

## Optimisation of transient green fluorescent protein (GFP) gene expression in *Phalaenopsis violacea* Orchid mediated by *Agrobacterium tumefaciens*-mediated transformation system

### ABSTRACT

Numerous transformation factors were successfully optimised to develop a reliable and highly efficient *Agrobacterium*-mediated transformation into the protocorm-like bodies (PLBs) of *Phalaenopsis violacea*. The optimisation of factors influencing stable transformation efficiency in new species is very important as it can reduce the costs in labor and materials in the future. Hypervirulent *Agrobacterium tumefaciens* strains, EHA 101 and 105, harboring the pCAMBIA 1304 plasmid which contains *gusA* gene and *gfp* gene as the reporter markers, were used for transformation study. Transient *gfp* gene expression was used to evaluate the efficiency of T-DNA delivery in transformants due to its simple, non-destructive and cell autonomous procedure. *Agrobacterium* strain EHA 105 was proved to be better in transforming the targeted PLBs than EHA 101, based on the notably high transient expression of *gfp* gene in all the parameters tested. Different temperatures during cocultivation period, the concentration of L-cysteine, calcium (CaCl<sub>2</sub>) and silver nitrate (AgNO<sub>3</sub>) in cocultivation medium as well as pH and light and dark conditions during cocultivation period were identified to be major factors in enhancing the percentage of transient *gfp* gene expression. Increased T-DNA delivery efficiencies were obtained when *P. violacea* PLBs were co-cultivated with *Agrobacterium tumefaciens* strain EHA 105 in half-strength MS medium supplemented with 5% of banana Mas extract containing 200 mg.L<sup>-1</sup> L-cysteine, 60µM silver nitrate, without calcium, adjusted to pH 5.5 and incubated in the dark at 24°C. The results from transient transformation of PLBs suggested that *Agrobacterium*-mediated transfer of T-DNA to the naturally recalcitrant *P. violacea* is feasible and is highly efficient. Consequently, by combining the best treatments, an efficient and reproducible *Agrobacterium*-mediated transformation protocol could be continued to facilitate the insertion of any desirable traits for the production of transgenic *Phalaenopsis violacea* orchid.

**Keyword:** Transient Green Fluorescent Protein; *Phalaenopsis violacea*; *Agrobacterium tumefaciens*