Transcriptional Analysis of the Relationship of Proanthocyanidins Biosynthesis to the Brown Pigmentation in Cotton Fiber

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With increased concern to environment and health in the modern society, naturally colored cotton becomes more and more attractive to textile industry and cotton production. Brown is the most common fiber color in naturally colored cottons. Traditional genetic analyses revealed that brown fiber was controlled by 6 loci ($Lc_{1\text{-}6}$) in cotton. But the exact structures and chemical properties of pigments in naturally colored fibers, and the molecular basis of pigment synthesis and deposition are essentially unknown. To analyze the molecular basis of pigmentation in cotton fibers, we performed cDNA amplified fragment length polymorphism (cDNA-AFLP) analysis to compare expression profiles between the brown and white fibers from a recombinant inbred line (RIL) population, which was derived from the cross of a white-fiber cultivar (Yumian No. 1) and a brown-fiber line (T586). By cDNA-AFLP analysis, we identified a differential fragment, highly homologous (63%～78% identity) to plant dihydroflavonol 4-reductase (DFR), an important enzyme in the flavonoid pathway. To determine whether the flavonoid pathway participates in the pigmentation in brown fibers, we employed dimethylaminocinnaldehyde (DMACA) staining method to detect proanthocyanidins (PA) in mature fibers with different colors from the RIL population. After DMACA staining, all the brown fibers (RILs) turned blue, while no significant color change was observed in the white and green fibers (each from 10 RILs), which suggested that PA was deposited in brown fibers, but not in white and green fibers. Real-time RT-PCR analyses indicated that 2 cinnamate 4-hydroxylase (GhC4H1 and GhC4H2), 1 4-coumarate:coenzyme A ligase (Gh4CL3), 2 chalcone synthase (GhCHS1 and GhCHS4), 1 chalcone isomerase (GhCHI), 1 flavanone 3-hydroxylase (GhF3H), 1 DFR (GhDFR1), 1 leucoanthocyanidin reductase (GhLAR1), 1 anthocyanidin synthase (GhANS1), 1 anthocyanidin reductase (GhANR1), 1 flavonoid 3′, 5′- hydroxylase (GhF35H1), and 1 TT12 (GhTT12-1) homologous unigenes were significantly up-regulated in brown fibers, and the high expression levels of these genes co-segregated with brown fiber in the RIL population of Yumian No. 1×T586. Along with the DCMAC staining, this observation indicated that the pigments in brown fibers belonged to PA, which might be transported into vacuoles by TT12 transporters. In these PA synthase genes, GhCHI had the lowest expression level, implying that the step from chalcones to flavanones may be the transcriptional regulatory step in the PA biosynthesis in cotton fiber. Furthermore, sequence alignment indicated that GhDFR was an Asp-type DFR which preferred dihydroflavonols with 2 or 3 hydroxyls on B ring as substrate. Along with the up-regulation of F35H in brown fiber, we proposed that the monomers of the brown pigment might have 2 or 3 hydroxyls on B ring. In conclusion, by expression analyses and DMACA staining, we demonstrated that PA was involved in the pigmentation in brown cotton fiber.

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