Cloning and Function Characteristic of *GhDWF* 4, an Ortholog of *Arabidopsis DWF* 4 from Upland Cotton (*Gossypium hirsutum* L.)

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As one of the longest cells characterized in plant kingdom, cotton fibers were regarded as an ideal material for studying plant cell growth and development. In recent years, several reports revealed that brassinosteroids (BRs) play an important role in the growth and development of cotton fiber. To further investigate the effect of BRs on fiber cell development and illuminate the mechanism of BRs action, we cloned *GhDWF*4, an ortholog of Arabidopsis *DWF*4 from upland cotton (*Gossypium hirsutum* L.). *DWF*4 is a rate-limiting enzyme in BR biosynthesis pathway, and its activity is correlated with the content of endogenous BRs. To clone the *GhDWF*4, a cotton EST (GenBank Accession # CO125422), with high homology to *AtDWF*4, was screened in dbEST database. Genome walking was performed to obtain the whole coding region of the *GhDWF*4. The resulting sequence was 3186 bp, including 7 extrons and 6 introns. The full length of cDNA was 1565 bp containing 1458 bp ORF, 86 bp 5-UTR, and 22 bp 3-UTR. The ORF encoded 485 amino acid residues with a predicted molecular mass 55 kDa. The deduced *GhDWF*4 shows high similarity to other *DWF*4 proteins, for instance, *GhDWF*4 and *AtDWF*4 share 74% identity and 84% similarity. *GhDWF*4 shares 70% identity and 83% similarity with *OsDWF*4. Furthermore, *GhDWF*4 possesses many conserved domains characterized in other *DWF*4 such as anchor region, proline, domain A, domain B, domain C, and heme-binding region. Quantitative real-time PCR assays showed that transcripts of *GhDWF*4 showed high activityin stem, hypocotyl, pistil, and 22 DPA fibers, while low activity was detected in other tissues. *GhDWF*4 activity was lowest in 12 DPA fibers, and compared to 12 DPA expression levels were slightly higher in the early stages of fiber development and far higher in the stage of secondary cell wall accumulation. However, the *GhDWF*4 mRNA peaked in 12 DPA ovules. These results showed that the products of *GhDWF*4 play important roles in secondary cell wall formation of cotton fibers and in ovule development. On the other hand, the BRs levels might be very high and be regulated strictly in rapid elongating fiber cell since the *GhDWF*4 was sensitive to BRs feedback regulation. To determine whether *GhDWF*4 is a functional gene, the cassette p6-35S::*GhDWF*4 was introduced into tobacco. Transgenic tobacco length of lamina of leaves increased greatly over wild-type f. Furthermore, length of transgenic petiole and fruit stalk were longer than that of wild-type. Bulk of fruits was bigger on transgenic plants than wild-type. Furthermore, bioassay of the extract from transgenic leaves shows more BR bioactivity compared with that from wild-type. These results indicated that over expression of *GhDWF*4 promoted transgenic tobacco growth and over expressing *GhDWF*4 could increase the content of BRs.

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