Development of a Pollen-mediated Transformation Method for Moth Orchids

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Abstract

Moth orchids (*Phalaenopsis* spp.) are one of the most economically important crops in Taiwan. Recently, several countries have paid more attention to them, since their cultivation can potentially bring high incomes. Plant transformation technology has become a basic method for plant breeding as well as for studying gene function in plants. In the present study, we processed a specific gene transfer method for *Phalaenopsis* based on pollen gene transformation. It is relatively easy to induce callus formation and regeneration through the seed culture of *Phalaenopsis*. Using pollen transformation is considered to be an efficient method of gene transformation in this genus. Furthermore, no chimeric transgenic plants were found based on this method. Tissue culture through a critical stage is required in most current transformation protocols to ultimately recover plants. Since different plants bear different cell types/genotypes in specific tissue culture systems, tissue culture-based regeneration systems are usually a limiting factor in plant transformation. At present, one of the most important challenges for plant transformation is to develop a technology that can eliminate the tissue culture step. A new approach, *in vivo* transformation of plants mediated by *Agrobacterium,* is introduced in the present study. Pollen was directly co-cultivated with a cell suspension of *Agrobacterium*. After infection by *Agrobacterium*, the pollen was used to pollinate stigmas. After development of embryogenesis, transgenic plants can be obtained from the seeds. The approach in the study requires no mediation of the plant tissue culture, which makes the method quite simple and rapid. In addition, non-chimeric-transformed plants were found. This transformation system is an easy technique for *Phalaenopsis* transformation.

Materials and Methods

Agrobacterium tumefaciens strain LBA4404 was transformed with the *mgfp*5-ER-containing pBIN121 derivative plasmid in this research. Pollenia of *Phalaenopsis* were the target cells for *Agrobacterium*-mediated transformation. The *Agrobacterium* strain was cultured in LB medium with specific antibiotics (G418 for pBIN121 and hygromycin for pCAMBIA) for 3 days. Bacterial pellets were collected and suspended in 5% sucrose, 400 mM aceto syringo(AS), and 0.05% L-77 Silwet. The final concentration of *Agrobacterium* was approximately 0.7~0.8 O.D. at a wavelength of 650 nm.

Seed germination and selection. Transgenic seeds were recovered from the ovaries of plants following *Agrobacterium* treatment. Those seeds were sterilized with 1% NaOCl for 15 min, and cultured in specific (kanamycin-free) medium to the seedling stage. After that, plantlets were transferred to specific medium with 10~50 mg/L kanamycin (depending on the different plant taxa) to select the transformed plants.

Microscopy. When seeds had germinated for 5 days, the Protocorm Like Bodies (PLBs) were inspected under microscopy with blue or white light. Fluorescent PLBs were counted under fluorescence microscopy.

A native species of *Phalaenopsis* (2n=38)

Seedings of *Phalaenopsis* sight under microscope with white light

Seedings of *Phalaenopsis* sight under microscope with blue light

Results and Discussion

The transformed PLB can be detected by the emission of the green fluorescent protein (GFP) under excitation with blue light (Fig. 3). The transformation efficiency in this study was estimated to be approximately 0.2%. There are hundreds of thousands of seeds per fruit in *Phalaenopsis*, and several transformed PLBs could be obtained even with the relatively low transformation efficiency in this study. Chlorophyll will eventually develop during seed germination, and this can interfere with detection of the emission of the stimulated GFP. Stimulated chlorophyll shows a red color under blue light. When the chlorophyll of the PLB is synthesized in great quantities, the green florescence of the GFP will be overcome by the red color. Therefore, it is very important to inspect transgenic PLBs at the proper time. Increased transformation efficiency of *Phalaenopsis* can be obtained by increasing the *Agrobacterium* concentrations or using YEB medium. Obviously, higher imbibition of pollinia will achieve higher transformation efficiencies. Therefore, the time course for the imbibition of pollen in the *Agrobacterium* cell suspension needs to be tested for different *Phalaenopsis* varieties.

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