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MASS PROLIFERATION OF MADONNA LILY (*LILIUM CANDIDUM* L) UNDER *IN VITRO* CONDITIONS

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Abstract

Mass proliferation from the lower half bulb scales and adventitious bulb regeneration from *in vitro* cultured leaf explants of Madonna Lilly (*Lilium candidum* L.) was achieved using various concentrations of BAP-IBA. Regenerated plantlets were transferred to greenhouse for adaptation where they flowered after two years.

Introduction

Madonna lily (*Lilium candidum* L.) with beautiful large white flowers (Bacchetta *et al*., 2003), occurs in apparently wild situations in Southern Turkey at low altitudes in maquis and light wood lands. It requires hot sun and good drainage for successful growth and flowering (Baytop & Mathew, 1984). It has been cultivated for centuries in the Middle East and Turkey for use in cut flower industry, obtaining perfumes and number of secondary products ranging from butanolic extracts from petals to saponins and pyroline derivatives from bulbs (Mucaji *et al*., 2000, Mimaki, 1999, Vachálková, 1999), which has increased its economic importance to many folds.

The plant is popularly multiplied using bulb scales and stems in Turkey under *in vitro* conditions without ensuring genetic uniformity by amateur workers, which results in production of undesired phenotypes and subsequently influence the quality and regeneration potential of the plants; as they select the plants randomly without taking necessary cares. This results in negative economic implications for mass production of this important ornamental and medicinal plant. Moreover, the propagation rate from bulb scales and stems is very low and produces 1-2 bulblets per bulb scale in one years time. This low propagation inhibits large scale cultivation of this plant.

Previous literature suggests that explants from flower organs to bulb scales of lilies could be easily manipulated and regenerated using tissue culture techniques (Robb, 1957; Hackett, 1969; Gupta *et al*., 1978; Stimart & Ascher 1981; Niimi, 1984; Kruczkowska, 1989; Nhut, 1998; Nhut *et al*., 2001; Varsheny *et al*., 2000). Conventional breeding in the species of different *Lilium* groups is difficult to carry out because of high heterozygosity and self incompatibility (Van Tuyl *et al*., 1990). Successful commercial application demands continuous improvement of flower color, morphology, longevity and size. Therefore, it was considered appropriate to manipulate growth by increasing productivity and reduce time for bulb production *in vitro*. This paper identifies suitable explants and reports improved and reliable protocol for rapid multiplication of madonna lily under *in vitro* conditions that might be helpful for rapid and large scale production of plants for greenhouse and field production.

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Materials and Methods

Young bulbs of *L. candidum* L., collected from Erzurum province of the Southern Turkey, were pre sterilized by washing them in running tap water for half hour to remove mud and traces of dirt etc. Using sterile sharp scalpel blade the roots were trimmed off from bulbs and the scales were gently teased off from the points of attachment. The scales were further sterilized in 60 % commercial bleach for 10 min followed by rinsing in sterile distilled water for four times to remove the traces of bleach. Thereafter, they were horizontally excised into upper and lower halves and cultured in regeneration medium in Petri dishes or Magenta boxes containing MS basal medium (Murashige & Skoog, 1962) supplemented with 0.50 mg dm^{-3} 6 benzylaminopurine (BAP) with 0.20-0.6 mg dm^{-3} indole 3 butyric acid (IBA; Table 1) adjusting pH to 5.6–5.8 with 1 N KOH or 1N HCl before autoclaving at 121°C, 1.4 kg/cm^2, for 20 min. All cultures were maintained at 24 ± 1°C under 16 h light photoperiod.

Rooting was achieved by transferring the regenerated bulblets on 35 ml of semi solid MS medium containing 0.8% agar (Sigma type A) and 0.5 mg dm^{-3} α Naphthalene acetic acid (NAA) in Magenta GA7® vessels at 24 ± 2°C under 16 h light photoperiod. After achievement of sufficient number of roots, the plants were transferred to pots containing sand and clay in ratio of 9:1 and placed in growth chamber for adaptation. At the initial stages humidity was maintained at 90%, which was reduced gradually to 40% in 15 d time. The temperature of the growth chamber remained 24 ± 2°C under cool white fluorescent light (35 µmol photons m^{-2} s^{-1}) with 16-h day and 8 hour dark photoperiod. Adapted plantlets were transferred to greenhouse, where ambient conditions of temperature and growth were maintained.

