Optimization of biolistic bombardment parameters for Dendrobium sonia 17 calluses using GFP and GUS as the reporter system

Abstract

Genetic transformation system of Dendrobium Sonia 17 was optimized using green fluorescent protein (GFP) and β -glucuronidase (GUS) gene as the reporter systems. The 35S-sgfp-TYG-nos (p35S) and pSMDFR, carrying sgfp and gusA gene, respectively, were co-bombarded into the calluses. Parameters optimized were acceleration pressure, target distance, gold particle size, pre-bombardment cultured time, plasmid DNA precipitation, total plasmid DNA and the ratio of the plasmids co-bombarded. Both reporter systems responded similarly to the bombardment parameters investigated. Based on the GUS/GFP spot counts, the GFP expression rate was higher than that for GUS under the control of the same promoter, CaMV 35S. GFP could be used as the reporter system for the co-bombardment as it was rapid and non-destructive system to monitor the transformed tissues. A combination of GFP and antibiotic resistance gene was used to select stable putative transformants. © Springer 2005.

Keyword: Bombardment; Dendrobium; Green fluorescent protein; Hygromycin