

Development of an efficient particle bombardment transformation system for the endemic orchid, *Phalaenopsis bellina*

ABSTRACT

Phalaenopsis bellina is an important indigenous orchid with high commercial value. In this study, we established an efficient particle bombardment transformation system for *P. bellina* using the protocorm-like-bodies (PLBs) as target tissues. Leaf derived PLBs were proliferated on ½ strength Murashige and Skoog (MS) medium supplemented with 0.8 µM 2,4 dichlorophenoxyacetic acid (2, 4-D). Both physical and biological parameters affecting the transformation system were optimised using the green-fluorescent protein (GFP) and β-glucuronidase (GUS) as reporter systems. Optimal bombardment conditions with 6 cm target tissues distance, 1100 psi acceleration pressure, 1.0 µm gold particle size, 27 mmHg chamber vacuum pressure, single bombardment time, spermidine as DNA precipitation agent, 72 h post bombardment incubation time, 2 µg plasmid DNA in 0.15:0.12 pmol ratio (pSMCHS:p35SGFP) were successfully determined. Surviving PLBs transformants were successfully recovered from the hygromycin selection medium and verified using genomic PCR analysis. The established system is not only useful for a simple and reliable transient gene analysis but as well as generating stable transformants for selective traits improvement in orchids.

Keyword: β-glucuronidase (GUS); Green fluorescent protein (GFP); Particle bombardment; *Phalaenopsis bellina*; Protocorm-like-bodies (PLBs)