Statistical analysis

Each treatment was replicated 4 times and contained 5 explants in both regeneration and rooting experiments and was repeated twice. Changes in stages of development were recorded periodically with evaluation of phenotypic changes. The data was analyzed by comparing means using one way ANOVA and the significance was determined by Duncan's Multiple Range Test using SPSS for windows (v. 11. SPSS Inc USA). Data given in percentages were subjected to arcsine (√X) transformation (Snedecor & Cochran, 1967) before statistical analysis.

Table 1. Effect of different concentrations of BAP-IBA on regeneration of bulblets from lower half bulb scale of *L. candidum*.

BAP (mg dm^{-3})	IBA (mg dm^{-3})	Frequency (%) of callus regeneration	Number of embryo/explant
0.5	0.2	100	0.50 c[1]
0.5	0.3	100	1.25 bc
0.5	0.4	100	1.25 bc
0.5	0.5	100	3.25 ab
0.5	0.6	100	5.75 a

Each value is the mean of 4 replicates each with 5 explants.

[1]Values with in a column followed by different lowercase letters are significantly different at 0.05 level using Duncan's Multiple Range Test.

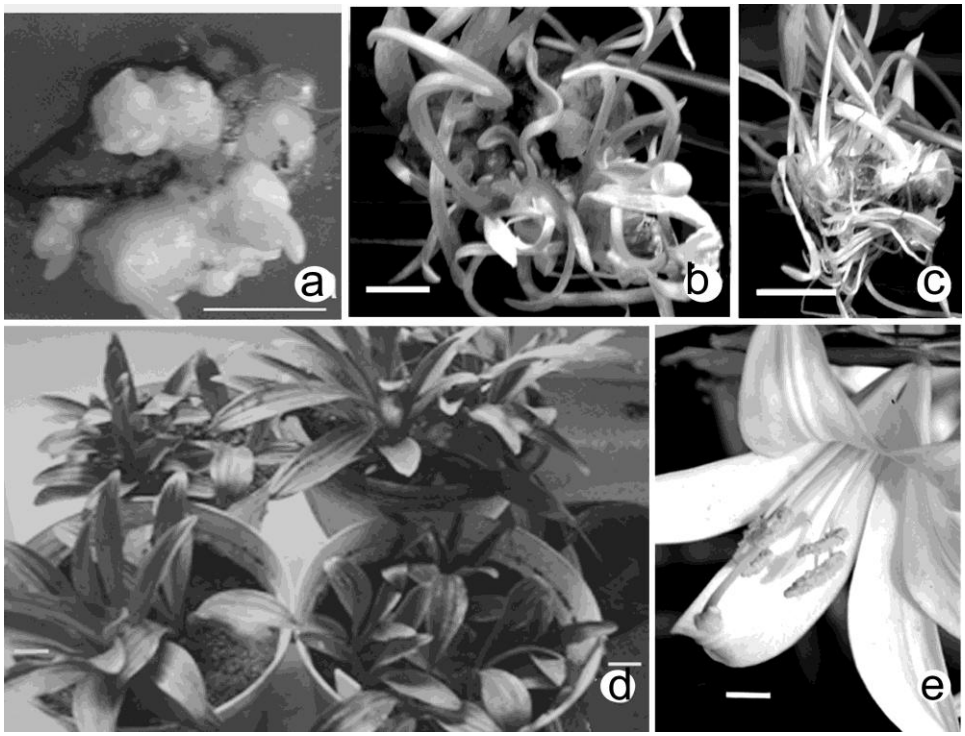

Fig. 1. Mass proliferation of Madona Lilly (*Lilium candidum* L) from lower halve bulb scales of *L. candidum* L under *in vitro* conditions (a) development of shoot primordia after 10 d of culture (b) development of clusters of bulblets with leggy appearance (c) rooting on medium containing 0.5 mg l^{-1} NAA (d) adaptated plants under greenhouse conditions (e) and flowering after two years of culture. Bar a = 1 cm, b, c = 1.25 cm, d, e = 4 cm.

Results and Discussions

Lower and upper halves of bulb scales (hereafter called lower and upper halves), greenhouse grown leaves and *in vitro* grown leaves showed distinct variations in their regeneration potential.

