Available online at www.sciencedirect.com
ScienceDirect
J. Genet. Genomics 35 (2008) 357–363
JOURNAL OF GENETICS AND GENOMICS
www.jgenetgenomics.org

Cloning and expression of two sterol C-24 methyltransferase genes from upland cotton (*Gossypium hirsuturm* L.)

Ming Luo*, Kunling Tan, Zhongyi Xiao, Mingyu Hu, Peng Liao, Kuijun Chen

Key Laboratory of Biotechnology and Crop Quality Improvement, Ministry of Agriculture; Biotechnology Research Center, Southwest University, Chongqing 400716, China

Received for publication 20 December 2007; revised 2 January 2008; accepted 4 January 2008

Abstract

Brassinosteroids (BRs) are an important class of plant steroidal hormones that are essential in a wide variety of physiological processes. Two kinds of intermediates, sitosterol and campesterol, play a crucial role in cell elongation, cellulose biosynthesis, and accumulation. To illuminate the effects of sitosterol and campesterol on the development of cotton (*Gossypium hirsuturm* L.) fibers through screening cotton fiber EST database and contigging the candidate ESTs, two key genes *GhSMT2-1* and *GhSMT2-2* controlling the sitosterol biosynthesis were cloned from developing fibers of upland cotton cv. Xuzhou 142. The full length of *GhSMT2-1* was 1,151 bp, including an 8 bp 5′-untranslated region (UTR), a 1,086 bp open reading frame (ORF), and a 57 bp 3′-UTR. *GhSMT2-1* gene encoded a polypeptide of 361 amino acid residues with a predicted molecular mass of 40 kDa. The full length of *GhSMT2-2* was 1,166 bp, including an 18 bp 5′-UTR, a 1,086 bp ORF, and a 62 bp 3′-UTR. *GhSMT2-2* gene encoded a polypeptide of 361 amino acid residues with a predicted molecular mass of 40 kDa. The two deduced amino acid sequences had high homology with the SMT2 from *Arabidopsis thaliana* and *Nicotiana tabacum*. Furthermore, the typical conserved structures characterized by the sterol C-24 methyltransferase, such as region I (LDVGCGVGGPMRAI), region II (IEATCHAP), and region III (YEWGWGQSFHF), were present in both deduced proteins. Southern blotting analysis indicated that *GhSMT2-1* or *GhSMT2-2* was a single copy in upland cotton genome. Quantitative real-time RT-PCR analysis revealed that the highest expression levels of both genes were detected in 10 DPA (day post anthesis) fibers, while the lowest levels were observed in cotyledon and leaves. The expression level of *GhSMT2-1* was 10 times higher than that of *GhSMT2-2* in all the organs and tissues detected. These results indicate that the homologue of sterol C-24 methyltransferase gene was cloned from upland cotton and both *GhSMT2* genes play a crucial role in fiber elongation. The role of GhSMT2-1 may be more important than that of GhSMT2-2.

Keywords: cotton fiber; sitosterol; campesterol; sterol C-24 methyltransferase; *GhSMT2-1*; *GhSMT2-2*

Introduction

Cotton (*Gossypium hirsuturm* L.) is the leading fiber crop and one of the mainstays of the economy in the world. Cotton fibers, as a main production of cotton plants, are unicellular, linear structures derived from the epidermis of the ovule. Cotton fiber development consists of four overlapping developmental stages: fiber initiation, cell elongation, secondary wall deposition, and maturation. Fiber cells from upland cotton generally grow up to 30–40 mm in length and about 15 μm in thickness at full maturity. Therefore, their lengths are 1,000 to 3,000 times longer than their diameter (Basra and Malik, 1984; Tiwari and Wilkins, 1995). In addition to being among the longest plant cells ever characterized, a single cell wall biopolymer, cellulose, accounts for over 95% of the dry weight of the mature cotton fiber (Kim and Triplett, 2001). The highly elongated structure and exceptional chemical composition of fiber cell establishes itself as an ideal experimental model for studying plant cell elongation, synthesis of cellulose, and cell wall accumulation (Kim and Triplett, 2001). Since the fiber quantity and quality are established during fiber development, the yield and quality of cotton

* Corresponding author.
E-mail address: luo0424@126.com; luomingyuan@swu.edu.cn

fibers are closely related to the fiber growth. Therefore, studies on fiber development will have theoretical and practical values.

