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BRIEF C0MMUNICATION

In vitro regeneration and bulblet growth from lily bulbscale explants as affected by retardants, sucrose and irradiance

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Abstract

Bulbscales of oriental lily hybrid Star Gazer were used as the explants. Bulblets were formed on the basal portion of the excised bulbscales on MS medium supplemented with growth retardants, different sucrose concentrations and exposed to continuous light or dark. *Alar*, *Cycocel* and *Paclobutrazol* in concentration 1 mg dm^{-3} produced higher number of bulblets as compared to the control. The number of bulblets, however, decreased with the increase in concentration of the growth retardants. The number of bulblets was higher at 90 than at 60 g dm^{-3} sucrose and when the bulbscales were exposed to continuous light than to darkness. The growth retardants, higher sucrose concentration and continuous dark stimulated fresh mass of bulblets. The number of bulblets having roots and leaves decreased in medium with *Alar*, *Cycocel* and *Paclobutrazol* as compared to the control. A few bulblets produced roots and leaves in medium with 90 g dm^{-3} sucrose and none of the regenerated bulblets produced leaves under continuous dark.

Additional key words: *Lilium*, *Alar*, *Cycocel*, *Paclobutrazol*, bulblet size.

Lily is one of the leading cut flower crops in the world. Several attempts have been made to multiply lily through tissue culture. However, the bulblets raised through tissue culture, although have shown high multiplication rate remained small in size for commercial use (Niimi and Onozawa 1979, Tanaka *et al.* 1991, Maesato *et al.* 1994, Niimi 1995, Kumar *et al.* 2001). In bulbous crops, vigour and growth of the plants are directly correlated with size of the underground organs. Certain minimum size of the bulb is essential for the plant to flower. Plant growth regulators, sucrose as well as various environmental factors are known to play an important role on *in vitro* bulblet size and rate of multiplication in various bulbous crops (Kim and De Hertogh 1995, Maesato *et al.* 1994, Lian *et al.* 2003). In the present investigation an attempt has been made to study the effects of growth retardants, sucrose and irradiance on bulblet size and plant regeneration from bulbscale explants in oriental lily hybrid Star Gazer.

Pre-cooled bulbs (2 °C for 8 weeks) of oriental lily hybrid (*Lilium auratum* × *Lilium speciosum* × *Lilium rubellum*) Star Gazer were procured from the Department of Floriculture and Landscaping, University of Horticulture and Forestry, Solan. The bulbscales were excised from the bulbs and the lower portion (3 × 4 mm) of the inner most scales was used as the explant. The explants were washed thoroughly in running tap water, surface sterilized with 0.1 % $HgCl_2$ for 3 - 4 min, rinsed with sterile distilled water and divided into three groups. The first group was cultured on Murashige and Skoog (1962; MS) medium supplemented with 8 g dm^{-3} agar, 30 g dm^{-3} sucrose and 1, 2.5 and 5 mg dm^{-3} each of *Alar* (succinic acid-2,2-dimethyl hydrazine), *Cycocel* (2-chloroethyl-trimethylammonium chloride) and *Paclobutrazol* (N-dimethylaminosuccinamic acid) (P_{333}), the second group was cultured in MS medium containing 60 and 90 g dm^{-3} sucrose and the third group was exposed to continuous light (50 - 60 μmol m^{-2} s^{-1}) or darkness in MS medium having 30 g dm^{-3} sucrose. The cultures maintained on MS medium supplemented with 30 g dm^{-3} sucrose and exposed to 16-h photoperiod served as control. Ten bulblets were kept for each treatment with one bulblet in each culture tube (25 × 150 mm). The experiment was repeated thrice with similar trends of results. The pH of the medium was adjusted to 5.8 before autoclaving at 121 °C, at a pressure of 1.1 kg cm^{-2} for 15 min. The cultures for the first and second group were maintained in culture room at 24 ± 2 °C under 16-h

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Abbreviations: MS medium - Murashige and Skoog medium; P_{333} - *Paclobutrazol*.
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photoperiod and irradiance of 50 - 60 μmol m^{-2} s^{-1} provided by cool white fluorescent tubes.

The regeneration percentage was recorded after 30 d whereas average fresh mass, diameter, number of scales per bulblet and number of bulblets with leaves and roots after 90 d of incubation. The data were analyzed statistically using analysis of variance (*ANOVA*).