Effect of BAP-IBA on callus induction and regeneration from lower halves

Lower halves were quite favorable to induce callus on MS media containing 0.5 mg dm^{-3} BAP with various concentrations of IBA. Statistically, similar frequency of callus induction was observed on all media; however at concentrations lower than 0.4 mg dm^{-3} IBA (with 0.5 mg dm^{-3} BAP), the callus formation was not extensive. It was extensive thereafter on media containing 0.5 mg dm^{-3} IBA or more (with 0.5 mg dm^{-3} BAP). The bulblet formation started with single bulblet formation (Fig. 1a) followed by the additional bulblets subsequently. The highest number of bulblets were obtained on MS medium containing 0.5 mg dm^{-3} BAP - 0.6 mg dm^{-3} IBA. These could be regenerated in

to full plantlets when teased off aseptically. Calli in general were nodular with various shades of white and green. A concentration higher than 4 mg dm^{-3} IBA, though was favorable for callus induction but was accompanied with bulblets having leggy leaves. When grown after teasing off from the callus, these bulblets (with leggy leaves - Fig. 1b) were not difficult to root on MS medium containing 0.5 mg dm^{-3} NAA. Initially, it was considered that these plantlets (Fig. 1c,d) had gone morphological changes on the media containing BAP-IBA and would reflect when transferred to greenhouse. However, when transferred to the greenhouse they shed the *in vitro* grown leaves; regenerated new leaves (Fig. 1e). The plants returned to normal robust conditions soon and flowered favorably after two years.

Portions of the callus induced on MS medium containing 0.5 mg dm^{-3} BAP-0.6 mg dm^{-3} IBA were subcultured on MS medium and MS medium containing 0.5 mg dm^{-3} BAP and 0.6 mg dm^{-3} NAA. Sub culturing on MS medium was non responsive and non regenerative. However, sub culturing on later medium not only encouraged callus growth and development but also produced 8-10 slender bulblets on each of the explant. Each of which was separated for further growth and development. They did not had leggy leaves. The bulblets were rooted without failure on MS medium containing 0.5 mg dm^{-3} NAA. The differences in cultural requirements among different explants may be attributed to the various levels of endogenous plant growth regulators at different points of the same plant that control and regulate the growth pattern of the explants.

Effect of BAP-IBA on upper halves

No bulblet formation or callus regeneration was recorded from upper halves either under 16 h light or complete darkness even after four months of culture with periodic subculture on MS medium containing 0.5 mg dm^{-3} BAP with 0.2-0.6 mg dm^{-3} IBA throughout. Wounding with sterile scalpel blade or needle was also ineffective to induce any regeneration. The possible explanation to this might be that concentrates of metabolites necessary for growth are placed into lower halves of the bulb scales, (which acts as storage organ) and applied concentrations of plant growth regulators were non stimulating for regeneration of the explant due to lack of these metabolites in the upper portions of the scales. This disruption resulted in difficulty of the movements of these metabolites with no regeneration from the upper halves. The results supports findings of Bacchetta *et al*., (2003) who observed no regeneration from upper parts of leaf explants of cv. Star Gazer (*L. longiflorium*) and Elite (*Lilium* of Asiatic group).

Effect of BAP-NAA or BAP-IBA on leaf explants

Leaf explants obtained from greenhouse grown plants responded poorly on MS, MS containing BAP or 0.5 mg dm^{-3} BAP with any concentration of IBA. Contrarily the same obtained from bulblets regenerated on MS medium containing 0.5 mg dm^{-3} BAP-0.6 mg dm^{-3} IBA initially formed shoot primordia followed by 1-3 bulblets, which could be separated and maintained on MS medium containing 0.5 mg dm^{-3} BAP without any problem. Bulblets were rooted on MS medium containing 0.5 mg dm^{-3} NAA successfully.

Previously bulblet regeneration has been achieved by Lian *et al*., (2003), *Lilium* oriental hybrid Casablanca under 16 h photoperiod. Similarly Varshney *et al*., (2000) also

achieved *in vitro* micropropagation of *Lilium* Asiatic Hybrid and Maesato *et al*., (1994) of *Lilium japonicum* and Stimart & Ascher (1978) and Lesham *et al*., (1982) for micropropagation of *Lilium longiflorium.* Our results show variations in shoot regeneration pattern of different explants and this variation might be due to the regeneration potential of the explant or culture conditions etc. in line with the results of Stimart & Asher (1981), Lesham *et al*., (1982), Niimi (1985) and Mesato *et al*., (1994) emphasizing variation due to genotype, age, time of culture and culture conditions etc.

Acclimatization

All rooted bulblets were not difficult to acclimatize in the growth chamber and were transferred to the field for flowering; where, they flowered after two years. The experiment was completed in 20-26 weeks starting from *in vitro* culture to transfer to the greenhouse/fields. As such this paper reports a successful and reliable protocol for mass proliferation of meadow lily under *in vitro* conditions and meet the objectives of the study. It is hoped the protocol would be helpful for rapid and large scale production of plants for commercial production.

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