EXPRESSION OF A PROMOTER FROM A FIBER-SPECIFIC ACYL CARRIER PROTEIN GENE IN TRANSGENIC COTTON PLANTS

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Abstract

Based on the cDNA sequence of a fiber-specific acyl carrier protein gene isolated by differential display, the promoter sequence (Gh10) of this gene was isolated by inverse PCR. A gene cassette was constructed by combining Gh10 promoter with β-glucuronidase (GUS) reporter gene and a 35s terminator. This gene construct was used to generate transgenic cotton plants by *Agrobacterium* mediated transformation. Transgenic plants regenerated from 3 independent cell lines were analyzed for the expression of GUS gene in different tissues and developing fibers. Histochemical staining indicated that GUS gene was strongly expressed in young developing tissue such as shoot, anther, petal, and developing fibers, but not in stripped ovules (fibers removed). GUS activity was significantly higher in fibers of transgenic plants than in nontransgenic plants. Therefore, the Gh10 promoter has the potential in the modification of cotton fiber properties through genetic engineering.

Introduction

Cotton is the major source of natural fiber for textile industry in the world. The development of cotton fiber requires the expression of specific genes. Modification of cotton fiber properties by genetic engineering demands the availability of cotton fiber specific promoters. Recently, cotton fiber-specific promoters have been successfully used to express acetoacetyl-CoA reductase and polyhydroxyalkanoate synthase genes in fibers of transgenic cotton plants (John and Keller, 1996). These genes led to the synthesis of a thermoplastic polymer poly-D-(-)-3-hydroxybutyrate in fibers of transgenic plants and resulted in measurable changes of thermal properties of cotton fibers. Using differential display technique, we have isolated a fiber-specific acyl carrier protein cDNA (Song et al. 1995a, 1995b, 1997). Here we report the expression of the promoter from this fiber-specific acyl carrier protein (ACP) gene in transgenic cotton plants.

Materials and Methods

Inverse PCR

About 10 µg cotton (*Gossypium hirsutum* cv. Coker 312) genomic DNA was completely digested with *EcoR* I. The digested DNA fragments were ligated into circle molecules that were used as template for the further inverse PCR amplification. Based on the cDNA sequence of the fiber-specific acyl carrier protein gene, two sets of primers close to 5' and 3' end respectively were synthesized and used for the inverse PCR reaction. PCR products were separated on 1.1% agarose gel and subcloned into pGEM-T vector (Promega, Madison, WI) and sequenced.

Gene Construct

A 1100 bp DNA fragment (Gh10) containing the 5' upstream sequence of the translation start codon of the fiber-specific ACP gene was combined with β-glucuronidase (GUS) reporter gene and 35s terminator. This gene cassette was inserted into a binary plant transformation vector that was introduced into the disarmed *Agrbacterium tumefaciens* strain EHA101.

Cotton Transformation

The previously reported cotton transformation procedure was followed to generate transgenic plants (Bayley et al., 1992). The hypocotyl of Coker 312 was used for the inoculation of *Agrobacterium tumefaciens* containing the Gh10::GUS gene construct.

PCR Analysis

Genomic DNA were extracted from leaves of putative T_0 transgenic and nontransgenic plants grown in greenhouse. Primers covering the GUS coding sequence and the chimeric gene were used for PCR analysis to detect the insertion of the gene construct.

GUS Assay

Histochemical staining and fluorogenic assay were used for the analysis of GUS expression (Jefferson, 1987). Fresh tissues were directly used for detection of GUS expression with histochemical staining solution (0.02 M x-glucoronide, 1.0 M $NaPO_4$, 0.25 M EDTA, 0.005 M K Ferricyanide, 0.005 M K Ferrocyanide, 10% Triton X-100, pH 7.0). For the fluorometric assay, 0.2 g fresh tissues were grounded in 500 µl GUS extraction buffer (50 mM $NaPO_4$, 10 mM β-mercaptoethanol, 10 mM EDTA, 0.1% sodium lauryl sarcosine, and 0.1% Triton X-100), and 10 µl crude extraction was transferred into 05 ml GUS assay buffer (1 mM 4-methyl umbelliferyl β-D-glucuronide in the extraction buffer). After incubation at 37°C for 2 hours, samples were measured for fluorenscence with a spectrofluorimeter.

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Results and Discussion

Isolation of Gh10 Promoter

One DNA fragments about 4 kb from inverse PCR amplification was subcloned into pGEM-T vector. Sequence analysis indicated that this DNA fragment contained 5'end sequence of the fiber-specific ACP cDNA. A TATA box was located at 81 bp upstream of the translation start codon. The transcription initiation site mapped with primer extension method was located 21 bp upstream of the translation start codon. There is a putative GC box at 798 bp upstream of the transcription initiation site. A 1100 bp promoter (Gh10) sequence was subcloned by PCR amplification using two primers. One *Pst* I and *Nco* I site were created in the 5' and 3'end primer respectively. The Gh10 promoter sequence was used as a probe in Southern blot analysis of cotton genomic DNA. The hybridization pattern was the same with either Gh10 or the fiber-specific ACP cDNA as a probe (data not shown). This demonstrated that the correct 5' end upstream sequence was obtained by inverse PCR as expected. Then, the GUS reporter gene plus a 35s terminator was linked with Gh10 at Nco I site. This gene construct was introduced into the disarmed *Agrbacterium tumefaciens* strain EHA101 for the transformation of cotton.