When basal portions of the excised bulbscales were transferred to MS medium bulblets were formed even in the case without growth regulators (*i.e.* control). Out of the growth retardants used, *Cycocel* followed by *Alar*, at all the concentrations gave best (60 - 67 %) regeneration percentage, and maximum number of bulblets. Although, the regeneration response with P_{333} was at par with the control, 1 mg dm^{-3} was most effective in increasing the number of bulblets (Fig. 1*A*) The regeneration percentage and the number of bulblets decreased with the increase in concentration of the retardants (Table 1). The regeneration response and the number of bulblets per explant also increased with the increase in sucrose concentration and in explants exposed to continuous light in comparison with those exposed to darkness and 16-h photoperiod (Table 1).

The average fresh mass and diameter of bulblets increased with the treatments of *Alar*, *Cycocel* and P_{333} as compared to control. The highest fresh mass and diameter were recorded for bulblets with 5 mg dm^{-3} *Alar*. The fresh mass and diameter increased with sucrose concentration, with maximum mass at 90 g dm^{-3}. The regenerated bulblets had higher fresh mass and diameter under continuous darkness (Table 1).

The number of bulbscales per bulblet increased with the addition of *Alar* (5 mg dm^{-3}), *Cycocel*, P_{333} and high sucrose concentrations and in explants exposed to

Fig. 1. Regenerated bulblets with leaves from bulbscales cultured on MS medium supplemented with 1 mg dm^{-3} P_{333} (*A*). Regenerated bulblets without leaves from bulbscales cultured on MS medium supplemented with 5 mg dm^{-3} *Alar* (*B*), 90 g dm^{-3} sucrose (*C*) and exposed to continuous darkness (*D*).

Table 1. Effect of growth retardants, sucrose and irradiance on regeneration percentage after 30 d of incubation and number of bulblets per explant, fresh mass, diameter, number of scales, and bulblet number with leaves and roots after 90 d of incubation. Mean ± SE, $n = 10$.

Treatments [mg dm^{-3}]	Regenerated explants [%]	Number of bulblets explant^{-1}	Fresh mass [mg]	Diameter [mm]	Number of scales	Number of bulblets with roots and leaves
Control	48 ± 4.50	2.5 ± 0.10	98 ± 1.05	2.8 ± 0.50	6.8 ± 0.16	6.0 ± 0.35
Alar 1.0	65 ± 2.00	4.9 ± 0.25	106 ± 2.25	3.8 ± 0.55	8.4 ± 0.20	4.0 ± 0.40
Alar 2.5	61 ± 3.50	3.6 ± 0.08	148 ± 1.35	5.2 ± 0.42	7.8 ± .25	2.0 ± 0.25
Alar 5.0	55 ± 1.50	3.5 ± 0.15	193 ± 2.50	6.6 ± 0.60	12.6 ± 0.55	0
Cycocel 1.0	67 ± 2.50	5.4 ± 0.15	174 ± 2.45	5.8 ± 0.24	11.8 ± 0.24	4.5 ± 0.15
Cycocel 2.5	65 ± 2.45	3.8 ± 0.05	132 ± 1.75	5.0 ± 0.25	10.4 ± 0.25	5.0 ± 0.18
Cycocel 5.0	60 ± 0.20	3.4 ± 0.10	132 ± 1.70	5.0 ± 0.15	10.4 ± 0.26	4.0 ± 0.20
P_{333} *1.0*	52 ± I.54	6.0 ± 0.18	164 ± 2.50	5.4 ± 0.45	11.8 ± 0.3	5.2 ± 0.24
P_{333} *2.5*	50 ± 4.35	4.0 ± 0.12	136 ± 1.95	5.0 ± 0.60	10.2 ± 0.25	4.0 ± 0.32
P_{333} *5.0*	48 ± 3.50	3.6 ± 0.06	116 ± 2.00	4.0 ± 0.20	9.2 ± 0.35	3.5 ± 0.10
Sucrose 6000	54 ± 2.55	4.4 ± 0.13	154 ± 1.85	4.0 ± 0.16	7.6 ± 0.20	6.5 ± 0.25
Sucrose 9000	63 ± 1.54	6.0 ± 0.26	226 ± 1.55	5.8 ± 0.15	9.4 ± 0.24	4.5 ± 0.15
Continuous light	56 ± 2.45	5.8 ± 0.15	174 ± 2.50	6.0 ± 0.20	12.6 ± 0.16	3.8 ± 0.25
Continuous dark	48 ± 2.40	3.2 ± 0.08	200 ± 1.55	6.8 ± 0.30	12.6 ± 0.45	0
$CD_{0.05}$	1.05	0.54	1.03	0.43	0.73	0.35

continuous light and darkness in comparison with control (Table 1).

