Optimization of Agrobacterium-Mediated Transformation Parameters for Melastomatacea Spp. Using Green Fluorescent Protein(GFP) as A Reporter

Abstract

Agrobacterium-mediated transformation for both Melastoma malabathricum and Tibouchinu semidecanda were optimized using green fluorescent protein (GFP) as a reporter. The binary vector pCAMBIA1304 harboring the modified green fluorescent protein (mgfp) gene driven by the CuMV 35S promoter was used. Parameters optimized were bacterial strain, bacterial concentration, pre-culture period, co-cultivation period,immersion time, acetosyring concentration and wounding type. Results obtained obtained were based on the percentage of (GFP expression which was observed 3 days post-transformation. Agrobacterium tumefaciens starin LBA4404 and EHA105 at concentration 1 X 107 cfu ml -1 (OD 600mm 0.8) showed the highest virulence on M. malabathricum and T.semidecandra, respectively. Four days of pre-culture and 2 days of co-cultivation were optimum for M.malabathricum transformation, while 3 days of pre-culture and co-cultivation fot T, semidecandra, . result also showed that 60 min of immersion and addition of 200 p.M acetosyringone gave the highest percentage of positive transformants for both M. malabathricum and T.semidecandra. Mild wounding also significantly increased the efficiency 0f M.malabathricum transformation.

Keyword: Agrobacterium, Melastomataceae, Green fluorescent protein