BULBLET REGENERATION FROM *IN VITRO* ROOTS OF ORIENTAL LILY HYBRID

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A B S T R A C T

Root segments from the middle and distal zones of *in vitro* roots of oriental lily hybrid cultivar 'Rosato' were isolated and cultured on MS nutrient medium supplemented with IBA, NAA and 2,4-D alone or in combination with BA. Hormone-free MS medium was ineffective in inducing bulblet regeneration. Bulblets were induced when the medium contained either 1.0, 1.5 and 2.0 mg l^{-1} NAA, 2.0 mg l^{-1} 2.4-D or 1.5 and 2.0 mg l^{-1} BA. A significant increase in the percentage of explants producing bulblets and the number of bulblets per an explant was observed on medium containing 2 mg l^{-1} NAA in combination with 2 mg l^{-1} BA. After ninety days of culture, the weighty bulblets were recorded on medium supplemented with 1 mg l^{-1} NAA and 1.5 mg l^{-1} BA. The bulblets attaining the size of 14-16 cm flowered in two years in pots without any phenotypic variations

Key words: Bulblet regeneration, growth regulators, root explant, *in vitro*, oriental lily

INTRODUCTION

Lilium is one of important floricultural crops for bulbs and cut flower production. Among various types of lilies, asiatic, oriental and *L. longiflorum* hybrids have premium potential in floral trade. Oriental lilies are the most expensive among various lily forms as their bulbs are highly valuable and require special technology for their production. It has a wide applicability as a cut flower and potted plant in the floral industry.

Several attempts have been made on *in vitro* regeneration of bulblets

from lily bulbscales, nodal and internodal segments, shoot tips, flowers, stems, embryos, petals, and leaves (Kumar et al., 2006). Although many explants have been used, bulbscales remained the choicest to regenerate bulblets in *Lilium* because scales seemed to be the most productive (Stimart and Ascher, 1978; Takayama and Misawa, 1979; Lian et al., 2003; Kumar et al., 2001, 2005).

The use of roots as a source of explants for *in vitro* propagation is limited to a small number of species (Bhat et al., 1992; Carmi et al., 1997; Vinocur et al., 2000; Zobayed and Saxena, 2003). The root explant source offers obvious advantages such as ease of manipulation, availability and less oxidation after excision in comparison with other organ cultures (Son and Hall, 1990). Root cultures have also received a considerable attention due to their stable metabolite production ability (Carvalho and Curtis, 1998). The aim of the present study was to elaborate a new propagation protocol for bulblet regeneration from root explants in oriental lily.

MATERIAL AND METHODS

The roots (2.5 cm long) of oriental lily hybrid cultivar `Rosato' were excised from aseptic cultures derived from bulbscales cultured for 90 days on MS (Murashige and Skoog, 1962) medium supplemented with 0.5 mg l^{-1} NAA and 1 mg l^{-1} BA. Root segments (4-5 mm long) from the distal (containing root meristem) and middle zones were inoculated on MS medium supplemented with 30 g l^{-1} sucrose and solidified with 8 g l^{-1} agar. The pH of the medium was adjusted to 5.8 before autoclaving.

The following growth regulators, alone or in combination, were also added to the medium:

- indole-3-butyric acid (IBA): 1.0, 1.5 or 2.0 mg l^{-1};
- naphthaleneacetic acid (NAA): 1.0, 1.5 or 2 mg l^{-1};
- dichlorophenoxyacetic acid (2,4-D): 1.0, 1.5 or 2.0 mg l^{-1};
- benzyladenine (BA): 1.0, 1.5 or 2.0 mg l^{-1}.

Each treatment consisted of ten explants and the experiment was repeated three times. The explants were cultured in a growth room maintained at 25°C and a 16 hour photoperiod under artificial light at an intensity of 100 μmol m^{-2} s^{-1}. Explants cultured without growth regulators served as a control.

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

All data were elaborated statistically using a completely randomized design (CRD) as described by Gomez and Gomez (1984). The statistical analysis based on mean values per treatment was performed using analysis of variance (ANOVA) for CRD.

