CLONING AND CHARACTERIZATION OF A COTTON LIPID TRANSFER PROTEIN GENE SPECIFICALLY EXPRESSED IN FIBER CELLS

H.C. Liu and D.P. Ma
Department of Biochemistry and Molecular Biology
Mississippi State University
Mississippi State, MS

Abstract

A polymerase chain reaction (PCR) based method was used to construct a cotton (Gossypium hirsutum L. cv DES119) fiber cDNA library in lambda gt10 vector. One full-length cDNA clone (GH3) was isolated using a differential screening method and subsequently sequenced. The nucleotide and derived amino acid sequences indicate that GH3 encodes a lipid transfer protein (LTP) of 120 amino acids. The GH3 was then used as a hybridization probe to retrieve corresponding genomic clones from a cotton genomic library. A genomic clone (1.7 kb DNA insert) containing the Ltp gene was isolated and characterized. The Ltp6 contains an open reading frame of 360 bp, which is interrupted by a single intron (136 bp) located in the region corresponding to the C-terminal of the protein. The derived amino sequence of LTP6 is 64% homologous to that of GH3. Like the GH3 gene, the Ltp6 is specifically expressed in fiber cells in a temporal manner. However, its expression level is lower than that of GH3. A long distance PCR method was used for 5' genomic DNA walking to clone promoter and upstream regulatory elements of the GH3 Ltp gene. The GH3 cDNA was amplified by PCR and cloned into expression vectors pMAL and pQE. The expressed GH3 LTP protein was injected into rabbit to produce polyclonal antibody, which was then used in Western analysis to determine the protein levels during fiber development.

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