GUS Expression in Transgenic Plants

Putative T_0transgenic cotton plants were regenerated from 3 independent cell lines. These plants were grown to flowering in green house and used for GUS expression analysis. All the putative T_0 plants showed the expected bands when their genomic DNAs were PCR amplified using primers covering GUS coding sequence or the Gh10::GUS gene construct, while no bands were detected in nontransgenic plants (Coker 312, Stoneville 7A, and Express) (Figure 1).

Different plant tissues including slices of young leaves, shoots, petal, anther, and styles were histochemically stained for GUS expression. Tissues form non transgenic plants (Coker 312) did not appear any blue color after incubation in the histochemical staining solution at 37^{O}C for 16 hours. All the tissues from transgenic plants Gh10#4-9 and #4-8 produced dark blue color due to the high expression of GUS reporter gene driven by Gh10 promoter. However, the intensity of the blue color produced by transgenic plants Gh10#7-7 and #3-5 was not as high as appeared in Gh10#4-9 and Gh10#-8. GUS activities in tissues from Gh10#4-8 and #4-9 were also significantly higher than that from Gh10#7-7 and #3-5. This phenomena probably is the result of position effect caused by insertion of the Gh10::GUS gene construct in different regions of cotton genome.

Developing ovules and fibers from different days post anthesis (DPA) were analyzed for the expression of GUS reporter gene. Intact ovules of nontransgenic plants (Coker 312) at different DPAs did not produce blue color after histochemical staining. Intact ovules of transgenic plants at 0, 4, 8, 12, 16, 20, 25, and 30 DPA were stained blue. Similar to the result observed in the other tissues, the intensity of blue color in plants Gh10#4-8 and #4-9 was higher than that in Gh10#7-7 and #3-5. When intact ovules of 12 DPA from plant Gh10#4-9 was longitudinally sectioned and incubated for histochemical staining of GUS expression, fibers appeared dark blue color, but ovule portion did not. Fluorometric assay showed that GUS activity in fibers of Gh10#4-8 and #4-9 was significantly higher than that in Coker 312 (Figure 2). However, the difference of GUS activity in fibers between Gh10#7-7 and Coker 312 was not as significant as between Gh10#4-8 or #4-9 and Coker 312, which is likely caused by position effect of the inserted gene. Furthermore, there were no significant differences in GUS activity of stripped ovules (fibers removed) between transgenic and nontransgenic plants. These results indicated that GUS gene driven by Gh10 promoter was strongly expressed in developing fibers.

Northern blot analysis of different cotton tissues including cotyledon, shoots, leaves, and roots with the fiber-specific ACP cDNA as a probe indicated that its transcripts predominately accumulated in developing fibers (Song and Allen 1997). However, GUS gene derived by Gh10 promoter was expressed not only in developing fibers but also in various developing tissues. We used 1100 bp upstream sequence from the start codon of the fiber-specific ACP gene as a promoter sequence. Possibly, there are additional transcription regulatory elements located upstream of this 1100 bp sequence or post transcription regulation is involved the expression of this fiber-specific ACP gene *in vivo*. Although Gh10::GUS gene construct was not exclusively expressed in fibers of transgenic cotton plants, Gh10 promoter has the potential to be used in cotton biotechnology.

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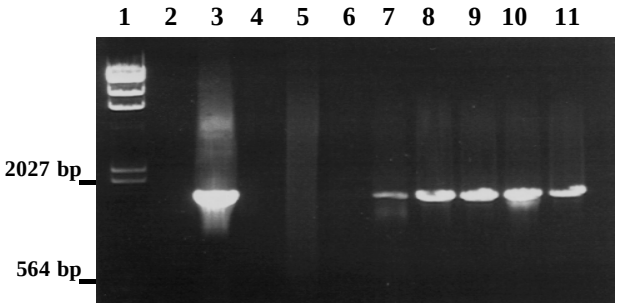

Figure 1. PCR analysis of cotton genomic DNA. Lane 1, λ phage *Hind* III DNA marker; Lane 2, water; Lane 3, plasmid DNA containing GUS report gene; Lane 4 - 6, *G.hirsutum* cv. Coker 312, Stoneville 7A, and Express; Lane 4-11, putative T_0 transgenic plants Gh10#4-3, #3-5, #7-7, #4-8, and #4-9.

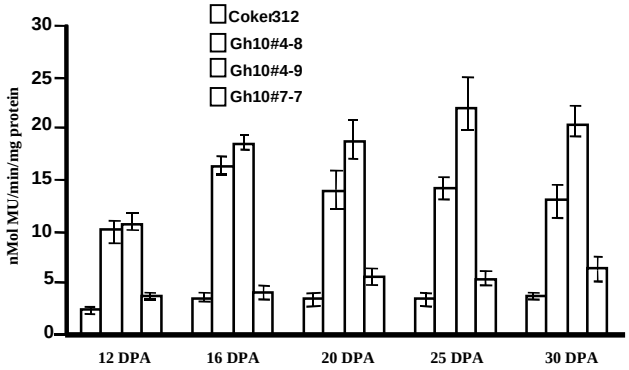

Figure2. Fluorometric assay of Gh10/GUS expression in developing fibers from different days post anthesis (DPA) in T_0 transgenic plants.