The *Journal of Cotton Science* 4:217-223 (2000)
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Molecular Biology

Expression of Two Tissue-Specific Promoters in Transgenic Cotton Plants

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INTERPRETIVE SUMMARY

Efficient genetic modification of cotton for agronomic traits will require the use of regulatory sequences, known as promoters, to drive the expression of transgenes in specific plant tissues or at certain developmental stages. The pattern of expression provided by different promoters must be analyzed before they can be used to generate transgenic cotton plants commercially. In this study, the expression patterns of two tissue-specific promoters were analyzed in transgenic cotton plants. These promoters were *Gh-sp*, derived from a seed protein gene, and *Gh-rbcS*, obtained from a nuclear-encoded chloroplast gene. These promoters were attached to a bb-glucuronidase (GUS) reporter gene. Tissues that express the GUS gene stain blue when provided with a specific substrate, thus providing a convenient means to identify the expression patterns determined by each promoter. As expected, GUS expression under control of the *Gh-sp* promoter was detected only in maturing seeds; GUS expression controlled by the *Gh-rbcS* promoter was seen predominately in leaves. These results indicate these two tissue-specific promoters can be used to target the tissue-specific expression of important genes in transgenic cotton plants.

ABSTRACT

In order to evaluate the functions of putative transcriptional regulatory regions of cotton genes, the upstream regulatory sequences (promoters) of two cotton genes, *Gh-sp* (seed protein) and *Gh-rbcS* (ribulose-1,5-bisphosphate carboxylase small subunit), were fused with a bb-glucuronidase (GUS) reporter gene and the cauliflower mosaic virus (CaMV) 35S terminator. These two gene constructs were transferred into cotton (*Gossypium hirsutum* L. cv. Coker 312) by *Agrobacterium*-mediated transformation. Transgenic plants from the T_0 generation were analyzed for expression of the GUS reporter gene in different tissues and developmental stages of cotton. Qualitative and quantitative analyses indicated the GUS gene driven by the *Gh-sp* promoter were expressed only during seed maturation, beginning approximately 25 d postanthesis. Expression of the GUS reporter gene driven by the *Gh-rbcS* promoter was detected primarily in leaf tissue from transgenic plants. Levels of GUS expression in leaves of *Gh*-rbcS/GUS transgenic plants was comparable to that in transgenic cotton plants containing a GUS gene construct controlled by the CaMV 35S promoter. These results indicate that these two tissue-specific promoters have the potential to be used to differentially express interesting genes in developing seeds or leaves of transgenic cotton.

In the past few years, genetically modified cotton carrying insect- and herbicide-resistant genes has been commercialized successfully. Transgenic cottons are likely to play an increasingly important role in worldwide cotton production by conferring useful agronomic and fiber traits. Typically, the beneficial gene is expressed in transgenic plants under control of a regulatory DNA sequence known as a promoter. Although the strict definition of the term promoter refers only to the core DNA sequences necessary for basal transcription, for the purposes of this report, a promoter is defined as the DNA sequences required to determine the appropriate spatial and temporal expression pattern. The primary regulatory sequences generally are located within 1000 base pairs upstream (5') of the transcription start site in plant genes, although there are cases where regulatory sequences are found

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Abbreviations: CaMV, cauliflower mosaic virus; EDTA, ethylenediaminetetraacetic acid; GUS, ß-glucuronidase; PCR, polymerase chain reaction.

further upstream or are downstream (3') of the coding sequences (Dietrich et al., 1992; Zhang et al., 1996).

