Mesocarp-specific metallothionien-like gene promoter for genetic engineering of oil palm.

ABSTRACT

Primers from within the coding region were used to capture the 5' regulatory sequence of the mesocarpspecific metallothionein-like gene, MT3-A, via PCR-based genome walking. The amplified 1040 bp genomic fragment was cloned and sequenced. The sequence of the genomic clone showed total homology with the MT3-A cDNA sequence within their overlapping regions. Rapid amplification of 5'-cDNA ends (5'-RACE) was used to determine the full length cDNA sequence and the putative transcription site of the gene. The adenine residue at the 5'-end of the RACE product was chosen as the likely transcription start site. The 986 bp promoter region upstream of the adenine contains putative regulatory elements including a TATA box, an ethylene responsive element in reverse orientation and two Iboxes. Functional analysis of the MT3-A promoter was performed using a transient assay system. Transient expression of ß-glucuronidase (GUS) examined using qualitative histochemical GUS assay can be detected in both oil palm mesocarp and leaf tissue slices bombarded with the pBI221 transformation vector which contains the GUS reporter gene under the control of the constitutive cauliflower mosaic virus (CaMV) 35S promoter. However, when the CaMV-35S promoter was replaced with MT3-A promoter in the transformation vector and used for bombardment, transient expression of GUS was detected in the oil palm mesocarp slices only and not in the leaf tissue. This suggests that the MT3-A promoter can be used to target specific gene expression into oil palm mesocarp tissues.

Keyword: Oil palm genetic engineering; Mesocarp-specific promoter; Metallothionein-like gene.