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Genetic Transformation of a *phalaenopsis* Orchid through *Agrobacterium tumefaciens*

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Objectives

We have tried to make transformation of *phalaneopsis* orchid through *Agrobacterium tumefaciens* and successfully obtained transgenic *phalaneopsis* by tissue culture system.

Materials and Methods

1. Material
   - Plant - *phalaneopsis* - *Phal.* Taipei Gold, *Phal.* Crystal Veil, *Phal.* Wataboushi.
   - *Agrobacterium* strain - LBA4404/pBI121 and EHA101/pIG121Hm
2. Methods: Callus were obtained from flower stalk cuttings of *phalaneopsis*. Liquid culture was maintained by callus in 20S NDM media containing phytohor mone (0.1 mg/L Naphthaleneacetic acid and 1.0 mg/L benzyladenine)

Results and Discussion

Phalaenopsis orchid were transformed via *Agrobacterium tumefaciens* that EHA101/pIG121Hm harbored genes for β-glucuronidase(GUS), hygromycin phophotransferase(HPT) and neomycin phosphotransferase gene (NPT Ⅱ) and LBA4404/pBI121. The infection timing of efficient transformation was used for 2hr cocultivation of cell clumps with *Agrobacterium* that had been cultured on YEP media. Plant transformation was optimal when the cell clumps were cultured on 20S NDM (New Dogashima medium) composed of 0.1 mg/L Naphthaleneacetic acid (NAA), 1.0 mg/L benzyladenine (BA) and 20 g/L sucrose. Each cell clumps had been changed to form PLBs(protocorm-like bodies) in 20S NDM and transfer to 20S NDM. After 2 month of infection, hygromycin resistance cell clusters were appeared from infected cell cluster dump on 8 g/L solid NDM medium containing 20 g/L sucrose, 0.1 mg/L Naphthaleneacetic acid (NAA), 1.0 mg/L benzyladenine (BA), 30 mg/L hygromycin and 375 mg/L augumentin. To induce the greenish callus, the carbon source was changed from sucrose to maltose moreover we use 10M NDM supplemented with 0.1 mg/L abscissic acid to induce the PLBs.

To confirm the putative transgenic plant, PCR analysis and GUS analysis were established. Each shoots of putative transgenic orchid presents intense blue staining in each tissue sections and whole shoot of putative transformants also showed GUS activity. PCR was carried out on genomic DNA of putative transformants using gene specific primer and putative transgenic plants gave the expected PCR product.

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