Quantitative real-time PCR for determination of Transgene in Callus of Jatropha curcas

Abstract

Jatropha curcas is an important plant belonging to the family Euphorbiaceae which is a potential candidate for biofuel production. Genetic transformation protocol for J. curcas callus mediated by Agrobacterium tumefaciens were optimized using a pCAMBIA1303 plasmid which carries green fluorescent protein (GFP) gene as a reporter. Results obtained were based on the highest percentage of GFP expression which was observed three days post-transformation. Immersion of callus into 1×105 cfu ml-1 (OD600nm 0.6) of A. tumefaciens LBA4404 with addition of 300 µM of acetosyringone for 45 min, two days of pre-culture and three days of co-cultivation periods were determined to be ideal for J. curcas callus transformation. Putative transformants were selected in the presence of 25 mg/l hygromycin. Surviving calli were transferred into proliferation media (MS with 1 mg/l NAA and 1 mg/l BAP) to proliferate the callus for further molecular analyses and to confirm the presence of the target GFP transgene in the putative transformants. Polymerase chain reaction (PCR) was carried out using a 35S specific primer pair confirmed the presence of the 454 bp of 35S promoter region from the transformed callus. Quantitative real-time PCR (qRT-PCR) was carried out to demonstrate the integration and copy number of the 35S promoter in the putative tranformants. The 35S promoter gene (178 bp) as a target gene and J. curcas actin gene (179 bp) which functions as reference gene was designed to detect the positive transformants and control sample in real-time PCR reaction analysis. The results indicated that the actin specific PCR product was present in both the control and transformed calli, however the 35S PCR product was found only in the positive transformants. The similarity in CT values confirmed that both the genes were present as single copy thus confirming a single integration event.