The number of bulblets with roots and leaves decreased with the addition of *Alar, Cycocel* and P_{333} in comparison with control. In fact, none of the bulblets produced leaves with 5 mg dm^{-3} *Alar* (Fig. 1*B*). The number of bulblets producing roots was higher with 90 g dm^{-3} sucrose (Fig. 1*C*). The number of bulblets with roots and leaves both was lower under continuous light and none of the bulblets under continuous darkness produced leaves (Table 1, Fig. 1*D*).

In micropropagation of bulbous crops, it is desirable to produce large bulblets for their ornamental value. The present study has shown that the incorporation of different growth retardants at various concentrations enhanced *in vitro* bulblet size and fresh mass. The increase in bulblet size and mass by these chemicals may be due to the fact that extension growth was arrested and the available sugars, normally utilized to sustain the growth, have formed storage sugars (Stoddart 1964), thereby increasing size and mass of the bulblets. Uptake of nutrients by the explant and their transport from explant to the bulblets may also result in increased size. Lim *et al*. (1998) reported that most of the retardants did not affect bulblet growth except for a high concentration of B_9, which strongly suppressed the formation of scaly leaves under light and enhanced bulblet growth in lily cv. Casa Blanca.

The number of bulblets per explant increased at 1 mg dm^{-3} *Alar*, *Cycocel* and P_{333}, but decreased at higher concentrations. There are reports demonstrating the role of ABA in bulb formation in *Lilium* (Klerk 1992, Kim *et al*. 1994). Also, role of P_{333} in enhancing bulblet formation has earlier been reported (Klerk 1992, Thakur *et al*. 2002). However, there was no report about the role of *Alar* and *Cycocel* in bulblet growth in *Lilium* under *in vitro* conditions. The present study has shown that growth regulators play an effective role in bulblet multiplication as well as growth *in vitro* in oriental hybrid lily.

Sucrose and irradiance also play a significant role in increasing the size of *in vitro* bulblets. Incorporation of sucrose at various concentrations in the medium enhanced bulblet size. Sucrose at higher concentration was more effective in increasing average fresh mass and diameter of *in vitro* bulblets confirming the previous studies (Klerk *et al*. 1992, Bonnier and Van Tuyl 1997, Marinengeli and Curvetto 1997). The increase in bulblet size in high concentration of sucrose was mainly due to an increase in starch and total carbohydrates (Langens-Gerrits *et al*. 1997).

Incubation in light enhanced the bulblet number; number of bulbscales and number of leaves as well as roots per bulblet. In addition, leaf emergence and its growth from the regenerated bulblets was observed only in light. Leshem *et al*. (1982) and Takayama and Misawa (1979) also reported such a stimulatory response of light in leaf emergence. Similarly, fresh mass increased under continuous light and continuous dark than under 16-h photoperiod. Leshem *et al*. (1982) reported that the mean fresh mass was significantly greater in dark than in light in *Lilium longiflorum*. The difference in fresh mass between light and dark may be due to increased water uptake due to etiolated growth. More number of bulblets was produced in continuous light. These results are in agreement with those of Leshem *et al*. (1982) but contrary to the observations of Stimart and Ascher (1978) who reported that light suppressed bulblet formation.

However, the bulblets produced under light were smaller in size. The difference was also observed in the growth habit of the bulblets, in dark, only fewer bulblets produced leaves. Maesato *et al*. (1994) reported that the number of bulblets per explant was lower in dark. It could be expected that competition for nutrients among bulblets would be lower in dark (as only few bulblets were formed in dark) than in the bulblets formed in light. Hence the bulblets in dark had higher fresh mass (Niimi 1995).

From the above results, it is concluded that growth retardants and higher sucrose concentration in the medium and incubation of cultures in continuous dark can be used to increase the average fresh mass and diameter of the bulblets *in vitro*, which is the basic need for vigour and growth of the plant for bigger size of the underground storage organ which in turn produces quality flowers.

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