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In-vitro propagation of asiatic hybrid lily from bulb scales*

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The genus *Lilium* belongs to family Liliaceae and is characterized by annual thermoperiodism. Among the various groups, hybrids of asiatic, oriental and longiflorum being exceptionally beautiful, are commercially grown for their wide applicability in floral industry, mainly as cut-flowers. This is one of the leading cut-flowers among various geophytes all over the world, because of its long vase-life and capacity to rehydrate after long transportation. The most commercially grown cultivars are propagated through vegetative means like stem bulblets, bulbils and adventitious bulblet formation on bulb scales. However, due to inadequate rate of multiplication through conventional propagation techniques, it is not possible to meet the growing demand of industry. Therefore, keeping in view the potentialities of tissue-culture techniques and the problems associated with conventional methods, an attempt was made to propagate *in-vitro* 2 cultivars of asiatic hybrid lily from bulb scales.

Scales (inner) from 2 pre-cooled (2°C for 8 weeks) asiatic hybrid cultivars 'Gran Paradiso' and 'Apeldoorn' of *Lilium* were removed from the bulbs and the basal portion (4–5 mm) of the scales was used as explant. The explant was thoroughly washed with water and surface sterilized with 0.1% $HgCl_2$ containing Tween 20, for 3–4 min. They were washed 3–4 times with sterilized distilled water and cultured on MS medium containing 0.5 and 1 mg/litre each of Benzyl amino purine (BAP) and NAA. The cultures were maintained on these media for 1 month and individual bulblets formed were transferred to the multiplication medium consisting of MS supplemented with 0.5 and 1 mg/litre BAP and NAA alone as well as in combination. The cultures were maintained in the culture room at 25 ± 2°C under 16 hr photoperiod provided with white-cool-fluorescent tubes with a light intensity of 1.5 K lux. The data on the number of days for establishment of culture, number of bulblets formed/explant and number of leaves/bulblets were recorded after 3 months of incubation.

The individual bulblets were separated, leaves were cut and transferred to the MS medium without growth regulators. When the leaves were dried, the bulblets were removed, washed thoroughly and dried in air at room temperature. The bulblets were treated with Bavistin (0.1%,w/w) and stored at 2°C for 8 weeks in peat for meeting cold requirement.

The `Gran Paradiso' took lesser time (25.5 days) for the establishment of cultures than `Apeldoorn' (37.6 days). The early establishment of `Gran Paradiso' may be attributed to the varietal character. In both the cultivars, earlier culture establishment was noted when MS medium was supplemented with 1 mg/litre NAA (Table 1). `Gran Paradiso' took 20.8 days and `Apeldoorn' 35 days (Table 1). However, when BAP was used in the MS medium, the number of days taken for establishment was slightly higher in both the cultivars compared with NAA. Dilta *et al.* (2000) showed BA at 1 mg/litre responsible for earlier establishment of cultures in 2 hybrid lily cultivars `Pollyana' and `Star Gazar'. Novak and Petru (1981) also reported earliness in Lilium Oriental hybrid `Crimson Beauty' on MS medium containing 1 mg/litre BA and 0.1 mg/litre NAA.

'Gran Paradiso' produced higher number of bulblets than 'Apeldoorn'. The number of bulblets/explant increased in both the cultivars when the medium was supplemented with 0.5 mg/litre NAA (Fig 1 *top left*). Similar observations were also recorded by Niimi (1984) and Priyadarshi and Sen (1992). BAP at 1 mg/litre also produced higher number of bulblets in both the cultivars which was close to 1 mg/litre NAA and may be due to the fact that BA promotes formation

Table 1 Effect of BAP and NAA on days for culture establishment

Growth regulator (mg/litre)	Varieties	
	`Gran Paradiso'	`Apeldoorn'
Control	25.5 ± 1.07	37.6 ± 0.80
BAP,0.5	23.5 ± 0.84	35.7 ± 0.76
BAP,1	22.8 ± 0.55	36.0 ± 0.45
NAA,0.5	22.0 ± 0.36	34.4 ± 0.62
NAA,1	20.8 ± 1.12	35.0 ± 1.14

*Short note

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Table 2 Effect of BAP and NAA on bulblet and leaf formation

Growth regulator (mg/litre)	Bulblets/explant		Leaves/bulblet	
	'Gran Paradiso'	'Apeldoorn'	'Gran Paradiso'	'Apeldoorn'
Control	5.0 ± 0.23	2.5 ± 0.22	4.0 ± 0.21	2.5 ± 0.11
BAP,0.5	5.5 ± 0.35	3.0 ± 0.12	4.2 ± 0.12	2.0 ± 0.14
BAP,1	6.3 ± 0.43	3.8 ± 0.15	3.8 ± 0.21	2.3 ± 0.15
NAA,0.5	8.0 ± 0.64	4.5 ± 0.25	4.0 ± 0.30	2.6 ± 0.18
NAA,1	6.5 ± 0.33	4.0 ± 0.35	5.3 ± 0.25	3.5 ± 0.20
BAP,0.5+NAA,0.5	10.0 ± 0.56	5.8 ± 0.55	4.2 ± 0.32	2.3 ± 0.21
BAP,0.5+NAA,1	6.0 + 0.35	5.0 ± 0.25	3.9 ± 0.15	2.4 ± 0.25
BAP,1+NAA,0.5	6.6 ± 0.32	5.2 ± 0.43	4.1 ± 0.23	3.0 ± 0.16
BAP,1+NAA,1	5.8 ± 0.26	5.0 ± 0.52	4.2 ± 0.31	2.9 ± 0.41

