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A tissue culture protocol for propagation of a rare plant, *Lilium speciosum* Thunb. var. *gloriosoides* Baker

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Abstract. Floret explant of a local accession of *Lilium speciosum* Thunb. var. *gloriosoides* Baker produced subculturable totipotent calli on a Murashige and Skoog basal medium with a supplement of 3 mg/l 2,4-dichlorophenoxyacetic acid and 0.25 mg/l benzyladenine. The calli were able to form bulblets, which subsequently developed into plantlets on the MS basal medium supplemented with 0.1 mg/l naphthalene acetic acid, 1 g/l active charcoal and 170 mg/l NaH_2PO_4. The rare lily was proliferated in vitro by a scale-bulblet cycling propagation method with a multiplication rate of eight times at three-month intervals. Finally, 6000 plantlets have been produced within nine months. We established 200 plants in the greenhouse under misty conditions for a four-week period with a 98% survival rate. These plants grew well and elongated with normal flowers in the second year.

Keywords: Bulblet; Callus; *Lilium speciosum* Thunb. var. *gloriosoides* Baker; Rare plant; Scale.

Abbreviations: 2,4-D, 2,4-dichlorophenoxyacetic acid; **BA,** N^6-benzyladenine; **NAA,** naphthalene acetic acid.

Introduction

There are many techniques available for the conservation of plant genetic resources of rare and endangered species. These include micropropagation, seed germination, regeneration from callus, embryo rescue, micrografting, and cryopreservation (Nitzsche, 1983; Rick, 1984; Stanilova et al., 1994). Micropropagation in bulb plants as an alternative to the conventional methods for vegetative propagation attracts much attention, because of its advantages. It increases many times the multiplication level (Novak and Petru, 1981; Takayama and Misawa, 1982; Takayama and Misawa, 1983; Van Aartrijk et al., 1990; Van Aartrijk and Blom-Barnhoorn, 1981; Wickremesinhe et al., 1994) and enables material free from viruses and other diseases to be obtained (Blom-Barnhoorn and van Aartrijk, 1985; Van Aartrijk et al., 1990).

In our investigation we researched the species *Lilium speciosum* Thunb. var. *gloriosoides* Baker, a native perennial bulbous plant only known at altitudes of 150-600 m in northern Taiwan (Liu and Ying, 1978). The plant, which produces large white flowers with flush red spots on petals, is a high-value ornamental specimen. Unfortunately, almost all of the bulbs were gathered as Chinese medicine, and the indigenous endemic gradually disappeared. In 1991, the variety reached currently criterion as a rare plant with small population in Taiwan (Lai, 1991). The artificial propagation protocol is necessary to rescue the rare lily and maintain the germplasm.

Recently, we designed a protocol for propagation of this lily variety via bulblets morphogenesis from floret explant and mass proliferation by scales culture in vitro. The tissue culture seedlings were successfully transplanted to the greenhouse and plants bore normal flowers in the second year.

Materials and Methods

Callus Induction and Plant Regeneration

Young floret (Figure 1a) of *Lilium speciosum* Thunb. var. *gloriosoides* Baker derived from the donor plant grown in the greenhouse and was sterilized in 1% (v/v) sodium hypochlorite solution for 10 min. The floret was rinsed in sterile water three times, excised into 2 mm segments, and placed in test tubes on a 0.22% gelrite-gelled basal medium containing MS basal medium (Murashige and Skoog, 1962), myo-inositol (100 mg/l); niacin (0.5 mg/l); pyridoxine HCl (0.5 mg/l); thiamine HCl (0.1 mg/l); glycine (2.0 mg/l); casein hydrolysate (1 g/l); sucrose (30 g/l). Supplement with 2,4-D (3 mg/l) and BA (0.25 mg/l) and the pH of the media was adjusted to 5.7 with 1 N KOH or 1 N HCl prior to autoclaving at 121°C for 15 min. Cultures were maintained at 20°C in the darkness.

