

Agrobacterium-Mediated Transformation of *Phalaenopsis*Using Protocom-like Bodies

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Objectives

Phalaenopsis is one of the most important orchids grown for commercial production of cut flowers and potted plants. The production of *Phalaenopsis* has increased due to an increased demand.

To establish high frequency of transformation system for *Phalaenopsis*, various conditions for transformation were investigated. we report the production of putative transgenic plantlets obtained using *Agrobacterium*-mediated Transformation.

Materials and Methods

- 1. Plant material: PLB (protocom-like body) derived from lateral bud of *Phalaenopsis* variety Brother Lawrance.
- Agrobacterium starin: LBA4404 (pTOK233), AGL1 (pCAMB IA3301)
- Cocultivation Medium: VW basal medium+150 ml/L Coconut milk+500 μm Acetosyringone

 Selection Medium: VW basal medium + 150 ml/L Coconut milk + 50 mg/L Hygromycin or 0.5 mg/L PPT + 300 mg/L Cefotaxime

Results and Discussions

On medium supplemented with 30 and 50 mg/L hygromycin and 0.25 and 0.5 mg/L PPT, new PLBs were not produced and necrosis occurred. From this results, 50 mg/L hygromycin and 0.5 mg/L PPT was used to discriminate between transformed and non transformed PLB. Using LBA4404 (pTOK233), 9 % of PLB produced new PLBs and direct shoots, while on infection by AGL1 (pCAMBIA 3301), about 30% of PLB produced new PLBs and direct shoots. But Most of them showed motionless growth and only few regenerated to plantlet with root. From our transformation experiments, a total 11 hygromycin-resistant young plants and 32 PPT-resistant plants were obtained. Shoot section of those putative transgenic plants expressed GUS activity and showed blue staining in GUS assay.



Figure 1. Genetic Transformation of *Phalaenopsis* using *Agrobacterium tumefaciens*. A, PPT-resistant PLBs produced from *Agrobacterium*-infected PLB.; B, Shoot formation in shooting medium containing 0.5 mg/L PPT; C, Putative transgenic plantlets with roots.

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