Molecular Detection of Foreign Pigment Gene-Dihydroflavonol 4-reductase in Genetic Transformed *Phalaenopsis* Cultivars

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Abstract

Transformed *Phalaenopsis* plants mediated from microprojectile bombardment cultivated in the greenhouse were taken for molecular analysis. Seven out of 30 transformed plants selected from hygromycin screening presented a 600 bp bend of dihydroflavonol 4-reductase gene for PCR detection with only 4-6 out of them presented one or more copy number of gene in the southern blot analysis by different restriction enzyme digestion. Most of flowers of transgenic lines showed normal color with only one plant had mosaic appearance.

Introduction

Phalaenopsis orchids, including cultivars of *Phalaenopsis* and its inter-genetic hybrid of *Doritis* and *Doritaenopsis*, are commercially important plants cultivated as cut flowers and pot plants around the world due to their beautiful flowers. Genetic transformation is expected to be an efficient tool for introducing a trait such as flower color, which has been difficult through conventional breeding techniques based on sexual crossing. Appropriate procedures for genetic transformation must be established for individual target plant materials. In the present study, we establish a detection procedure for particle gun transformation of *Phalaenopsis* orchids.

Materials and Methods

Plant material

Protocorm like bodies (PLB) of various *Phalaenopsis* cultivars were taken for bombardment using DuPont/BioRad PDS1000 Helium microprojectile system. Shoot regenerated on hygromycin containing medium and survived after at least 3 times subculture (4wks/subculture) were cultivated for the following confirmation.

GUS assay, DNA isolation and southern blot analysis

β-glucuronidase (GUS) expression was assayed histochemically with 5-bromo-4-chloro-3-indolye glucuronide (X-GLU). The presence of *dfr* gene sequences in genomic DNA was assayed using a polymerase chain reaction (PCR) procedures.

Genomic DNA was extracted from leaf tissues (1.0 g fresh weight) using CTAB method. After quantifying by gel electrophoresis, 40 ug of each genomic DNA was digested with *Eco*RI and *Hind*III and fractionated on a 2.0% agarose gel at 30V for 6 hours. Southern hybridization and detection were carried out using a digoxigenin-labeled *dfr* probe following the manufactures instructions (Roche).

The PCR amplification was carried out using the following primer1, 5'-GTAAACATGAAGAAGGTGAAGCATT-3'; primer2, 5'-AGAGATCATCTAAGTGGACGAATTG-3' at conditions: 5 min at 95°C, 30 cycles of 1min at 95°C, 1min at 66°C and 1min at 72°C.

Result

Some PLBs were subjected to GUS expression analysis 48 hrs after particle bombardment. Blue spots represented the transient GUS expression (Fig. 1).

Fig. 1. .Histochemical assay for β-glucuronidase (GUS) of PLB explants 48 hrs after particle bombardment.

All survival regenerates from transformation after hygromycin selection were analyzed with PCR analysis and southern hybridization. A total 30 lines were investigate with only 7 lines presenting a 600 bp single bend by PCR analysis (Fig. 2A), and these 7 PCR products were further hybridized with *dfr* probe (Fig. 2B) .

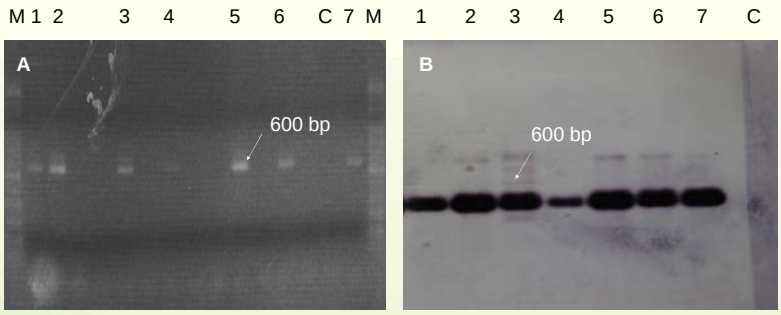

Fig. 2. (A) PCR analysis of transformed *Phalaenopsis* plants using primers for *dfr* (600 bp product), lane C is control with DNA from untransformed plants, lane M is 100 bp ladder, lane 1-7 were transformed plants T1-T7. (B) Southern blots of PCR products in Frame A using digoxigenin-labeled probe of DFR gene, lane C is control with DNA from untransformed plants, lane 1-7 were transformed plants T1-T7.

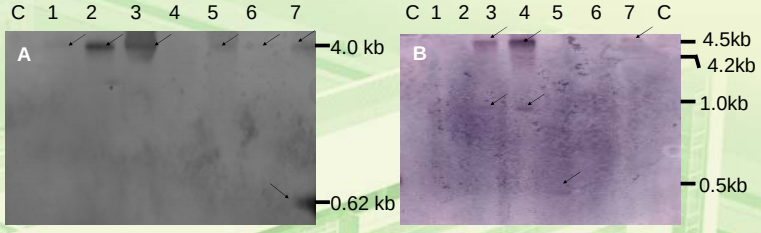

Fig. 3. Southern blots of DNA from transformed *Phalaenopsis* plants. Genomic DNA was digested with *Eco*RI (A) or *Hind*III (B) and probed with a fragment of the *dfr* gene, lane C is control with DNA from untransformed plants, lane 1-7 were transformed plants T1-T7.

Fig. 4. Flowers of transformed *Phalaenopsis* plants (T1-T7) show normal or mosaic color,C1: untransformed control for T1-T4; C2: untransformed control for T5-T7.

Conclusion

Molecular detection of foreign gene(s) in the transformed plants provides a strong evidence for proving of transformation. However, much complicate mechanism is predicted for gene expression than that of presence of foreign gene(s) itself. There are 6 transformed lines showed the presence of *dfr* gene however only one plant with slightly mosaic color appearance. The high ploidy level of commercial *Phalaenopsis* cultivars is suspected for interferecing a single foreign gene to express in the complicate genetic background.