MANIPULATION OF FIBER QUALITY IN TRANSGENIC COTTON VIA ECTOPIC EXPRESSION OF FIBER GENES

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Abstract

Advances in cotton biotechnology and gene discovery are making the selection and manipulation of fiber genes for the improvement of yield and fiber quality a reachable goal . To test the potential for altering fiber properties, the expression of expansin, a cell wall enzyme involved in fiber elongation, was altered in transgenic cotton plants. Four different gene constructs were assembled, in which the expansin gene was cloned in the sense or antisense (RNAi) orientation under the control of two different promoters – a cotton promoter or the CaMV 35S constitutive promoter. The genes were introduced into *G. hirsutum* L. cv. Coker-312 by *Agrobacterium*-mediated transformation. Variations in the number and density of leaf and stem hairs (trichomes), flower morphology (anthers and stigma), leaf size and shape, and fiber quality were observed relative to control plants, depending on the gene construct. Transgenic cotton plants containing the 35S::expansin gene construct exhibited increased fiber strength, fiber tenacity and fiber weight/seed (yield).

Introduction

Cotton is the world's leading natural fiber in textile manufacturing (Harig, 1992). Rising production costs, stagnant market prices, and no significant gains in yield threaten cotton's competitiveness in the marketplace. The identification of fiber genes important to production and manufacturing offers an opportunity to improve fiber yield and fiber quality using molecular approaches. A molecular model for fiber development has been proposed based on the spatial and temporal regulation of several fiber-enriched genes, including tubulin isoforms (Dixon et. al., 1994), a GTPase (Delmer et.al., 1995), a lipid transfer protein (Ma et. al., 1997; Orford and Timmis, 1998) and an acyl carrier protein (Song and Allen, 1997).

Expansin proteins were first isolated in 1992 as the mediators of "acid growth". Since expansins were first cloned (Shcherban et. al., 1995), many homologous sequences have been identified from a wide range of plants (Michael, 1996; Rose et. al., 1996; Cho and Kende, 1997; Shimizu et. al., 1997; Chen and Bradford, 1998; Orford and Timmis, 1998). A biological role assigned to expansins is the loosening of the cell wall during cell expansion (Campbell and Braam, 1998; Cosgrove, 1999,2000).

The goal of this study was to determine if fiber quality could be altered by ectopic expression of an expansin gene during fiber development. Four different gene constructs were introduced into cotton in which expression of a fiber expansin gene, in either the sense or antisense (RNAi) orientation, was driven by a cotton vacuolar H^+-ATPase (V-ATPase) or 35 S CaMV promoter. Transgenic cotton plants were generated using A*grobacterium*-mediated transformation and somatic embryogenesis (Trolinder and Goodin, 1987). Depending on the promoter and orientation of the expansin gene, changes in development and morphology were observed that were consistent with a functional role of expansin in cell expansion and fiber elongation. Most importantly, transgenic cotton plants bearing the *35S::expansin* gene construct produced stronger fibers with increased tenacity and increased yield. These results provide compelling evidence for the genetic improvement of fiber yield and quality through the manipulation of single fiber genes.

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Materials and Methods

Gene Constructs

A fiber expansin cDNA was placed under the transcriptional control of a cotton V-ATPase promoter in the sense (TW 317) or anti-sense (TW 318) orientation and cloned into the binary vector pBI101.1. The chimeric gene construct was introduced into *Agrobacterium tumefaciens* strain LBA4404 by electroporation. Bacterial colonies were selected using 50 µg/ml kanamycin (plasmid selection) and 100µg/ml streptomycin (A*grobacterium* selection).

A second set of expansin constructs were assembled using the 35S CaMV promoter to produce sense (TW 321) and anti-sense (TW 319) chimeric genes, which were subsequently cloned into the binary vector pDVS572 and transformed *Agrobacterum* LBA4404 using electroporation. Bacterial colonies were selected using 15µg/ml tetracycline (plasmid selection) and 100µg/ml streptomycin (A*grobacterium* selection).

Plant Material and Transformation

Coker-312 seed were generously provided by Dr. N.L. Trolinder (GenesPlus, TX). Surface sterilized seeds were germinated on Stewart's germination medium in 150-mm test tubes. Hypocotyl explants from 8-10 day-old germinated seedlings were used for co-cultivation with an overnight culture of *Agrobacterium* grown under the appropriate selection. Explants were incubated for 24 hrs at 28^0 C, washed and placed on selection medium containing 50 µg/ml kanamycin. Kanamycin-resistant callii were selected and placed in induction medium to promote embryogenesis. Heart-shaped or globular embryos were recovered, dehydrated, and germinated. Embryos successfully producing roots and shoots were transferred to pint jars. Plantlets were transferred to soil and hardened off in a growth chamber prior to transfer to the greenhouse.