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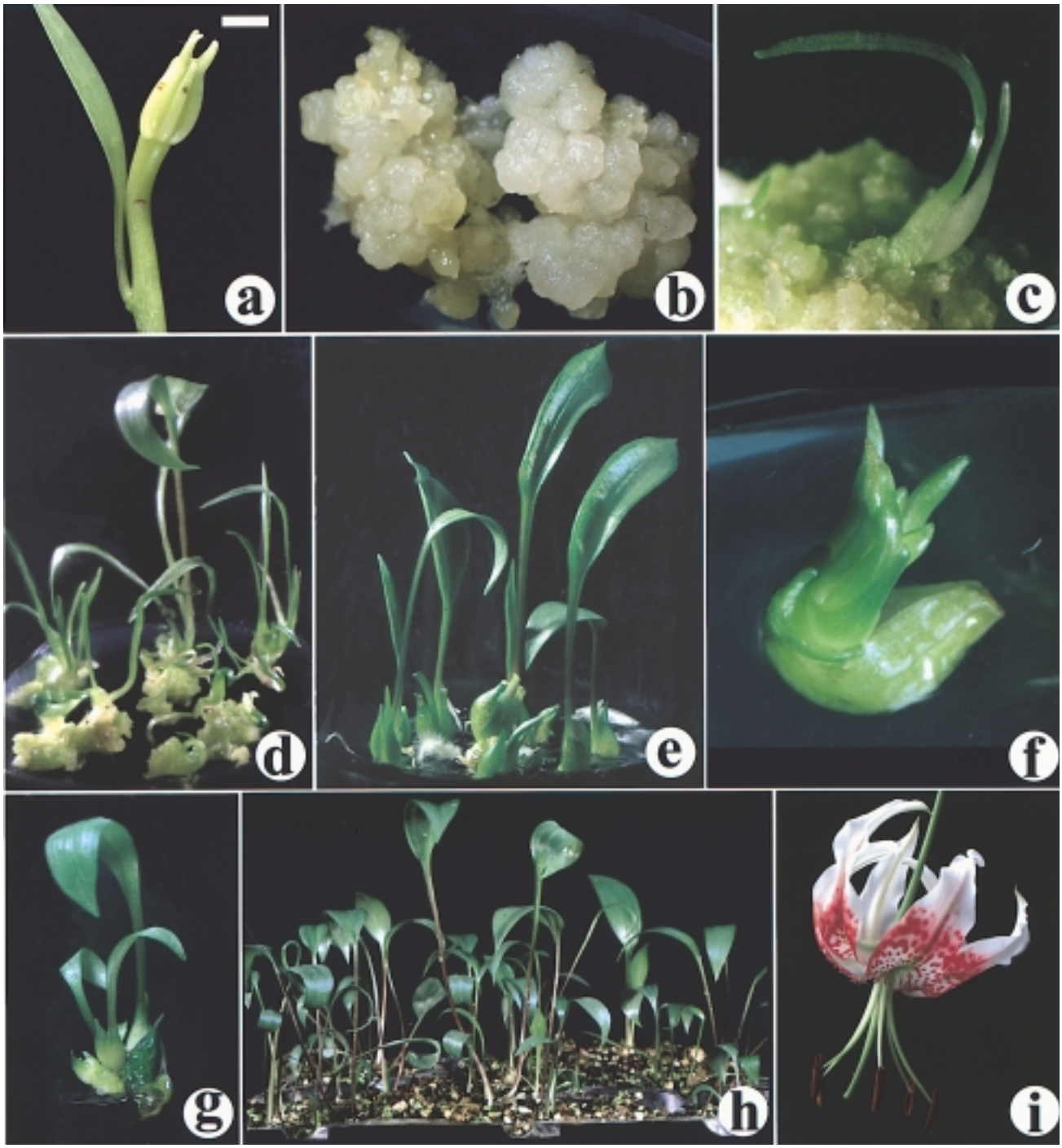

Figure 1. Callus induction and plant regeneration of *Lilium speciosum* Thunb. var. *gloriosoides* Baker. a, Floret explant for callus induction. (bar = 6 mm); b, Callus derived from explant of floret on basal medium plus 3 mg/l 2,4-dichlorophenoxyacetic acid 0.25 mg/l benzyladenine for 3 months. (bar = 0.7 mm); c, bulblet found on floret-derived callus. (bar = 0.5 mm); d, More adventitious bulblets formed one month after transfer to the basal medium supplemented with 0.1 mg/l NAA, 1 g/l active charcoal and 170 mg/l NaH_2PO_4. (bar = 4 mm); e, Bulblet-derived plants of about 5 cm in height were obtained after three months. (bar = 5 mm); f, Bulblets regenerated from the adaxial basal part of scale in the basal media plus 0.1 mg/l NAA. (bar = 8 mm); g, Plantlets derived from the bulblets induced from scale. (bar = 2.7 mm); h, Plantlets were transplanted into the tray and acclimated well in the greenhouse. (bar = 12 mm); i, Normal flower morphology of the tissue culture seedling. (bar = 18 mm).

