ABSTRACT

The objectives of this research were to improve regeneration of *Dendrobium draconis* Rchb.f and *Dendrobium Miss Singapore*, to select the suitable antibiotic for elimination of *Agrobacterium tumefaciens* strain LBA4404 (pCAMBIA1305.1) while maintaining high levels of shoot regeneration and to establish the optimal conditions for the transformation of both orchid species by using *A. tumefaciens* and microprojectile bombardment technique. The modified VW (Vacin and Went, 1949) medium supplemented with 100 mg/l myo-inositol, 0.5 mg/l pyridoxine, 0.1 mg/l thiamine, 2 mg/l glycine, 2% (w/v) sucrose and 8 g/l agar (pH 5.2) was suitable for protocorm proliferation of *D. Miss Singapore*. The suitable medium for protocorm proliferation of *D. draconis* was VW medium containing 1 mg/l benzylamino purine (BAP). The protocorms of *D. draconis* and *D. Miss Singapore* were successfully regenerated on the modified VW medium supplemented with 100 mg/l myo-inositol, 0.5 mg/l pyridoxine, 0.1 mg/l thiamine, 2 mg/l glycine, 15% (v/v) coconut water and 8 g/l agar (pH 5.2). Hygromycin was tested for its effect on the regeneration of both orchid species. It was found that the protocorms of *D. draconis* and *D. Miss Singapore* were completely inhibited by hygromycin at concentrations of 40 and 30 mg/l, respectively. The optimal concentration of cefotaxime for elimination *A. tumefaciens* strain LBA4404 was 200 mg/l. Transformations of *D. draconis* and *D. Miss Singapore* protocorms were investigated by using *A. tumefaciens* and microprojectile bombardment technique. The plasmid pCAMBIA1305.1 containing antisense ACC oxidase gene, hygromycin resistant (hpt) gene and β-glucuronidase (gus) gene was used. The percentages of *D. draconis* and *D. Miss Singapore* transformed by *A. tumefaciens* strain were 2.17% and 8.15%, respectively. Microprojectile bombardment was also used to deliver antisense ACC oxidase gene into the both orchid species. Gold particles (1 μm) and tungsten particles (1.1 μm) were coated with plasmid DNA and introduced into the protocorms of the two orchid species. The result showed that the transformation efficiency of gold particles was higher than that of tungsten particles. The highest transformation percentages of *D. draconis* were 15%, whereas it was 3.13% for *D. Miss Singapore*. The optimal distances from the stopping screen to the target protocorms of *D. draconis* and *D. Miss Singapore* were 12 and 9 cm., respectively. The transformant protocorms and plantlets of *D. draconis* and *D. Miss Singapore* were verified by GUS assay. The GUS assay revealed the GUS activity while PCR method indicated the successful integration of transgenes.