Currently, the most widely used promoter for expression of foreign gene constructs (transgenes) in dicot plants is the cauliflower mosaic virus (CaMV) 35S promoter (Ow et al., 1986). The CaMV 35S promoter provides strong constitutive expression in most dicot plants, including cotton. However, to develop transgenic cottons with specialized agronomic traits such as fiber quality and seed nutrition components, a larger arsenal of constitutive and tissue-specific promoters will be required. The characteristic expression patterns provided by these promoters must be analyzed to determine whether they can be used to express beneficial genes in specific target tissues or developmental stages at maximum levels. Although such promoter tests can be conducted with transient expression assays or in model plant systems such as transgenic tobacco and *Arabidopsis*, gene expression analysis in stable transgenic cotton plants provides confirmation that these promoters can be used for development of transgenic cotton for commercial production. Although several fiber-specific promoters have been tested in transgenic cotton plants (Dang et al., 1995; John and Crow, 1992; Rinehart et al., 1996), the isolation of constitutive and tissue-specific promoters (Song and Allen, 1997; Song et al., 1998) is also of interest. Here we report the expression patterns of two promoters from cotton that direct seed-specific and leaf-specific expression in transgenic cotton plants. We anticipate that these promoters can be used to direct expression of transgenes in cotton and other plants.

MATERIALS AND METHODS

DNA Extraction

Genomic DNA was extracted from fully expanded leaf tissues of greenhouse-grown cotton plants (*Gossypium hirsutum* L. cv. Coker 312) using the procedure of Guillemant and Laurence (1992) and further purified by phenol/chloroform/isoamyl alcohol (25:24:1) extraction and ethanol precipitation. DNA concentration was measured spectrophotometrically. Quality of DNA was determined by Hind III digestion of 2 mg genomic DNA followed by gel electrophoresis. Ethidium bromide staining was used to view the digested DNA.

Promoter Isolation

DNA sequences of a cotton ribulose-1, 5-bisphosphate carboxylase small subunitgene (*Gh-rbcS*, GenBank accession no. X54091), and a late embryogenesis abundant (*Lea*) seed protein gene (*Gh-sp*, GenBank accession no. M19389), were identified. For PCR amplification of the 5' regulatory region of the *Gh-rbcS* gene, the following oligonucleotides were developed and used as 5' and 3' primers, respectively: 5'-cgctcatgttaacaatta attcctataatc-3' and 5'-catcgtagtacgtgggtaagc tcgagtact-3'. For polymerase chain reaction (PCR) amplification of the 5' regulatory region of the *Gh-sp* promoter, the following oligonucleotides were used as 5' and 3' primers, respectively: 5'-gaaccaggtcgatag ttgaattagttatgtt-3'and 5'-ctcagctgtttgcatcat ggcagcatcttg-3'. About 1 mg of genomic DNA was used in 50 mL PCR. PCR was performed with TaKaRA Ex Taq polymerase (Takara Shuzo Co., Otsu Shiga, Japan) under conditions suggested by the manufacturer. PCR products were separated on 1.1 % agarose gels and stained with ethidium bromide. DNA fragments of the expected size, based on the designated primers, were cut from gels and subcloned into a TA cloning vector (pGEM-T System, Promega, Madison, WI). Subcloned PCR products were sequenced to verify the gene identity.

Gene Constructs

To build gene constructs including *Gh-rbcS* or *Gh-sp* promoters fused to the GUS reporter gene, *Pst* I sites were added to the 5' ends of the promoter sequences and the initiation codons were mutated to *Nco* I sites. This was done by PCR amplification of the subcloned promoter fragments with primers containing the respective restriction site changes, with the resulting PCR products being purified and digested with *Pst* I and *Nco* I. This resulted in a 1200 base pair DNA fragment containing the *Gh-sp* promoter and a 560 base pair fragment containing the *Gh-rbcS* promoter. These two promoter fragments were ligated into the *Pst* I and *Nco* I sites

in the binary vector pCGN1578-*Gh10* to replace the *Gh10* promoter (Song et al., 1998). pCGN1578 *Gh10* contains *Gh10*/GUS/CaMV 35S terminator-CaMV 35S promoter /NPT/CaMV 35S terminator cassette. These two gene cassettes, *Gh-sp*/GUS/CaMV 35S terminator-CaMV35 promoter /NPT/CaMV 35S terminator and *Gh-rbcS*/GUS/CaMV 35S terminator-CaMV35 promoter /NPT/CaMV 35S terminator, were introduced into the disarmed *Agrobacterium tumefaciens* strain EHA101 by the freeze-thaw method. For cotton transformation, positive clones were selected on LB medium containing 50 $\mu g\ mL^{-1}$ of kanamycin and gentamycin. Restriction digests and southern blot analysis of plasmid DNA extracted from positive clones were used to verify the integration of the GUS expression cassette.

