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*Full Length Research Paper*

# *In vitro* propagation and organogenesis of *Lilium* 'Prato'

**Hany El-Naggar[1]*, Amira Osman[2] and Eman Sewedan[2]**

[1]Department of Floriculture, Faculty of Agriculture, Alexandria University, Egypt.
[2]Department of Horticulture, Faculty of Agriculture, Damanhour University, Egypt.

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***Lilium*** **consists of more than 80 species native to the Northern Hemisphere. It is widely used as cut flowers, flowering potted and garden plants. Since** ***Lilium in vitro*** **production, as an alternative to the conventional vegetative propagation methods is becoming an important way to increase shoot proliferation rates; therefore, the purpose of this study was to establish a protocol for** ***in vitro*** **production of** ***Lilium*** **Asiatic hybrid 'Prato' and to compare between two explants for shoot proliferation and organogenesis. Bulb scales and leaf segments as explants of** ***Lilium*** **'Prato' were cultured on Murashige and Skoog (MS) basal medium supplemented with benzyl adenine (BA) at 0.25, 0.5, 1.0 and 2.0 mg/l and naphthalene acetic acid (NAA) at 0.25, 0.5 and 1.0 mg/l. Callus was formed over the bulb scales before shoot organogenesis occurred, while shoot organogenesis occurred directly from the leaf segments without callus formation. It was found that the bulb scales gave higher percent of shoot regeneration than leaf segments when used as explants and was 96.67 and 64.67%, respectively. BA at 0.5 mg/l gave the highest percentage of shoot formation, shoot height and the lowest number of days to proliferation, while BA at 2.0 mg/l caused a delay in shoot organogenesis and reduced shoot height in both explants.**

**Key words:** *Lilium* hybrid, benzyl adenine, naphthalene acetic acid.

## INTRODUCTION

*Lilium*, a monocotyledonous species belonging to the *Liliaceae*, is one of the most important cut-flower species, mainly because of its large, attractive flowers and its long vase life.

The genus *Lilium* comprises about 80 species, classified into seven sections (De Jong, 1974). *Lilium* 'Prato' belongs to the Asiatic hybrids which originated from interspecific crosses between species of the *Sinomartagon* section and the Oriental hybrids, from crosses within the *Archelirion* section. Most cultivars are grown for bulb and cut flower production (Van Tuyl et al., 1991). *Lilium* 'Prato' has clear orange flowers without spots on petal, used as cut flower, pot plant and in flowering beds.

*Corresponding author. E-mail: hanyelnaggar@hotmail.com. Tel: 002-0100-7340409.

Conventional breeding in the lily is hampered by its heterozygous state present among the species of the different *Lilium* groups (Van Tuyl et al., 1990).

Cytokinins act as cell division mediating factors and upon the differentiation and arrangement of parenchyma cells and procambium necessary for shoot development and regulation, plant meristem activity as the apical meristem decreased in size with reduction of cytokinin levels and are generally known to promote formation of buds and shoot differentiation in tissue cultured organs (Nitsch et al., 1967; Werner et al., 2001; Takateru et al., 2007).

Benzyl adenine (BA) is one of the synthetic cytokinin that enhances plant growth and development and it is used in *Lilium* species due to its effect on the adventitious shoot formation (Takayama and Misawa, 1983; Maesato et al., 1994).

Takateru et al. (2007) mentioned that BA concentration plays an important role in the transition from juvenile to

**Table 1.** Effects of BA and NAA on shoot formation from bulb scales of *Lilium* 'Prato' cultured on MS media.

| Concentration | Percentage of shoot formation | Number of shoots | Shoot height (cm) | Fresh weight (g) | Days to proliferation |
|---|---|---|---|---|---|
| **NAA (mg/l)** | | | | | |
| 0.25 | 85.50[a] | 3.67[ab] | 4.88[b] | 874.25[a] | 56.25[a] |
| 0.5 | 85.75[a] | 4.08[a] | 6.57[a] | 804.58[ab] | 52.25[b] |
| 1.0 | 80.00[a] | 3.08[b] | 6.33[a] | 757.25[b] | 55.75[a] |
| **BA (mg/l)** | | | | | |
| 0.25 | 78.00[b] | 1.56[c] | 7.01[a] | 498.44[c] | 52.33[b] |
| 0.5 | 96.67[a] | 3.78[b] | 6.43[a] | 765.67[b] | 48.89[b] |
| 1.0 | 94.00[a] | 4.00[ab] | 5.61[b] | 1020.56[a] | 57.22[a] |
| 2.0 | 66.33[c] | 5.11[a] | 4.64[c] | 963.44[a] | 60.56[a] |

vegetative adult phase in *Lilium formolongi* 'White Aga' cultured *in vitro*. BA is the most effective cytokinin for adventitious shoot regeneration from leaf and nodal-stem segments explants of Asiatic *Lilium* genotype 'Elite' (Loretta et al., 2003).

