Enhancers of Agrobacterium-mediated transformation of Tibouchina semidecandra selected on the basis of GFP expression

Abstract

Genetic engineering is a powerful tool for the improvement of plant traits. Despite reported successes in the plant kingdom, this technology has barely scratched the surface of the Melastomataceae family. Limited studies have led to some optimisation of parameters known to affect the transformation efficiency of these plants. The major finding of this study was to optimise the presence of selected enhancers [e.g., monosaccharides (D-glucose, D-galactose and D-fructose), tyrosine, aluminium chloride (AlCl3) and ascorbic acid] to improve the transformation efficiency of Tibouchina semidecandra. Agrobacterium tumefaciens strain LBA4404 harbouring the disarmed plasmid pCambia1304 was used to transform shoots and nodes of T. semidecandra. Different concentrations of the transformation enhancers were tested by using green fluorescent protein (GFP) as a reporter. The results obtained were based on the percentage of GFP expression, which was observed 14 days post-transformation. A combination of 120 μM galactose and 100 μM tyrosine supplemented with 600 μM AlCl3 in the presence of 15 mg/l ascorbic acid gave the highest percentage of positive transformants for T. semidecandra shoots. Whereas 60 μM galactose and 50 μM tyrosine with 200 μM AlCl3 in the presence of 15 mg/l ascorbic acid was optimum for T. semidecandra nodes. The presence of the hygromycin phosphotransferase II (hptII) transgene in the genomic DNA of putative T. semidecandra transformants was verified by PCR amplification with specific primers.