The regenerated bulblets were separated and transferred to the rooting medium consisted of MS salts supplemented with 1 mg l^{-1} IBA. The bulblets with leaves and adequate number of roots were removed from

the flasks, washed thoroughly with tap water and transferred to plastic pots (10 cm in diameter) containing a mixture of cocopeat and sphagnum peat mixed in the ratio 1:1. The bulblets were hardened by covering them with jars to maintain high humidity at the initial stages. The jars were removed gradually and bulblets watered regularly. The survival rate of the bulblets was recorded thirty days after transferring to pots. The hardened bulblets were stored at 2°C before transferring to pots filled with barnyard manure and sand mixed in the ratio 1:1. The process was repeated till required size of the bulblets for flowering was achieved.

RESULTS AND DISCUSSION

The root explants isolated from the distal zones of 2.5 cm long *in vitro* roots did not produce bulblets on growth regulator-free medium or when the medium was supplemented with growth regulators alone or in combinations. The bulblets were formed on explants from the middle zone of the roots cultured on medium supplemented with NAA, 1.5 mg l^{-1} 2,4-D or 1.5 and 2 mg l^{-1} BA (Tab. 1; Fig. 1A). Only 20-70% of the explants regenerated 2-3 bulblets, depending upon the concentration of growth regulators. The highest percentage of explants producing bulblets was observed in the culture medium supplemented with 2 mg l^{-1} NAA. The lowest percentage of explants producing bulblets was recorded with 1.5 mg l^{-1} BA. In the previous study, Maesato et al. (1994) reported that supplementation of medium with cytokinins, alone or in combination with NAA, induced lower response than that supplemented with NAA alone because the excised bulbscales have sufficient endogenous cytokinin-/cytokinin-like substances and, when supplemented with auxins, they might attain critical cytokinin-auxin balance required for organ regeneration. In another study, Niimi (1985) reported that NAA at 0.05 and 0.1 mg l^{-1} stimulated bulblet formation in *L. rubellum* and the addition of BA had little effect. Hardly any report on bulblet regeneration from root explants in *Lilium* is available in the literature. Cavallini and Natali (1989) reported somatic embryogenesis from root explants of *Brimeura amethystina*.

Auxins in combination with BA did not affect significantly the percentage of explants producing bulblets and the number of bulblets formed, except for medium supplemented with 2 mg l^{-1} NAA and 2 mg l^{-1} BA, where the average number of bulblets produced by an explant increased to four. In the previous study on bulbscale, Maesato et al. (1994) observed as many as seven bulblets per an explant on medium with 0.1 mg 1^{-1} NAA and 1 mg l^{-1} 2-IP in *Lilium rubellum* and Kumar et al. (2007) recorded four bulblets in oriental lily cultivar 'Marco Polo' on medium with 0.5 mg l^{-1} NAA and 1 mg l^{-1} BA. In another study, mass multiplication by indirect organogenesis in young stem, leaf and root explants on medium with2.5 mg l^{-1} BA and 1.5 mg l^{-1} NAA was reported in

Table 1. Effect of growth regulators on bulblet regeneration from the middle zone of *in vitro* roots of lily oriental hybrid