Characterization

The presence of the selectable marker and expansin gene in independently transformed transgenic lines were confirmed by PCR using gene-specific primers. The transgenic plants were monitored for changes in growth and morphology relative to control (untransformed) Coker-312 plants. Fiber properties were measured (length, strength and tenacity) by Dr. You-Lo Hsieh (UC-Davis) using the Mantis single-fiber testing instrument. T1 Seeds were collected from T0 plants and germinated on kanamycin-selection medium. The presence of the transgene in kanamycin-resistant T1 plants was confirmed by PCR using gene-specific primers.

Results

Ectopic expression of expansin in transgenic cotton plants produced changes in growth and morphology, as well as fiber properties, depending on the gene construct.

Trichomes

Transgenic plants expressing the *35S::expansin* (TW 321) gene produced significantly more trichomes on the stem, leaf and leaf petioles compared to control plants. The 35S antisense (TW 319) plants produced fewer trichomes than control plants.

Leaves

Plants containing the V-ATP::expansin (TW 317) construct exhibited much smaller leaves than control plants.

Flowers

Flowers in V-ATP::expansin (TW 317) plants failed to completely unfurl on the day of anthesis. In contrast, flowers of the antisense (TW 318) transgenic plants opened normally, but produced abnormally short filaments, resulting in male sterility. In addition, the stamens remain tightly

packed against the style. The stigma of TW318 transgenic plants is also abnormally club-shaped.

Germination Test

Germination of *35S::expansin* (TW321) T1 seeds on kanamycin-selection medium showed 3: 1 segregating ratio of kanamycin-resistant to kanamycin-sensitive plants, indicating a single insertion of the transgene in the cotton genome. The taproot of germinating kanamycin-resistant seedlings grew down into the kanamycin-selection medium, while taproots of non-surviving kanamycin-sensitive seedlings did not enter the selection medium.

Fiber Quality

Single fiber analysis data revealed that T0 fibers of *35S::expansin* plants exhibit increased strength and fiber tenacity compared to control fibers (Table 1). In addition, the T0 transgenic fibers exhibited an increase in fiber wt /seed (yield) (Table 2). The increased fiber strength and fiber yield of the T0 generation was transited to T1 progeny.

Discussion

Expansin proteins play a critical role in cell expansion during plant growth and development (Brummell et al., 1999; Cho and Kende, 1997; Cosgrove, 1999). Expression of expansin genes is highest in growing and differentiating cells, particularly in those cells having thickened cell walls, although additional functional roles have been postulated (Cosgrove, 2000). During cell differentiation and morphogenesis, the cell wall requires major re-modeling, a process that is likely facilitated by expansins (Baluska et al., 2000; Cosgrove, 2000).

Ectopic expression of a fiber expansin gene in transgenic cotton plants altered the growth and development of various tissues and organs, depending on the promoter and whether the gene was expressed in the sense or antisense orientation. The number and density of trichomes in stems, leaves, and leaf petioles of transgenic plants bearing the 35S CaMV gene constructs were significantly different relative to wild-type control plants. The sense construct produced an increase in trichome number, while the antisense construct significantly decreased the number of trichomes.

Fiber yield (fiber weight/seed) of transgenic *35S::expansin* (TW321) plants was significantly greater than control plants (Table 2). In addition, fiber strength also increased in the transgenic fibers. However, despite the role of expansins in cell wall loosening and expansion (Cosgrove, 1999), there was no significant difference in fiber length. These data are consistent with reports indicating that there may be only limited correlation between expansin gene expression and elongation growth rates (Caderas et. al., 2000). It has been hypothesized that elongation rates may, in fact, be controlled by expansin acting in concert with other factors that may limit growth under some physiological conditions (Caderas et. al., 2000).

Summary

Ectopic expression of the fiber gene expansin in transgenic cotton increased fiber yield, fiber strength and fiber tenacity. In conjunction with the biological activity associated with expansins in other plant systems, the results presented here define a new role for expansin as a molecular determinant for fiber yield and fiber strength. We propose a model in which expansins play a critical role in the re-modeling of trichome/fiber initials, that in turn, leads to an increased number of cells that fully develop into mature trichomes/fibers. Further studies are underway to test this working hypothesis by determining if the yield increase observed in transgenic plants is due to an increased number of fibers and/or an increase in fiber diameter.

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Table 1. Fiber strength measurements from *35S::expansin* (TW 321) transgenic and control plants.

	TW 321	**Control**
Breaking Force (Strength)	8.1 gf	5.5 gf
Tenacity	42 mtex	38 mtex
Fiber length	24.0 mm	25.4 mm

Table 2. Fiber yield of *35S::expansin* (TW 321) transgenic and control fibers.

		TW 321	**Control**
Fiber weight/ Seed weight	Mean	0.9719	0.8705
	S. D.	0.1869	0.1162
Fiber weight (g)/ Seed	Mean	0.2089	0.0513
	S.D.	0.0536	0.0138