

Optimization of Agrobacterium-mediated transformation parameters for Melastomataceae spp. using green fluorescent protein (GFP) as a reporter

Abstract

Agrobacterium-mediated transformation for both *Melastoma malabathricum* and *Tibouchina semidecandra* 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 CaMV 35S promoter was used. Parameters optimized were bacterial strain, bacterial concentration, pre-culture period, co-cultivation period, immersion time, acetosyringone concentration and wounding type. Results obtained were based on the percentage of GFP expression which was observed 3 days post-transformation. *Agrobacterium tumefaciens* strain LBA4404 and EHA105 at concentration 1×10^7 cfu ml⁻¹ (OD_{600nm} 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 for *T. semidecandra*. Result also showed that 60 min of immersion and addition of 200 μ M acetosyringone gave the highest percentage of positive transformants for both *M. malabathricum* and *T. semidecandra*. Mild wounding also significantly increased the efficiency of *M. malabathricum* transformation. © 2006 Elsevier B.V. All rights reserved

Keyword: Agrobacterium; Green fluorescent protein; Melastomataceae