Transgenic Plants

Transformation of cotton plants with the *Gh-sp*/GUS/CaMV 35S term-CaMV 35S promoter /NPT/CaMV 35S terminator and *Gh-rbcS*/GUS/CaMV 35S term-CaMV35 promoter /NPT/CaMV 35S terminator gene constructs was carried out by inoculation of hypocotyl segments from cotton (*G. hirsutum* L. cv. Coker 312) seedlings grown under sterile conditions with *Agrobacterium tumefaciens* strain EHA101 and regeneration of plant via somatic embryogenesis as previously reported (Bayley et al., 1992; Payton et al., 1997). T_0 plants were analyzed for GUS expression.

GUS Assay

Histochemical staining and fluorometric assays were used for analysis of GUS expression (Jefferson, 1987). Fresh tissues were used for detection of GUS expression with histochemical staining solution (0.02 M 5-bromo-4-chloro-3-indolyl-bb-D-glucuronide, 0.1 M NaH_2PO_4, 0.25 M ethylenediaminetetraacetic acid (EDTA), 5 mM potassium ferricyanide, 5 mM potassium ferrocyanide, 1.0% (v:v) Triton X-100, pH 7.0). Tissues from mature flowering plants in the greenhouse were collected. All individual plants in each independent transgenic line (two to four lines) were analyzed. Three emerging leaves, flowers, and shoots and developing seed from three bolls from each plant were used for histochemical staining. Slices of emerging leaves, longitudinal sections of shoot and developing seeds, intact anthers, pollen, and petals were incubated in GUS staining solution overnight at 37°C. Stained tissues were cleared with 70% ethanol.

For the fluorometric assay, 0.2 g of fresh tissues were ground in 500 mL GUS extraction buffer (50 mM phosphate buffer, pH 7.4;10 mM ß-mercaptoethanol, 10 mM EDTA, 0.1% sodium lauryl sarcosine, and 0.1% Triton X-100). The samples were centrifuged at 12 000 × g for 15 min at 4°C. Ten microliters of supernatant were transferred to 0.5 mL GUS assay buffer (1 mM 4-methyl umbelliferyl ß -D-glucuronide in extraction buffer). After incubation at 37°C for 2 h, the reactions were stopped by the addition of 2 mL of 0.2 M Na_2CO_3 and the fluorescence was measured with a fluorometer (Turner Model 112, Sequoia-Turner Corp., Mountain View, CA). Tissue collection was the same as for histochemical staining. Three samples were taken from each plant and each sample was measured three times. Mean GUS activities for each independent line were compared.

RESULTS AND DISCUSSION

Seed Protein Promoter (*Gh-sp*)

The *Gh-sp* promoter sequence is from the late embryogenesis abundant (*Lea*) class of seed proteins (Baker et al., 1988). Analysis of *Lea* gene expression in cotton indicated that most *Lea* mRNAs start to accumulate in developing seeds at about 30 d postanthesis (Hughes and Galau, 1991). Sequence analysis of the *Gh-sp* promoter indicated a conserved sequence motif located 89 base pairs upstream of the TATA-box that is similar to the G-box core motif (ACGT). Because similar elements are required for seed-specific gene expression in several plant species (Stalberg et al., 1993; Vincentz et al., 1997; Wu et al., 1998), we anticipated that the 1200-base pair *Gh-sp* promoter would provide seed-specific expression of the reporter gene.

