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 NaH₂PO₄. 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, N⁴-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 Petr, 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 bulbets 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.