Cloning, Characterization, and Gene Annotation of Cellulose Synthase Genes from *Arabidopsis thaliana*

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The mechanistic basis of cellulose biosynthesis in plants has gained ground during last decade or so. The isolation of plant cDNA clones encoding cotton homologs of the bacterial cellulose synthase catalytic subunit was a significant achievement, which promises the elucidation of cellulose biosynthesis. Two cDNA clones of the bacterial celA genes that encode the catalytic subunit of cellulose synthase are GhcesA-1 and GhcesA-2 that are highly expressed at the onset of secondary wall synthesis when the rate of cellulose synthesis in vivo raises over 1000 fold. These genes were believed to possess four conserved sub domains critical for catalysis and/or binding of substrate UDP glucose. New insights into biochemistry of cellulose biosynthesis and identifying of different subunits of GhCesA-1 and GhCesA-2 complex are likely to stem from *Arabidopsis* cellulose biosynthetic mutants and their homologues of cotton. In the present investigation, we partially cloned cellulose related genes viz., Ath A (AtCeSA-2) and Ath B (AtCeSA-3) from *Arabidopsis*, which brings about rapid conversion of carbon to UDP glucose to facilitate the synthesis of cellulose and/or callose as an energy-efficient process. Based on the cDNA database of *Arabidopsis*, AthA and AthB primers were designed using FastPCR. Total RNA from *Arabidopsis thaliana* was isolated by Qiagen RNeasy Plant Mini kit. The cDNA was prepared by using Qiagen long range 2 Step RT-PCR kit. The two-step protocol was used because it is more sensitive than one-step method, with yields of rare and longer targets. This protocol is designed for amplification of cDNA targets up to approximately 10 kb. The amplified amplicons were separated by 1.5% agarose gel electrophoresis. There was only one amplicon of 3.2 kb consistently amplified with AthA primer, and three amplicons (1.0, 2.0, and 3.4 kb) were amplified with AthB primer. The PCR amplified fragments were cloned in pDrive vector. All the amplicons were ligated in pDrive vector separately. The ligated recombinant plasmids were incorporated in to competent *E. coli* cells (DH5α). The bacteria were cultured in the selection medium. The non-transformants and transformants were characterized as blue/white colonies, respectively. The transformed white colonies were selected, and automated sequence analysis was carried out. The nucleotide sequence data were subjected to BLAST analysis, and sequence information revealed the similarity of AthA and AthB genes in the *Arabidopsis* data bank.