Non conventional way of breeding such as transformation requires a protocol for *in vitro* regeneration of *Lilium*; therefore, the objective of the present research was to establish a protocol to optimize *in vitro* cultural conditions and regeneration of *Lilium* Asiatic hybrid 'Prato' using cytokinin (BA), NAA and different explants.

## MATERIALS AND METHODS

This work was done at the Tissue Culture and Biotechnology Laboratory of the Horticulture Department, Faculty of Agriculture, Alexandria University, Egypt.

### Plant material

Bulbs and leaves (fully unfolded leaves from the 4$^{th}$ and 5$^{th}$ node from the growing point) of the cultivar 'Prato' were brought from commercial nursery. All explants were first washed with detergent and distilled water, then disinfected with 15% bleach plus two drops of Tween 20 for 20 min, then placed in ethanol 75% for one minute and rinsed three times with autoclaved distilled water, to ensure that the under soil parts and leaf segments are totally sanitized.

### Shooting medium

Bulb scales and leaf segments (1 cm$^2$ from middle leaf section containing the main midrib) were cultured on solidified media containing 4.43 g/l MS with vitamins (Murashige and Skoog, 1962) supplemented with 40 g/l sucrose and 7 g/l agar, the pH was adjusted at 5.8 ± 0.1 and poured in tubes (2.5 cm diameter × 15 cm height).

For shoot proliferation, four different concentrations of BA (0.25, 0.5, 1.0 and 2.0 mg/l) and three different concentrations of NAA (0.25, 0.5 and 1.0 mg/l) were added to the basal medium.

### Rooting medium

Rooting was achieved by transferring the regenerated shoot after 3 months (5 to 7 cm height) to rooting medium containing 4.43 g/l MS with vitamins + 40 g/l sucrose + 5 g/l agar + 0.5 g/l NAA and pH was adjusted to 5.8 ± 0.1. 25 ml of medium was poured in tubes (2.5 cm diameter × 15 cm height). All media were autoclaved at 121°C and 15 bar/cm$^2$ for 20 min. Plants were cultured in tubes and placed under cool white florescent light at intensity of 66 to 52 µmol/m$^2$/s for 16 and 8 h in the dark at 25 ± 1°C.

### Data collected

Data collected were percentage of formed shoots, number of shoots/explant, shoot height, the fresh weight of all aerial parts (shoots, leaves and bulblets were weighted during transfer of the regenerated shoots to the rooting medium) and the days to shoot proliferation.

### Statistical analysis

Two experiments, depending on the type of explants, were done; one for the bulb scales and one for the leaf segments, each was designed as factorial design with two factors (BA at 0.25, 0.5, 1.0 and 2.0 mg/l and NAA at 0.25, 0.5 and 1.0 mg/l) each with 3 replicates. Data were analyzed for significance by analysis of variance (ANOVA) using the SAS program (2002) and Tukey's multiple comparisons method for least significant difference at confidence level of $P<0.05$ (Tukey, 1994).

## RESULTS

From the results presented in this study, it appeared that NAA had no effect on the percentage of successful shoots formed from bulb scales and leaf segments, while the frequency of shoot formation from bulb scales and leaf segments increased by increasing BA concentration from 0.25 to 1.0 mg/l; then this percent declined after that at the highest BA concentration (2.0 mg/l) (Tables 1 and 3). The number of shoots increased by increasing BA concentration in the media (Tables 1 and 3), while NAA at high concentration (1.0 mg/l) caused a reduction in the number of shoots regenerated from bulb scales (Table 1) but it had no effect on the number of shoots formed from leaf segments (Table 3). The highest number of shoots

**Table 2.** Effects of interaction between BA and NAA on shoot formation from bulb scales of *Lilium* 'Prato' in culture on MS media.

