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 (CaCl2) and silver nitrate (AgNO3) in cocultivation medium as well as pH and light and dark conditions during co-cultivation 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-1 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