Transgenic cotton plants (T_0) were regenerated from two independent cell lines transformed with the *Gh-sp*/GUS gene construct. The presence of the GUS reporter gene in these transgenic plants was detected by PCR amplification, using GUS-specific

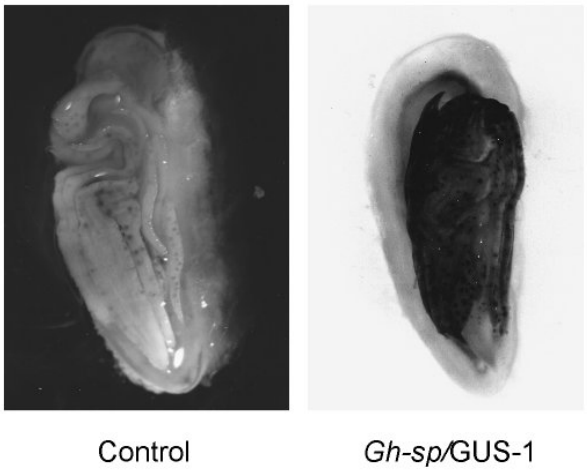

Fig. 1. Immature seeds (25 d postanthesis) from nontransformed (control) cotton plants and a transgenic cotton plant containing a reporter gene construct with a promoter from a cotton seed protein gene (*Gh-sp*/GUS-1) were stained for ß-glucuronidase (GUS) activity. No detectable GUS activity was seen in control seeds. Embryos of seeds that contained the *Gh-sp*/GUS-1 reporter stained intensely blue with strongest signal in the cotyledons, indicating high levels of GUS activity in these organs.

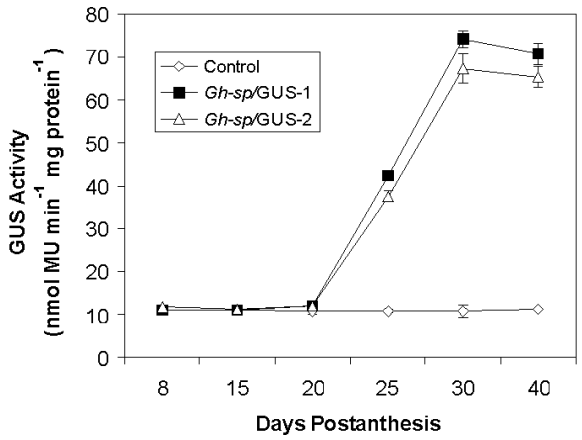

Fig. 2. Quantitative assays for GUS-specific activity in extracts of developing ovules from untransformed cotton plants (control) and two independent transgenic cotton lines (each has two plants) that contain the *Gh-sp* reporter gene construct (*Gh-sp*/GUS-1 and *Gh-sp*/GUS-2). Background levels of GUS activity were seen in both control and transgenic plants through 20 d postanthesis. A rapid increase in GUS activity occurred in transgenic seeds during the next 10 d of maturation, reaching maximum levels at about 30 d postanthesis.

primers. The expected band was not observed in nontransgenic plants (data not shown). Although this assay indicates the presence of the transgene, it does not indicate whether the transgene is expressed. To analyze expression, these T_0 plants were grown to flowering in a greenhouse and analyzed for GUS expression. Different plant tissues - including sections of emerging leaves, longitudinal sections of shoots, roots, petals, mature anthers, and styles - were stained for GUS activity. After incubation in the staining solution overnight at 37 °C, no blue stain indicative of GUS activity was apparent in any tissues from these primary transgenic (T_0) plants, suggesting that the GUS gene was not expressed in these tissues (data not shown). Also, no GUS staining was detected in any of the nontransgenic control plants.

To determine the expression pattern of the *Gh-sp/GUS* construct in seed development, bolls from two T_0 plants from each independent transgenic line were tagged and ovules (T_1) were harvested at different days postanthesis and stained for GUS activity. GUS activity was not detected in developing seeds before 25 d postanthesis, but very strong staining was observed in the developing seeds from all of the transgenic lines at 30 d postanthesis or later (Fig. 1). Staining was limited to embryonic tissues, with strongest activity in the cotyledons. Results from quantitative fluorometric assays of GUS activity matched the histochemical staining pattern in maturing seeds. GUS activity in ovule extracts increased dramatically at 25 d postanthesis relative to background levels in non-transgenic control plants (Fig. 2).