To investigate the growth and development mechanism of cotton fibers, a few genes that are preferentially or specifically expressed in the fiber developmental stages have been identified and cloned (John and Crow, 1992; John and Keller, 1995; Qxford and Timmis, 1998; Loguercio et al., 1999; Wilkins and Jernstedt, 1999; Ruan et al., 2003; Shi et al., 2006; Luo et al., 2007a). However, the fiber growth and development were not affected in transgenic cotton plants through either up- or down-regulating expressions of most cloned genes. Therefore, the mechanism of fiber development is hitherto largely unknown. Recently, a few studies have demonstrated that brassinosteroids (BRs) play pivotal roles in the growth and development of cotton fibers. In the system of cotton ovule culture, the treatment of cultured ovules of Coker312 with brassinolide (BL), the most bioactive BRs, leads to a 30%–38% increase of the mean fiber length. In the same way, the treatment of ovules of Ligon mutant, a lintless mutant, results in a 16.5% increase of the fiber length and a 26.1% increase of the fiber production compared to the control samples (Kasukable et al., 2001). The exogenous application of BL results in a 6.1% and 9.5% increase of the fiber length and strength in a field test. Contrarily, exogenous application of BR biosynthesis inhibitor brassinazole2001 (Brz) inhibited fiber elongation (Sun et al., 2004; Sun and Allen, 2005; Shi et al., 2006). In addition, increasing reports indicated that the genes responsible for various biosynthetic enzymes in the BR biosynthetic pathway and the genes involved in BR perception/signaling such as *GhDET2*, *GhDWF1*, *GhSMT1*, *GhBRI1*, and *GhBIN2*, showed the highest expression profiling during fiber initiation and/or elongation (Sun et al., 2004; Sun and Allen, 2005; Shi et al., 2006; Luo et al., 2007b). These suggest that BR is an important growth promoting hormone required for fiber cell development. However, either the final production or intermediates in BR biosynthetic pathways perform different bioactivities and physiological functions in the complicated network of plant developmental regulations. For example, sitosterol is a primer in cellulose biosynthesis (Peng et al., 2002), and the ratio of sitosterol to campesterol dramatically influences plant cell growth and development (Schaeffer et al., 2001). Therefore, the effect of one gene involved in BR biosynthesis on the fiber growth and development needs to be further investigated. In this study, we cloned the *SMT2* (sterol C-24 methyltransferase 2) homologue from upland cotton based on screening the cotton EST database and contigging the candidate EST sequences to illuminate the effect of sitosterol and/or the ratio of sitosterol to campesterol on fiber cell development. Gene *SMT2* is involved in BRs upstream biosynthetic pathway, which confers to the plant cell the capability to synthesize 24-ethyl sterols from a 24-methylene sterol precursor (Benveniste, 1986; Kagan and Clarke, 1994; Bouvier-Nave et al., 1997). The expression patterns of *GhSMT2* gene in various organs and at various stages of fiber development were investigated. The results revealed that *GhSMT2* gene is closely associated with the growth and development of cotton fibers.

Materials and methods

Plant materials

Gossypium hirsuturm cv. Xuzhou 142 was provided by the Cotton Research Institute, Chinese Academy of Agricultural Sciences, and was grown in field with normal administration.

Isolation of cotton genomic DNA and total RNA

Genomic DNA was isolated from young, expanding cotton leaves using the CTAB (Cetyltriethyl ammonium bromide) method as described previously (Xiao et al., 2002). For isolation of total RNA from various organs and tissues in cotton plant, roots, cotyledons, and hypocotyls were collected from 7-day-old seedlings grown in sand, and leaves, ovules, and fibers of different developmental stages were sampled from mature plants. Isolation of total RNA was carried out following the protocol described previously (Luo et al., 2003).

Synthesis of the first-strand cDNA

Five micrograms of total RNA from various organs and tissues were used for each sample to synthesize the first-strand cDNA by the instructions of the first strand cDNA synthesis kit (TaKaRa, Dalian, China).

Screening cotton EST sequences with high homology to Arabidopsis SMT2 gene.

The amino acid sequence of SMT2 (NP173458) was found in the GenBank database and was used as a probe to screen the cotton EST database in the GenBank. The cotton ESTs with high sequence homology to *Arabidopsis SMT2* were identified using the BLAST (Basic Local Alignment Search Tool) program (http://www.ncbi.nlm.nih.gov/blast).

Cloning the full length of cDNA of the GhSMT2-1 and GhSMT2-2 genes

The candidate EST sequences identified were subjected to contig analysis with the SeqMan program (DNAStar, Madison, WI, USA). BLASTX analysis (http://www.ncbi.nlm.nih.gov/blast) was performed to determine the puta-

Download English Version:

https://daneshyari.com/en/article/2787978

Download Persian Version:

https://daneshyari.com/article/2787978

Daneshyari.com