Treatment [mg l^{-1}]				Explants producing bulblets [%]*	The number of bulblets per an explant	Bulblet fresh weight [mg]
Control				0	0	0
IBA	1.0			0	0	0
	1.5			0	0	0
	2.0			0	0	0
NAA	1.0			50.0 (45.00)	2.0	122.5
	1.5			50.0 (45.00)	2.0	165.6
	2.0			70.0 (57.00)	3.0	200.0
2,4-D	1.0			0	0	0
	1.5			0	0	0
	2.0			40.0 (39.23)	2.2	160.9
BA	1.0			0	0	0
	1.5			20.0 (26.56)	2.5	126.4
	2.0			50.0 (45.00)	2.0	144.3
IBA	1.0			0	0	00
	1.5	BA	1.0	0	0	0
	2.0			0	0	0
NAA	1.0			0	0	0
	1.5	BA	1.0	0	0	0
	2.0			0	0	0
2,4-D	1.0			0	0	0
	1.5	BA	1.0	0	0	0
	2.0			0	0	0
IBA	1.0			20.0 (26.56)	2.5	120.2
	1.5	BA	1.5	40.0 (29.23)	3.2	110.6
	2.0			30.0 (33.21)	3.3	161.8
NAA	1.0			30.0 (33.21)	3.0	216.0
	1.5	BA	1.5	30.0 (33.21)	3.3	200.0
	2.0			20.0 (26.56)	3.5	125.9
2,4-D	1.0			20.0 (26.56)	2.5	100.7
	1.5	BA	1,5	30.0 (33.21)	3.0	99.7
	2.0			40.0 (39.23)	2.2	99.5
IBA	1.0			40.0 (39.23)	3.3	125.8
	1.5	BA	2.0	60.0 (50.77)	36	104.0
	2.0			0	0	0
NAA	1.0			50.0 (45.00)	3.2	125.8
	1.5	BA	2.0	60.0 (50.77)	3.0	196.3
	2.0			80.0 (63.43)	4.0	165.3
2,4-D	1.0			0	0	0
	1.5	BA	2.0	20.0 (26.56)	3.0	103.6
	2.0			30.0 (33.21)	3.3	108.2
$LSD_{0.05}$				6.13	0.30	7.56

Figures within parentheses are arc sin transformed values
*Mean of ten explants

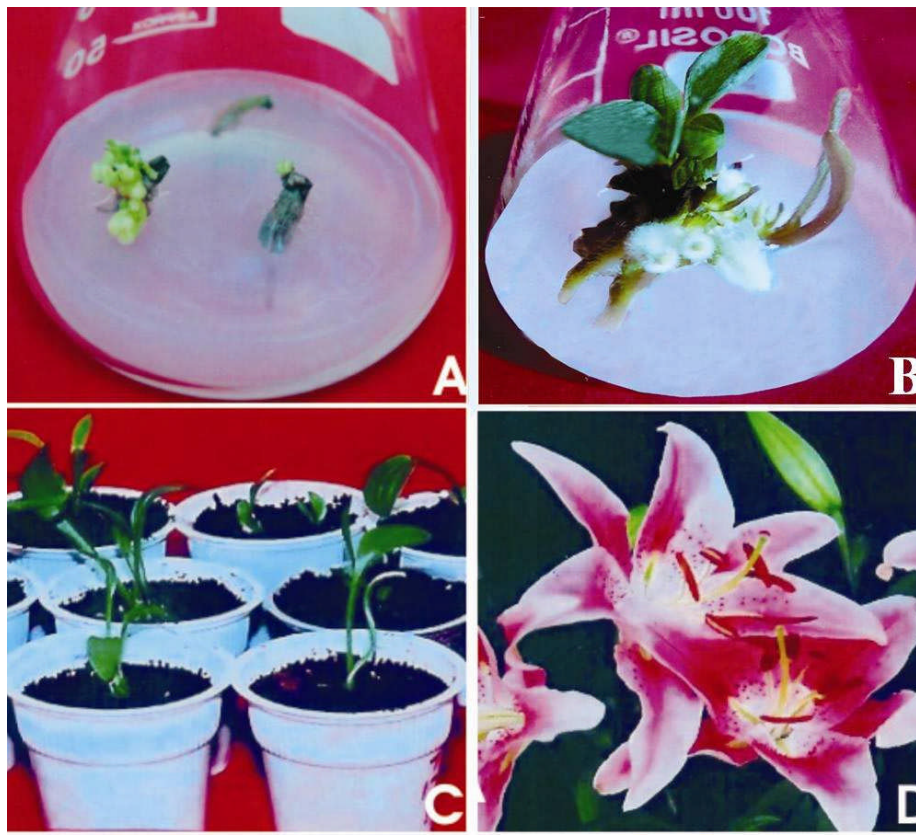

Figure 1. **A.** Bulblet formation on root explant on MS medium supplemented with 2 mg l^{-1} NAA; **B.** Rooting of bulblets on MS medium supplemented with 1 mg l^{-1} IBA; **C.** Acclimatized and hardened *in vitro* rooted bulblets thirty days after they were transferred to pots and **D.** Flowering plant in earthen-ware pot (25 cm diameter) in the second year of growing period

Plumbago rosea (Satheeshkumar and Seeni, 2003).

