00
BA (1)	0.00	0.00	0.00	0.00	0.00	0.00
BA (1.5)	0.00	0.00	0.00	0.00	0.00	0.00
NAA + BA (1 +1.5)	144.30	150.30	114.30	121.30	114.00	128.80
NAA + BA (1.5 + 1.5)	126.70	160.30	103.30	115.00	134.00	127.80
NAA + BA (2 +1.5)	164.70	162.00	147.30	143.70	134.00	150.30
NAA + BA (1.5 +1)	153.30	143.70	142.00	135.00	122.70	139.30
NAA + BA (1.5 +2)	152.70	123.30	135.70	128.70	152.00	138.50
NAA + BA (1.5 +2.5)	124.00	142.00	133.00	137.00	153.00	137.80
Mean	78.70	80.15	70.48	70.97	73.61	

LSD (P = 0.05); treatment (A) = 0.48; cultivar (B) = 0.32; A × B = 0.70

Table 4. Per cent survival of bulblets in coco peat and soil:FYM

Potting mixture	Asiatic hybrids			Oriental hybrids		
	Alaska	Apeldoorn	Beartix	Siberia	Marco Polo	Mean
Cocopeat	82.00	81.37	82.30	82.00	82.30	82.00
	(64.90)	(64.42)	(65.16)	(64.96)	(65.16)	(64.90)
Soil:FYM	72.00	62.51	63.00	62.00	62.00	64.20
	(58.06)	(51.95)	(52.04)	(51.95)	(51.95)	(53.29)
Mean	77.00	71.67	72.67	72.00	72.17	
	(61.48)	(58.18)	(58.85)	(58.42)	(58.55)	

LSD (P = 0.05); treatment (A) = 0.60; cultivar (B) = 0.90; A × B = 1.20
Figures within parenthesis are arc sine transformed values

the bulblets were regenerated along the entire length of segment without any visible callus formation as an intermediate step.

The number of bulblets per explant was significantly higher (3.17) with 2 mg/dm^3 NAA in combination with 1.5 mg/dm^3 BA as compared with other treatments (Table 2). Niimi (1984) reported 3.1 bulblets from leaf explant with 1 mg/dm^3 NAA and 0.1 mg/dm^3 BA in *Lilium rubellum*. A combination of 0.01 mg/dm^3 NAA and 0.01 mg/dm^3 BA produced 1.6 bulblets per bulb scale in *Lilium japonicum* (Maesato et al. 1994). Azadi and Khosh (2007) obtained 5.41 bulblets from bulb scale explant with 0.1 mg/dm^3 NAA and 0.1 mg/dm^3 BA in *Lilium ledebourii.*

A different effect of growth regulators, under similar environment was observed on fresh weight of regenerated bulblets. The highest fresh weight mean of 150.30 mg was observed with a combination of 2 mg/dm^3 NAA and 1.5 mg/dm^3 BA, followed by 1.5 mg/dm^3 NAA and 1–2.5 mg/dm^3 BA (Table 3). Niimi and Onozawa (1979) also observed relatively higher fresh weights with 1mg/dm^3 NAA and 1 mg/dm^3 BA in *Lilium rubellum*. Kumar et al. (2008) recorded highest fresh weight of 171.7 mg in *in vitro* root with 1.5 mg/dm^3 NAA and 2 mg/dm^3 BA in oriental hybrid cv. Marco Polo. No differences were observed among cultivars in bulblet regeneration of explants or bulblet number although more weighty bulblets were found in cv. Apeldoorn, followed by Alaska and Marco Polo.

The bulblets were rooted with 1 mg/dm^3 IBA and hardened in two potting mixtures. The survival of bulblets was significantly higher in coco peat (82%) as compared to soil:FYM. Bulblet survival was significant in the cultivar Alaska than in the other cultivars, which did not differ significantly from each other. Thakur et al. (2002) reported that for hardening *in vitro* rooted bulblets of *Lilium*, cocopeat, peat moss and cocopeat + peat moss (1:1) gave 100% survival whereas sand:soil:FYM (1:1:1) was the least effective, yielding only 62% surviving plantlets. Gong et al. (1996) obtained 80–90% survival rate of tissue culture plants of *Lilium* × Connecticut King when transplanted in potting mixture of sand, peat moss and humus soil (1:1:1).

The present work was the first attempt to develop a protocol for bulblet regeneration from *ex vitro* root in *Lilium*. The regenerated bulblets were successfully rooted and hardened with about 82% success. The technology developed can be exploited for bulblet regeneration and may be extended to other lily cultivars.

References

ANONYMOUS, 1996. International flower trade show. Action Market, Aalsmeer, Holland, 8–12 November.

AZADI P., KHOSH K., 2007. Micropropagation of *Lilium ledebourii* (Baker) Boiss as affected by plant growth regulators, sucrose concentration, harvesting season and cold treatments. Journal of Biotechnology, *10*: 582–589.

BETTIES D.T., WHITE J.W., 1993. *Lilium* hybrids and species. In: DE HERTOGH A.A., LE NARD M. (eds), Physiology of Flower Bulbs. Amsterdam, London, New York, Elsevier: 423–454.

CARMI T., ZIV M., ALTMAN A., 1997. Bud regeneration, growth and proliferation of aspen (*Populus tremula*) roots in liquid cultures. Acta Horticulturae, *447*: 669–670.

DILTA B.S., SEHGAL O.P., PATHANIA N.S., CHANDER S., 2000. *In vitro* effect of NAA and BA on culture establishment and bulblet formation in lily. Journal of Ornamental Horticulture, *3*: 67–70.

GOMEZ K.A., GOMEZ A.A., 1984. Statistical Procedures for Agricultural Research. New York, John Wiley and Sons.