The GUS expression pattern controlled by the *Gh-sp* promoter in transgenic cotton plants was similar to that reported for endogenous cotton *Lea* genes (Hughes and Galau, 1991). Therefore, this *Gh-sp* seed-specific promoter will be useful in the genetic modification of seed properties during the later stages of seed maturation such as protein quality, fatty acid composition, and gossypol content. In fact, the *Gh-sp* promoter has been linked to an antisense cDNA for cadinene synthase, one of the key enzymes in the gossypol synthesis pathway, in an effort to reduce gossypol synthesis in cottonseeds (C.R. Benedict, personal communication). Introduction of this gene cassette into transgenic cotton is underway.

Rubisco Small Unit Promoter (*Gh-rbcS*)

The small subunit of ribulose-1,5-bisphosphate carboxylase (*rbcS*) is encoded by gene families in most plants. Promoters from one group of these genes contain two *cis*-acting elements, the I-box and the G-box, that are important for tissue-specific expression (Donald and Cashmore, 1990; Manzara et al., 1991). Analysis of transgenic tomato plants expressing a *rbcS* -promoter/GUS fusion gene confirmed that promoter fragments ranging from 0.6 to 3.0 kb of *rbcS1*, *rbcS2*, and *rbcS3A* genes were sufficient to confer the temporal and organ-specific expression pattern (Manzara et al., 1993; Meier et al., 1995). In these genes, the I-box and G-box are located within -600 to -100 bp upstream of the transcription initiation site. The 560-bp promoter fragment from the cotton *rbcS* gene used to assemble the GUS reporter gene construct reported here includes putative I-box (-287 to -274 base pair) and G-box (-260 to -252 base pair) sequences.

Three transgenic (T_0) cotton plants were regenerated from each of five independent cell lines transformed with the *Gh-rbcS*/GUS gene cassette. The presence of the transgene in these plants was confirmed by PCR amplification of the GUS DNA fragment from genomic DNA using GUS-specific primers; corresponding bands were not amplified from DNA of nontransgenic plants (data not shown). Analysis of transgene expression was carried out by analysis of GUS activity. Leaf sections from emerging leaves of plants with four to five true leaves and flowering plants were incubated with GUS staining solution at 37°C overnight. GUS expression was detected in all 15 transgenic plants tested and no blue color appeared in nontransgenic (control) plants (Figs. 3, 4). Other tissues - including roots from regenerated transgenic plantlets, longitudinal sections of shoots, intact petals and anthers, and 8-d postanthesis-developing ovules from flowering transgenic plants - were tested for GUS expression. GUS activity was not detected in these samples except for the shoot, which showed light staining primarily in the outer parts of the cortex (data not shown). These results demonstrate the GUS gene expression under control of the *Gh-rbcS* promoter is expressed in chlorophyll-containing tissues, primarily leaves.

Fig. 3. Segments of expanding leaves from nontransformed (control) cotton plants and transgenic cotton plants that contained a reporter gene with a promoter from a cotton *rbcS* gene (*Gh-rbcS*/GUS-1) were stained for ß-glucuronidase (GUS) activity. While GUS activity was not detected in leaves of control plants, leaves from the transgenic plant stained intensely blue, indicating high levels of GUS activity.

Fluorometric assays of leaf tissues from the 15 transgenic plants indicated that GUS activity in leaves of *Gh-rbcS*/GUS transgenic plants was significantly higher than in nontransgenic plants (data not shown). There was considerable variation in the level of GUS expression among transgenic plants regenerated from different cell lines (Fig. 4), while the plant-to-plant variation observed within the same transgenic line was small. Expression in the transgenic lines with the highest levels of GUS activity (*Gh-rbcS*/GUS-1 and *Gh-rbcS*/GUS-2) were only slightly lower than that of a 35S/GUS transgenic plant tested. The variation in expression between different cell lines could have been caused by "position effects" that depend on the chromosomal location of the transgene insertion, by co-suppression, and/or the presence of multiple copies of transgenes. Because we have not yet analyzed the number of transgene inserts in our transgenic plants, we cannot distinguish among these possible mechanisms at this time.