The weighty bulblets with an average fresh weight of 216 mg were obtained on medium supplemented with 1.0 mg l^{-1} NAA and 1.5 mg l^{-1} BA. These results agree well with those of the previous study, in which the bulblets of *Lilium rubellum* had relatively high fresh weight on MS medium supplemented with 1 mg l^{-1} BAP and 1 mg l^{-1} NAA (Niimi and Onozawa, 1979).

Bud regeneration in lily was also affected by the position of the isolated segments on the root. In the present study, the middle section of the root was found to be more suitable for bulblet induction. These results agree well with those of the previous studies on Asiatic and oriental hybrid lilies (Kapoor et al.,

2008). In another study, Carmi et al. (1997) also observed higher number of buds formed on explants from the middle zone of 2.5 cm long *in vitro* roots in *Populus tremula*. Bulblets were not observed when distal section (containing root cap) was used as an explant. The distal section grew further to produce new roots, indicating that the actively growing distal zone had limited number of competent responding cells that failed to regenerate bulblets. It may be possible that BA or NAA affect only competent cells present in proximity of dividing meristematic cells (Christianson, 1998). Thus, regeneration potential of the isolated segments of *in vitro* roots is largely dependent on the location of the segment isolated from the main root and level and type of the growth regulator. The bulblets were regenerated without any callus formation as an intermediate stage. These results agree well with those of previous studies on *Comptania peregrina* (Goforth and Torrey, 1977) and *Populus alba* x *Populus grandidentata* (Son and Hall, 1990).

The bulblets were separated and rooted *in vitro* (Fig. 1B). After thirty days, about 90% of the bulblets were still alive (Fig. 1C). The *in vitro* bulbs attained the size of 14-16 cm after repeated storage at 2°C and transfer to soil. The bulbs flowered after second year of growing period without any phenotypic variations (Fig. 1D).

From the above results it may be concluded that, similarly to other explants of *Lilium*, roots has also the ability to regenerate bulblets which can be used in raising genetically identical plants for propagating individual genotypes as clones.

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REGENERACJA *IN VITRO* CEBUL LILII ORIENTALNYCH HYBR. Z KORZENI

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

Eksplantaty korzeni lilii orientalnych hybr. cv. 'Rosato' długości 4-5 mm były izolowane z korzeni długości 2,5 cm otrzymanych z łusek przetrzymywanych na pożywce Murashige-Skooga (MS) z dodatkiem NAA (0,5 mg l^{-1}) i BA (1,0 mg l^{-1}). Eksplantaty korzeni utrzymywano na pożywce MS uzupełnionej IBA, NAA i 2,4-D lub z dodatkiem BA. Regenerację cebul otrzymano z korzeni na pożywce MS z dodatkiem NAA (1,0; 1,5; 2,0 mg l^{-1}), 2,4-D (2,0 mg l^{-1}) i BA (1,5; 2,0 mg l^{-1}). Na pożywkach zawierających NAA, 2,4-D IBA łącznie z BA zachodziła regeneracja cebul z korzeni. Najwięcej cebul tworzyło się na pożywce MS zawierającej łącznie NAA w stężeniu 2,0 mg l^{-1} i BA w stężeniu 2,0 mg l^{-1}. Po 2 latach uprawy w doniczkach cebule osiągały obwód 14-16 cm, a kwiaty nie wykazywały zmian fenotypowych.

Słowa kluczowe: regeneracja cebul, regulatory wzrostu, eksplantaty korzeni, *in vitro*, lilie orientalne