GONG X.K., ZHAO X.Y., CHEN X.L., HOU F.M., GAO F.Y., 1996. Studies on improvement of survival rate of lily

plantlets from tissue culture transplanted into soil. Acta Horticulturae Sinica, *23*: 73–77.

JANA B.K., ROYCHOUDHARY N., 1989. *Lilium*. In: BOSE T.K., YADAV L.P. (eds), Commercial Flowers. Calcutta, Naya Prokash: 601–602.

KUMAR S., KANWAR J.K., SHARMA D.R., 2006. *In vitro* propagation of *Lilium*. Advances in Horticultural Science, *20*: 181–188.

KUMAR S., AWASTHI V., KANWAR J.K., 2007. Influence of growth regulators and nitrogenous compounds on *in vitro* bulblet formation and growth in oriental lily. Horticultural Science, *34*: 77–83.

KUMAR S., CHAUDHARY V., KANWAR J.K., 2008. *In vitro* propagation of oriental hybrid lily from root explant. Advances in Horticultural Science, *22*: 63–65.

KUMAR S., SHARMA D.R., SHARMA, Y.D., PATHANIA N.S., 2001. *In vitro* propagation of asiatic hybrid lily from bulb scales. Indian Journal of Agricultural Sciences, *71*: 463–465.

LIAN M.L., CHAKRABARTY D., PAEK K.Y., 2003. Bulblet formation from bulbscale segments of *Lilium* using bioreactor system. Biologia Plantarum, *46*: 199–202.

MAESATO K., SARMA K.S., FUKUI H., HARA T., 1991. *In vitro* bulblet induction from shoot apices of *Lilium japonicum*. HortScience, *26*: 211–219.

MURASHIGE T., SKOOG F., 1962. A revised medium for rapid growth and bioassay with tobacco tissue cultures. Physiologia Plantarum, *15*: 473–479.

NIIMI Y., 1984. Bulblet productivity of explants from scales, leaves, stem and tepals of *Lilium rubellum* Baker. Scientia Horticulturae, *22*: 391–394.

NIIMI Y., ONOZAWA T., 1979. *In vitro* bulblet formation from leaf segments of lilies, especially *Lilium rubellum* Baker. Scientia Horticulturae, *11*: 379–398.

OSTAZEKI A., HENSON P.P., 1965. Effect of morphology of propagules on performance of birdsfoot trefoil clones. Crop Science, *5*: 253–254.

SANKHOLA D., SANKHOLA N., DAVIS T.D., 1995. Promotion of *in vitro* shoot formation from excised roots of silktree (*Albizzia jublibrissin*) by an oxime ether derivative and other ethylene inhibitors. Plant Cell Reports, *15*: 143–146.

SON S.H., HALL R.B., 1990. Multiple shoot regeneration from root organ cultures of *Populus alba* × *Populus grandidentata*. Plant Cell, Tissue and Organ Culture, *20*: 53–57.

STIMART D.P., ASCHER P.D., 1978. Tissue culture of bulbscale sections for asexual propagation of *Lilium longiflorum* Thunb. Journal of American Society for Horticultural Sciences, *103*: 182–184.

TAKAYAMA S., MISAWA M., 1979. Differentiation of *Lilium* bulbscales grown *in vitro*: Effect of various cultural conditions. Physiologia Plantarum, *46*: 184–190.

THAKUR N., KAUR R., SHARMA D.R., GAUTAM H., MANGAL M., 2002. Effect of certain chemicals and sucrose on enhancement of *in vitro* bulblet size of *Lilium* sp. (Asiatic hybrids) cv. Toscana. Plant Cell Biotechnology and Molecular Biology, *3*: 69–72.

VINOCUR B., CARMI T., ALTMAN A., ZIV M., 2000. Enhanced regeneration in aspen (*Populus tremula* L.) roots cultured in liquid media. Plant Cell Reports, *19*: 1146–1154.

Received for publication March 3, 2008
Accepted after corrections March 19, 2008

Regenerace hlíz z *ex vitro* kořenových explantátů u kříženců lilie

ABSTRAKT: Studovali jsme vliv růstových regulátorů na *in vitro* tvorbu hlíz u *ex vitro* kořenů asijských a orientálních hybridů lilie. Kořenové segmenty (3–4 mm) izolované ze střední části 2–3 cm *ex vitro* kořene byly kultivovány na Murashige a Skoog (MS) médiu obsahujícím 1 nebo 1,5 mg/dm^3 kyseliny naftyloctové (NAA) nebo benzyladeninu (BA). Hlízy se netvořily v přítomnosti samotného NAA nebo BA. Průkazné zvýšení procenta explantátů bylo pozorováno s 1,5 mg/dm^3 NAA a 1 mg/dm^3 BA. Maximální počet hlíz a průměrná čerstvá hmotnost hlízy byla pozorována s 2 mg/dm^3 NAA a 1,5 mg/dm^3 BA po 90 dnech pěstování kultury. Žádné rozdíly v regeneraci nebo počtu hlíz mezi kultivary nebyly zjištěny, ačkoliv hlízy s vyšší čerstvou hmotností se objevily u cv. Apeldoorn. Po 30 dnech po přesazení do nádob přežilo kolem 82 % hlíz.

Klíčová slova: kříženci lilie; kultivary Alaska; Apeldoorn; Beartix; Siberia; Marco Polo; NAA; BA; IBA; *in vitro*; regenerace hlíz; *ex vitro* kořen; zakořenění; otužování

Corresponding author:

Prof. Surinder Kumar, Ph.D., University of Horticulture & Forestry, Department of Biotechnology, Solan – 173 230 (H.P.), India
tel.: + 91 01792 252 310, fax: + 91 01792 252 242, e-mail: skuhf07@yahoo.co.in