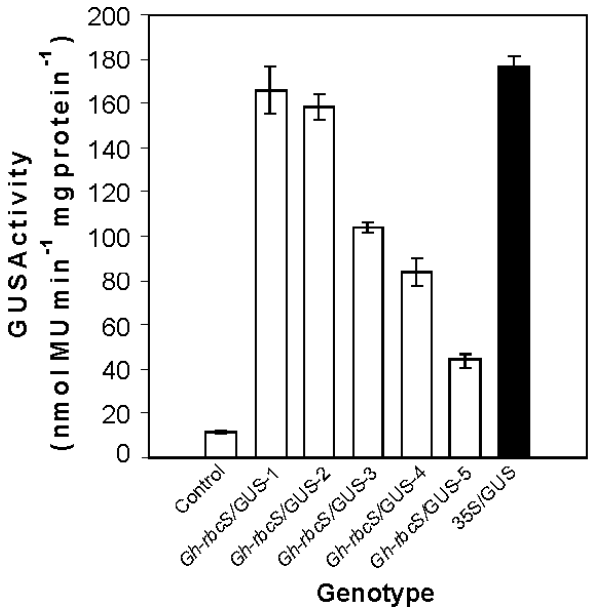

Fig. 4. Quantitative assays of GUS specific activity in extracts from expanding leaves from untransformed (control) cotton plants and five independent transgenic cotton lines containing the *Gh-rbcS*/GUS reporter gene construct (*Gh-rbcS*/GUS-1, two plants; *Gh-rbcS*/GUS-2, three plants; *Gh-rbcS*/GUS-3, three plants; *Gh-rbcS*/GUS-4, four plants; *Gh-rbcS*/GUS-5, three plants) and one transgenic cotton plant containing a GUS reporter gene controlled by a CaMV 35S promoter (35S/GUS, two plants). Levels of GUS activity in all of the Gh13/GUS lines were substantially higher than in control plants but activity varied by as much as 300% between transgenic lines. The two highest-expressing lines (*Gh-rbcS*/GUS-1 and *Gh-rbcS*/GUS-2) had activities similar to those in leaves of a typical 35S/GUS containing plants.

A tobacco tissue-specific *rbcS* gene promoter has been used successfully to generate herbicide-resistant transgenic plants (Stalker et al., 1988). Based on the GUS expression from this experiment, the *Gh-rbcS* promoter also could be used to express foreign genes at high levels in green tissues of transgenic cotton plants.

CONCLUSIONS

The patterns of GUS reporter gene expression under control of either the *Gh-sp* or *Gh-rbcS* promoters in transgenic cotton accurately reflect the expression of native genes. While we recognize that promoters from seed protein and *rbcS* genes from other species are also likely to be expressed in cotton, this work shows that promoters from native cotton genes can effectively mimic the expression of endogenous genes and do not appear to be greatly affected by gene silencing.

ACKNOWLEDGEMENT

Supported by Cotton Incorporated grant no. 96-334TX.

REFERENCES

Baker, J., C. Steele, and L.S. Dure III.1988. Sequence and characterization of 6 *Lea* proteins and their genes from cotton. Plant Mol. Biol. 11:277-291.

Bayley, C., N. Trolinder, C. Ray, M. Morgan, J.E. Quisenberry, and D.W. Ow.1992. Engineering 2,4-D resistance into cotton. Theor. Appl. Genet. 83:645-662.

Dang, P.M., J.L. Heinen, and R.D. Allen.1995. Expression of a cotton fiber gene promoter in tobacco. Proc. Beltwide Cotton. Conf., San Antonio, TX. 4-7 Jan. 1995. Natl. Cotton Counc. Am., Memphis, TN.

Dietrich, R.A., S.E. Radke, and J.J. Harada.1992. Downstream sequences are required to activate a gene expressed in the root cortex of embryos and seedlings. Plant Cell 4: 1371-1382.

Donald, R.G.K., and A.R. Cashmore. 1990. Mutation of either G box or I box sequences profoundly affects expression from the *Arabidopsis rbcS*-1A promoter. EMBO J. 9: 1717-1726.

Guillemant, P., and M.D. Laurence.1992. Isolation of plant DNA: A fast, inexpensive, and reliable method. Plant Mol. Biol. Rep.10(1):60-65.

