

Abstracts

In this study, a simple method using Murashige and Skoog basal medium (1962) supplemented with 6-BA alone was developed for direct multiple shoots induction from shoot base culture of *C. willisii*. Shoots proliferation and elongation were achieved on a single medium without the need of subculturing. The optimum concentration of 6-BA for direct shoots regeneration of *C. willisii* was investigated. It was found to be 6 mg/ L. Optimum media was then used for regenerate and propagate of transformed *C. willisii*. On the other hand, transformation of *C. willisii* with *Agrobacterium tumefaciens* strain LBA4404 harbouring pCAMBIA1304 was performed. Effects on different infection times (2, 4, 6, 8, 10 mins) and co-cultivation periods (1, 2, 3, 4 days) were assessed. Analysis of β -glucuronidase (GUS) reporter gene expression through fluorometric assay revealed that the highest transformation rate (582.09 ± 84.30 pmol 4MU/mg/min) can be achieved with 6 mins of infection and one day of co-cultivation with *A. tumefaciens* LBA4404 harbouring pCAMBIA1304. Green fluorescent proteins (GFP) reporter genes expression was visualized under ultraviolet light. Transformed explants appeared fluorescent green under UV excitation. Plantlets of *C. willisii* showed positive result in fluorescent assessment were then subjected to molecular analysis. Polymerase Chain Reaction (PCR) analysis was applied to confirm the presence of *mgfp5* gene in individual putative transformed *C. willisii*. Integration of the gene in the genome *C. willisii* was evidenced via Southern hybridization analysis.