| NAA (mg/l) | BA (mg/l) | Number of shoots | Shoot height (cm) | Fresh weight (g) | Days to proliferation |
|---|---|---|---|---|---|
| 0.25 | 0.25 | 1.33$^{e}$ | 5.60$^{ef}$ | 473.67$^{e}$ | 52.00$^{e}$ |
| 0.25 | 0.5 | 3.33$^{c}$ | 5.47$^{ef}$ | 883.00$^{c}$ | 51.33$^{ef}$ |
| 0.25 | 1.0 | 4.33$^{b}$ | 4.67$^{g}$ | 1125.67$^{a}$ | 54.67$^{d}$ |
| 0.25 | 2.0 | 5.67$^{a}$ | 3.80$^{h}$ | 1014.67$^{b}$ | 67.00$^{a}$ |
| 0.5 | 0.25 | 2.33$^{d}$ | 7.83$^{a}$ | 529.00$^{e}$ | 50.33$^{ef}$ |
| 0.5 | 0.5 | 3.67$^{bc}$ | 7.13$^{bc}$ | 735.00$^{d}$ | 46.00$^{g}$ |
| 0.5 | 1.0 | 4.33$^{b}$ | 6.13$^{de}$ | 975.00$^{b}$ | 55.00$^{d}$ |
| 0.5 | 2.0 | 6.00$^{a}$ | 5.13$^{fg}$ | 979.33$^{b}$ | 57.67$^{c}$ |
| 1.0 | 0.25 | 1.00$^{e}$ | 7.60$^{ab}$ | 492.67$^{e}$ | 54.67$^{d}$ |
| 1.0 | 0.5 | 4.33$^{b}$ | 6.70$^{cd}$ | 679.00$^{d}$ | 49.33$^{f}$ |
| 1.0 | 1.0 | 3.33$^{c}$ | 6.03$^{e}$ | 961.00$^{b}$ | 62.00$^{b}$ |
| 1.0 | 2.0 | 3.67$^{bc}$ | 5.00$^{fg}$ | 896.33$^{c}$ | 57.00$^{cd}$ |

**Table 3.** Effects of BA and NAA on shoot formation from leaf segments of *Lilium* 'Prato' in culture on MS media.

| Concentration | Percentage of shoot formation | Number of shoots | Shoot height (cm) | Fresh weight (g) | Days for proliferation |
|---|---|---|---|---|---|
| **NAA (mg/l)** | | | | | |
| 0.25 | 53.75$^{a}$ | 2.67$^{a}$ | 2.94$^{b}$ | 700.17$^{a}$ | 64.00$^{a}$ |
| 0.5 | 56.75$^{a}$ | 2.83$^{a}$ | 3.82$^{ab}$ | 690.50$^{a}$ | 64.42$^{a}$ |
| 1.0 | 50.25$^{a}$ | 2.42$^{a}$ | 3.97$^{a}$ | 634.33$^{a}$ | 68.08$^{a}$ |
| **BA (mg/l)** | | | | | |
| 0.25 | 47.00$^{b}$ | 1.22$^{b}$ | 4.62$^{a}$ | 390.22$^{c}$ | 61.22$^{bc}$ |
| 0.5 | 64.67$^{a}$ | 2.78$^{a}$ | 4.21$^{ab}$ | 645.00$^{b}$ | 59.00$^{c}$ |
| 1.0 | 63.33$^{a}$ | 3.11$^{a}$ | 3.32$^{bc}$ | 840.78$^{a}$ | 68.00$^{ab}$ |
| 2.0 | 39.33$^{b}$ | 3.44$^{a}$ | 2.14$^{c}$ | 824.00$^{a}$ | 73.78$^{a}$ |

from bulb scales and leaf segments were formed at 2.0 mg/l BA and 0.5 or 0.25 mg/l NAA (Tables 2 and 4). The shoot height produced from both bulb scales and leaf segments was reduced significantly by increasing BA concentration (Tables 1 and 3) and the highest shoot was reached at the lowest BA concentration (0.25 mg/l) combined with 0.5 or 1.0 mg/l NAA (Tables 2 and 4).

NAA concentration had no effect on the fresh weight of plants regenerated from leaf segments (Table 3), while fresh weight of formed shoots from scales and leaf segments increased by increasing BA concentration (Table 1 and 3), the fresh weight increased significantly at 1.0 and 2.0 mg/l BA combined with NAA at 0.25 mg/l (Table 2 and 4).

Shoot regeneration was delayed by increasing BA concentration, while NAA had no effect on days to proliferation (Tables 1 and 3). Number of shoots, shoot height and fresh weight were significantly higher in plants regenerated from bulb scales when compared with shoots regenerated from leaf segments (Tables 2 and 4), while the number of days to proliferation was higher in plants regenerated from leaf segments as compared to plants regenerated from bulb scales (Tables 2 and 4). Also, the percentage of shoot formation was higher in bulb scale plants than leaf segment plants (Tables 1 and 3).