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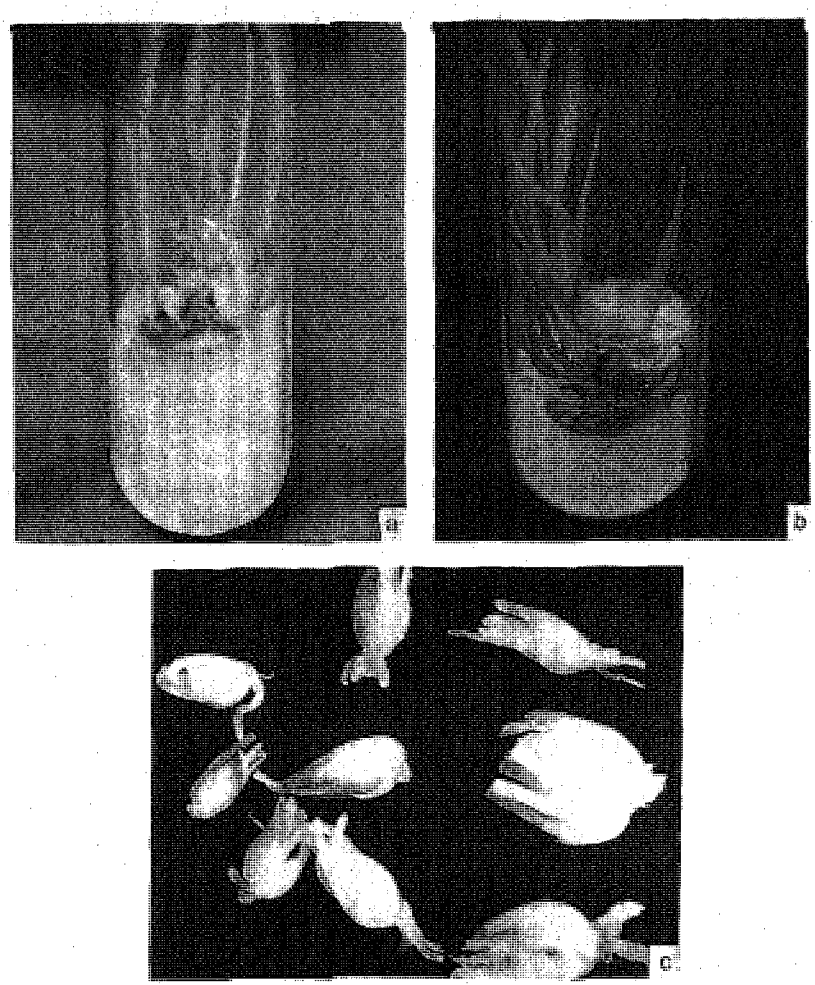

Fig 1. **a**, Bulblet formation in 'Gran Paradiso' on MS medium supplemented with 0.5 mg/litre NAA; **b**, bulblet proliferation in 'Gran Paradiso' on MS medium supplemented with 0.5 mg/litre BAP + 0.5 mg/litre NAA; **c**, bulblets formed after three months of incubation

of adventitious buds in excized organs and in-vitro cultured tissues.

The NAA is essential for the formation and growth of bulblets in scale culture (Stimart and Ascher 1978). Van Aatrijk *et al.* (1985) noticed that auxin and its distribution within the tissue are important factors in the process of bud

regeneration from lily scales. The number of bulblets increased further when medium was supplemented with 0.5 mg/litre each of BAP and NAA (Table 2, Fig 1, *top right*).

Maximum leaves/bulblet were recorded when MS medium was supplemented with 1 mg/litre NAA in both the cultivars (Table 2). The number of leaves varied in both the cultivars , 'Gran Paradiso' having 5.3 and 'Apeldoorn' 3.5. The number of leaves did not vary much when MS medium was supplemented with either BAP alone or in combination with NAA. However, Dilta *et al.* (2000) reported maximum leaves/ bulblet with 0.5 mg/litre NAA and 0.5 mg/litre BA. The variations in the results with respect to leaf number may be attributed to the genetic differences. The bulblets were separated and transferred to the basal MS medium. When the leaves started drying, these were taken out of the test tubes and washed thoroughly. The dried leaves were removed and the bulblets were stored as described earlier (Fig 1, *bottom*).

SUMMARY

Explants from basal bulb-scale segments (4-5 mm) of pre-cooled (2^0C for 8 weeks) asiatic hybrids 'Gran Paradiso' and 'Apeldoorn' of lily (*Lilium* sp) were established and proliferated on MS medium supplemented with 0.5 and 1 mg/litre each of NAA and BAP. Culture establishment was much earlier in 'Gran Paradiso' than 'Apeldoorn' when the medium was supplemented with 1 mg/litre NAA. Maximum litre bulblets were regenerated per explant in 'Gran Paradiso' with 0.5 mg/litre and 0.5 mg/litre BAP in the medium.

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