The floret-derived calli were transferred to MS basal medium supplemented with 0.1 mg/l NAA, 1 g/l active charcoal and 170 mg/l NaH_2PO_4 for plantlet regeneration. Specimens were cultured at 20°C and exposed to 28-36 µmol $m^{-2}s^{-1}$ of artificial light (daylight fluorescent tubes FL30D/29, 40W, China Electric Co, Taipei) with a light/dark cycle of 16/8 h.

Mass Propagation by Bulblet Proliferation

Scales of bulblet were inoculated on MS basal medium supplemented with 0.1 mg/l NAA in the flask at 25°C under cool white fluorescent light and a light/dark cycle of 16/8 h.

Establishment of Plantlets in the Greenhouse

There were 210 rooting plantlets washed with tap water to move the gelrite medium and transplanted in 35-cavity growing trays with perlite-peatmoss mix soil. In order to make these seedlings acclimate to the greenhouse, they were placed in misty conditions during the first 4 weeks. A total of 25 acclimatized plants were potted in the 5-inch individual pots containing perlite-vermiculite-peatmoss mix medium and placed under normal greenhouse conditions to bloom.

Result and Discussion

Callus Induction and Plant Regeneration

Callus was induced from the floret segments within three months in the medium plus 3 mg/l 2,4-D and 0.25 mg/l BA. Calli were nodular and pale yellow (Figure 1b) in color. They were subcultured to the same medium at six-week intervals. Bulblet (Figure 1c) and roots were observed in the cultures of both initial and subcultured callus. These calli were transferred into the medium containing 0.1 mg/l NAA, 1 g/l active charcoal, and bulblets subsequently formed within 4 weeks (Figure 1d). After two months, 5 cm tall plantlets (Figure 1e) were obtained and were able to be used for mass multiplication in vitro or transplantation to the greenhouse.

Mass Propagation by Bulblet Proliferation

Scales of bulb derived from callus were used as the explants for mass proliferation. Leafy adventitious bulbs were regenerated from the adaxial basal part of the scale (Figure 1f), and these subsequently rooted to form whole plantlets (Figure 1g). Two plantlets regenerated from one scale on average within three months, and every regenerated plantlet had four scales for the next proliferation culture. The proliferation rate by scale-bulblet method was 8-hold/ 3 months. Finally, 6000 plantlets were produced in a nine-month culture by the proliferation protocol, and these seedlings had1-2 leaves, 3-6 roots, and a 0.6-1.0 cm bulblet.

Establishment of Plantlets in the Greenhouse

The survival rate of the rooted plantlets placed in the trays and acclimatized over a 4-week period under misty conditions was 98%. This demonstrated that all these plants were easily and efficiently acclimatized from in vitro to greenhouse conditions (Figure 1h). None of the plants were dormant, and they continued to grow both in vitro and in the greenhouse.

From February to March of the second year, these plants elongated and differentiated more leaves under a natural day length with a 50% shading condition. In September and October, these plants grew trailing with 100-120 cm in length and had 25-35 leaves with the bulbils on the basal part nodes. Some of these plants fully bloomed with 2-11 flowers. The flower (Figure 1i) bloomed downward with the normal morphology, just as in the mother plants.

Our purpose is to perpetuate the rare lily germplasm, so we attempted to amplify the population by the tissue culture method. The results presented here demonstrate a practical process by which large numbers of true-to-type plantlets can be produced from floret explants of *Lilium speciosum* Thunb. var. *gloriosoides* Baker. The callus induction and high regeneration activity has already been observed in other species of the same genus (Arzate-Fernandez et al., 1997; Chou, 1976; Okazaki, and Koizumi, 1995; Stimart et al., 1980; Wickremesinhe et al., 1994). We modified earlier methods and designed the protocol for mass propagation of the rare lily in vivo and in vitro. We are investigating the horticultural habits of the cloning plants and will introduce the germplasm into our lily breeding program.

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組織培養法繁殖臺灣稀有植物－艷紅鹿子百合

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艷紅鹿子百合幼花苞培植體在含 3 mg/l 2,4-D 及 0.25 mg/l BA 的 MS 基本培養基中三個月可以產生可繼代培養、具分化全能性的癒傷組織。將癒傷組織移植到含 0.1 mg/l NAA, 1 g/l 活性碳及 170 mg/l NaH_2PO_4 可以形成小鱗莖，繼而發育成為小苗。豔紅鹿子百合可藉由鱗片-小鱗莖方法在試管內以 8 倍/三個月的速率大量增殖，經九個月的培養，已產生六千株試管內小苗。豔紅鹿子百合組織培養幼苗移植到溫室培養具 98% 存活率，栽培至第二年可抽莖及開花。

關鍵詞：稀有植物；鱗莖；癒傷組織；艷紅鹿子百合；鱗片。