Bulb scales formed callus first and then shoot organogenesis took place, while shoot organogenesis occurred directly from leaves without forming callus. Shoot regeneration mainly occurred from the cuts across the mid vein and the base of the leaf explants (Figures 1 and 2). Roots were formed after 15 to 20 days of transfer to rooting medium on all regenerated shoots (Figure 3).

## DISCUSSION

BA at 0.5 mg/l was the most effective concentration for shoot proliferation from scales and leaf segments reaching 96.67 and 64.67%, respectively (Tables 1 and 3)

**Table 4.** Effects of interaction between BA and NAA on shoot formation from leaf segments of *Lilium* 'Prato' in culture on MS media.

| NAA (mg/l) | BA (mg/l) | Number of shoots | Shoot height (cm) | Fresh weight (g) | Days for proliferation |
|---|---|---|---|---|---|
| 0.25 | 0.25 | 1.00$^{f}$ | 3.57$^{d}$ | 345.67$^{h}$ | 58.67$^{ef}$ |
| 0.25 | 0.5 | 2.33$^{de}$ | 3.47$^{d}$ | 717.67$^{d}$ | 58.00$^{ef}$ |
| 0.25 | 1.0 | 3.33$^{abc}$ | 2.67$^{ef}$ | 886.00$^{a}$ | 65.33$^{cd}$ |
| 0.25 | 2.0 | 4.00$^{a}$ | 2.06$^{fg}$ | 851.33$^{ab}$ | 74.00$^{a}$ |
| 0.5 | 0.25 | 1.67$^{ef}$ | 4.83$^{ab}$ | 439.00$^{g}$ | 60.33$^{def}$ |
| 0.5 | 0.5 | 3.00$^{bcd}$ | 4.67$^{bc}$ | 655.00$^{e}$ | 56.00$^{f}$ |
| 0.5 | 1.0 | 3.00$^{bcd}$ | 3.30$^{de}$ | 810.33$^{bc}$ | 67.67$^{bc}$ |
| 0.5 | 2.0 | 3.67$^{ab}$ | 2.47$^{fg}$ | 857.67$^{ab}$ | 73.67$^{a}$ |
| 1.0 | 0.25 | 1.00$^{f}$ | 5.47$^{a}$ | 386.00$^{gh}$ | 64.67$^{cd}$ |
| 1.0 | 0.5 | 3.00$^{bcd}$ | 4.50$^{bc}$ | 562.33$^{f}$ | 63.00$^{cde}$ |
| 1.0 | 1.0 | 3.00$^{bcd}$ | 4.00$^{cd}$ | 826.00$^{b}$ | 71.00$^{ab}$ |
| 1.0 | 2.0 | 2.67$^{cd}$ | 1.90$^{g}$ | 763.00$^{cd}$ | 73.67$^{a}$ |