Hughes, D.W., and G.A. Galau. 1991. Developmental and environmental induction of Lea and *LeaA* mRNAs and the postabscission rogram during embryo culture. Plant Cell 3:605-618.

Jefferson, R.A.1987. Assaying chimeric genes in plants: The GUS gene fusion system. Plant Mol. Biol. Rep. 5:387-405.

John, M.E., and L.J. Crow. 1992. Gene expression in cotton (*Gossypium hirsutum* L.) fiber: cloning of the mRNAs. Proc. Natl. Acad. Sci. USA 89:5769-5773.

Manzara, T., P. Carrasco, and W. Gruissem. 1991. Developmental and organ-specific changes in promoter DNA-protein interactions in tomato *rbcS* gene family. Plant Cell 3:1305-1316.

Manzara, T., P. Carrasco, and W. Gruissem. 1993. Developmental and organ-specific changes in DNA-protein interactions in the tomato *rbcS1*, *rbcS2,* and *rbcS3* promoter regions. Plant Mol. Biol. 21:69-88.

Meier, I., L. Kristie, J.A. Fleming, and W. Gruissem. 1995. Organ-specific differential regulation of a promoter subfamily for the ribulose-1, 5-bisphosphate carboxylase/oxygenase small subunit genes in tomato. Plant Physiol. 107:1105-1118.

Ow, D.W, K.V. Wood, M. DeLuca, J.R. de Wet, D.R. Helinski, and S.H. Howell. 1986. Transient and stable expression of the firefly luciferase gene in plant cells and transgenic plants. Science 234:856-859.

Payton, P., R.D. Allen, N.L. Trolinder, and A.S. Holaday. 1997. Over-expression of chloroplast-targeted Mn superoxide dismutase in cotton (*Gossypium hirsutum* L., cv. Coker 312) does not alter the reduction of photosynthesis after short exposures to low temperature and high light intensity. Photosyn. Res. 52:233-244.

Rinehart, J.A., M.W. Peterson, and M.E. John.1996. Tissue-specific and developmental regulation of cotton gene FbL2A. Plant Physiol. 112:1331-1341.

Song, P., and R.D. Allen. 1997. Identification of a cotton fiber-specific acyl carrier protein cDNA by differential display. Biochim. Biophys. Acta 1351:305-312.

Song, P., J.L. Heinen, T.H. Burns, and R.D. Allen. 1998. Expression of a promoter from a fiber-specific acyl carrier protein gene in transgenic cotton plants. Proc. Beltwide Cotton Prod. Res. Conf., San Diego, CA. 5-9 Jan. 1998. Natl. Cotton Counc. Am., Memphis, TN.

Stalberg, K., M. Ellerstrom, L.G. Josefsson, and L.Rask. 1993. Deletion analysis of a 2S seed storage protein promoter of *Brassica napus* in transgenic tobacco. Plant Mol. Biol. 23:671-683.

Stalker, D.M, K.E. McBride, and L.D. Maly. 1988. Herbicide resistance in transgenic plants expressing a bacterial detoxification gene. Science 242: 419-423.

Vincentz, M., A. Leite, G. Neshich, G. Vriend, C. Matter, L. Barros, D. Weinberg, E.R. de Almeida, M. Paes de Carvalho, F. Aragao, and E.S. Gander.1997. ACGT and vicilin core sequence in a promoter domain required for seed-specific expression of a 2S storage protein gene are recognized by the opaque-2 regulatory protein. Plant Mol. Biol. 34:879-889.

Wu, C.Y., A. Suzuki, H. Washida, and F. Takaiwa.1998. The GCN4 motif in a rice glutelin gene is essential for endosperm-specific gene expression and is activated by *Opaque-2* in transgenic rice plants. Plant J. 14: 673-683.

Zhang, J.Z., C.M. Santes, M.L. Engel, C.S. Gasser, and J.J. Harada. 1996. DNA sequences that activate isocitrate lyase gene expression during late embryogenesis and during postgerminative growth. Plant Physiol. 110:1069-1079.