Molecular cloning and characterization of the actin-depolymerizing factor gene in *Gossypium barbadense*

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The Sea Island cotton (*Gossypium barbadense* L.) has been highly valued in verticillium wilt resistance and many fiber qualities including fiber length, strength and fineness. To identify whether it had some special genes in fiber development in comparison with the Upland cotton (*G. hirsutum* L.), an actin-depolymerizing factor (ADF) gene was cloned and characterized in this research. A 420 bp open reading frame of the cloned gene, named *GbADF1*, encoded a protein of 139 amino acids, including 39.57% nonpolar amino acids, 17.27% acidic amino acids, 15.83% basic amino acids and 31.92% hydrophobic amino acids. Its molecular weight was about 15 kDa, and pI 5.04. *GbADF1* contained two conserved domains, 6-Ser and the PIP2/actin binding site. Its amino acid sequence was similar to the ADF/cofilin family of other plants. Compared with cDNA sequence, the *GbADF1* gene contained one intron near the 3' end in genomic sequence. Semi-quantitative RT-PCR result showed that *GbADF1* was a constitutive expression gene in cotton, and higher expression level was detected in fibers than in trophic tissues. The *GbADF1* was successfully expressed as a fusion protein in *Escherichia coli* BL21 (DE3). The molecular weight was firstly calculated by SDS-PAGE. Western blotting analysis confirmed the existence of a protein corresponding to *GbADF1*. The structure of *GbADF1* was different from that of other ADF genes in higher plant, although the coding sequences of all cloned ADFs were highly conserved.

Key words: ADF (actin-depolymerizing factor), semi-quantitative RT-PCR, Sea Island cotton, western blotting

INTRODUCTION

The plant actin cytoskeleton is known to play key roles in morphogenesis and function of highly specialized cell types, such as root hairs, trichomes (leaf hairs), pollen tubes and stomatal guard cells. Multiple actin binding proteins, such as actin-depolymerizing factors (ADF)/cofilins, profilins etc., regulate actin dynamics by maintaining the optimum equilibrium between unpolymerized actin molecules (G-actin) and assembled actin filaments (F-actin) in different cellular processes (Hussey et al., 2006). ADF/cofilin family, highly conserved in eukaryotic cells, is a large ubiquitous family with low molecular

mass (15–22 kDa) (Staiger et al., 1997). In eukaryotic cells, the ADF/cofilin family is an essential group of actin regulatory proteins that enhance the turnover of G-actin and F-actin by regulating the rate constants of dual functions, not only promoting the depolymerization, but also accelerating the polymerization of F-actin under certain conditions (Lappalainen and Drubin, 1997; Bamburg et al., 1999; Gibbon, 2001). ADF is more potent in depolymerization than cofilin (Giuliano et al., 1988).

In animals and yeast systems, genes encoding ADF/cofilins have been cloned and identified from a variety of organisms (Iida et al., 1993; Moon et al., 1993; Aizawa et al., 1995; Gunsalus et al., 1995). In higher plant, cDNAs encoding ADFs have been cloned and characterized from *Lilium longiflorum* (Kim et al., 1993), *Brassica napus* (Kim et al., 1993), *Zea mays* (Kim et al., 1993; Rozycka et al., 1995; Lopez et al., 1996; Hussey et al., 1998),

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Arabidopsis (Dong et al., 2001a; 2001b) and *G. hirsutum* (Zhang et al., 2006, 2007). Overexpression of *AtADF1* caused irregular tissue morphogenesis and reduced the growth of cells and organs in *Arabidopsis* (Dong et al., 2001a). Overexpression of *AtADF1*, *AtADF5* and *AtADF6* in different types of plant cells reorganized the actin cytoskeleton and reduced the length and number of filamentous actin structures (Dong et al., 2001b). The heterogenous expression of *AtADF4* gene in tobacco resulted in wavy hypocotyls of transgenic plants, especially in darkness (Peng and Huang, 2006). In contrast, reduced *AtADFs* expression promoted the formation of actin cables, stimulated cell expansion as well as organ growth, and resulted in a delay in *Arabidopsis* flowering (Dong et al., 2001a).