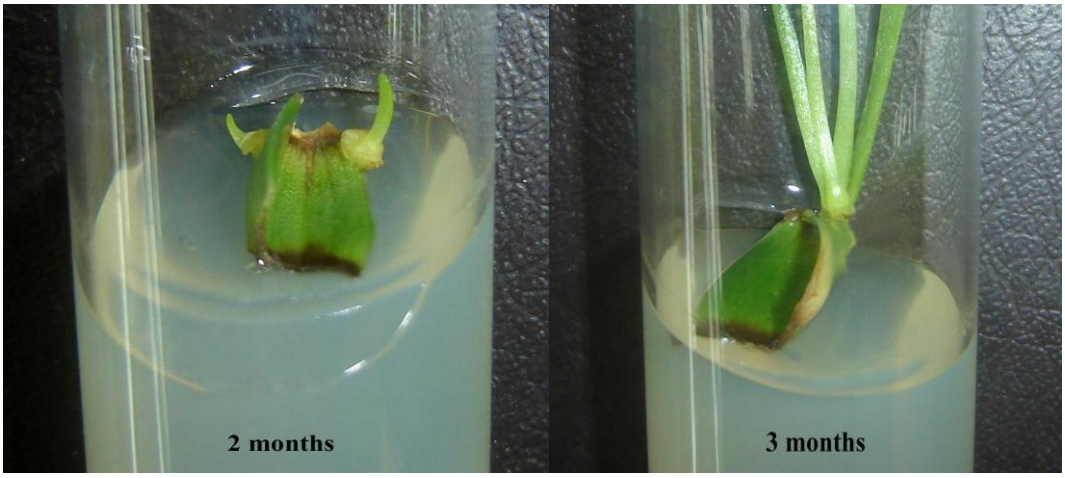


**Figure 1.** Shoot proliferation from leaf segments directly without callus formation after 2 and 3 months.

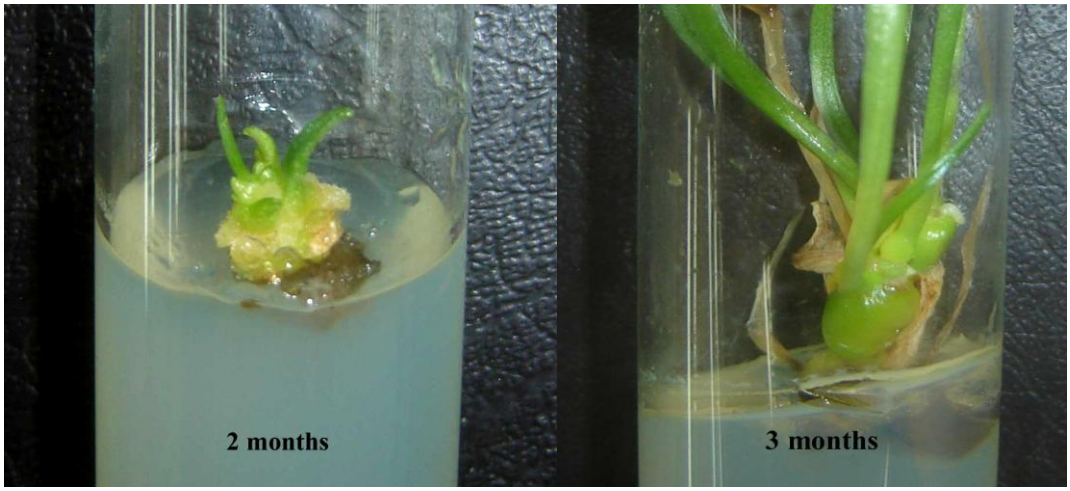


**Figure 2.** Shoot proliferation from bulb scales after callus formation after 2 and 3 months.

**Figure 3.** Root formation after transfer of shoots to rooting medium.

then declined at higher BA concentration and this is in agreement with the study of Han et al. (2004) and Loretta et al. (2003) who mentioned that BA and cytokinins in general at high concentration inhibited shoot formation from bulb scales. The percentage of shoot regeneration from leaf explants was low when compared with bulb scales, this was due to the high potential of bulb scales to regenerate adventitious bulbs when used as explants *in vitro* (Marijana et al., 2012); also it was due to the rapid browning and death of leaf segments and this was observed also by Loretta et al. (2003) since they mentioned that leaf explants from cv. Snow Queen rapidly became necrotic and there was no regeneration.

Godo et al. (1998) mentioned that the addition of BA to the solidified medium was effective for increasing the number of shoots induced from cell clumps of *Lilium* × *formolongi* and 1 mg/l BA was optimal, same results were obtained in this study and the highest number of shoots was obtained at 1.0 and 2.0 mg/l BA (Tables 1 and 3).

Shoot regeneration from leaf segments occurred at the cuts and the base of the leaf explants without callus formation, same observations were stated by Xu et al. (2009) working on *in vitro* shoot induction from leaves of *Lilium davidii* var unicolor.

Shoot height of plants formed from scales and leaf segments were reduced by increasing the BA concentration; these results were consistent with the study of Nhut et al. (2006) and Han et al. (2004) since they found that the shoot length declined significantly by increasing BA concentration in *Lilium in vitro* culture.

The fresh weight of regenerated shoots increased by increasing the BA concentration up to 1.0 mg/l; then declined after that in both scales and leaf segment explants. Han et al. (2004) also found that the fresh weight of shoots formed from bulb scales of *Lilium longiflorum* 'Georgia' declined significantly by increasing BA concentration from 0.4 to 22.2 µM.

Godo et al. (1998) mentioned that the frequency of shoot regeneration of *Lilium* × *formolongi* during one month of *in vitro* culture increased by increasing BA concentration; then declined at the highest BA concentration (1 mg/l). Results in the present study also showed that BA at 0.5 mg/l was the optimum concentration for number of days to proliferation, and the number of days increased by increasing the BA concentration at 2.0 mg/l, and the maximum number of days for shoot proliferation in leaf segments and in bulb scales were 74 and 67 days, respectively (Tables 2 and 4).

## Conclusion

The bulb scales of *Lilium* 'Prato' produced shoots more than the leaf segments when used as explants reaching 96.67 and 64.67%, respectively. BA at 0.5 mg/l was most effective on percentage of shoot formation and reduced the number of days to proliferation in both explants. BA at 0.5 mg/l if combined with NAA at 0.5 mg/l enhanced the formation of regenerated shoots in both bulb scales and leaf segments (46 and 56 days to proliferation, respectively).

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