Although *ADFs* in *Arabidopsis* had been well characterized, little such research was done about cotton *ADFs*. In cotton, eight *GhADFs* with completed coding sequences could be found in NCBI (the National Center for Biotechnology Information). *GhADF1* shared 100% identity with *GhADF8* in amino acids, but there were only sequences in NCBI and no more other information. Four of *GhADFs* were isolated from cotton fiber cDNA library and analyzed by the molecular evolutionary relationship (Zhang et al., 2007). *GhADF2* and *GhADF3* all encoded 139 amino acids, and shared 99% identity with each other. *GhADF4* deduced a protein with 143 amino acids and was closely related to *AtADF6*. *GhADF5* encoded a protein of 141 amino acids and was closely related to *AtADF5*. RT-PCR analysis showed that *GhADF2* was predominantly expressed in fiber, *GhADF3* and *GhADF4* in each tissue, and *GhADF5* mainly in cotyledons. Expression levels of different *GhADFs* showed a discrepancy in the same cell type (Zhang et al., 2007), suggesting that they might be involved in regulating the development of cotton fiber.

In this study, we want to identify and characterize the *GbADF* involved in fiber development of *G. barbadense*. As far as we are aware, this work represents the first published *ADFs* study of Sea Island cotton.

MATERIALS AND METHODS

Cotton materials The Sea Island cotton (cv. Pima 90-53) was planted in a field. Developing bolls were tagged from the starting day post anthesis (DPA) on. All developing fibers of 10–20 DPA were removed from the seeds of the tagged bolls. Cotyledons, hypocotyls and roots were collected from two-week-old young seedlings. The fibers and the collected tissues were immediately frozen in liquid nitrogen for extraction of nucleic acids.

Isolation and purification of cotton DNA and total RNA The genomic DNA (gDNA) of Pima90-53 was extracted from plantlets with two cotyledons according to

the published protocol (Paterson et al., 1993). Total RNA was extracted from fibers of 10–20 DPA, roots, cotyledons and hypocotyls with PlantRNA reagent (Tiagen, China) according to the manufacture's instruction, respectively. The isolated RNA was treated by RNase-free DNase I according to manufacture's protocol to remove any potential gDNA contamination. The purity and concentration of the total RNA were determined using gel electrophoresis and Beckman spectrophotometer.

Isolation of the full-length coding sequence encoding *GbADF* from cotton Bioinformatics methods and the RT-PCR procedure were combined to clone the full-length open reading frame (ORF) of *GbADF1* from Pima90-53. First, an EST fragment of 511 bp homologous to *ADF* gene was identified by sequencing 42 PCR products from cotton fiber. Four cotton ESTs, DW500081, DW482538, DW482537 and DR459201, were found in NCBI. A 729 bp sequence was assembled via *in silico* cloning. According to the sequence, full length ORF was amplified and sequenced. The primers PF: 5'-ATGGCA-AACGCGGCTTCAG-3' and PR: 5'-TCAATTGGCACG-GCTCCTTATG-3' were designed. Then the PCR was run as follows: one cycle of 1 min at 95°C, 30 cycles of 1 min at 94°C, 1 min at 58°C, 1.5 min at 72°C, and a final extension of 10 min at 72°C, held at 10°C. The *Taqplus* DNA polymerase (Tiagen, China) was used for fidelity. The products from RT-PCR of cDNA and PCR of gDNA were cloned into the pMD18-T (TaKaRa, China), and nine clones were sequenced. The PCR amplification of gDNA was performed with PF and RP in *G. hirsutum* and *G. arboreum*, respectively, and the products were cloned and sequenced.

Bioinformatics analysis The DNA sequences were analyzed with DNASTAR software and the BLAST program (<http://ncbi.nlm.nih.gov>). Conserved domain, PROSITE motifs and CAM (calmodulin) binding site were analyzed with NCBI cds Blast (<http://www.ncbi.nlm.nih.gov/structure/>), ProDom (<http://motif.genome.jp/motif-bin/>) and CTDB (<http://calcium.uhnres.utoronto.ca>), respectively. The ADF protein sequences of different species used for the construction of a phylogenetic tree were downloaded from GenBank. Homological comparison was made using ClustalW software. The phylogenetic tree was constructed with Treeview software and the DRAWGRAM program in PHYLIP (<http://evolution.genetics.washington.edu/phyliip.html>).

Gene expression analysis First-strand cDNA was synthesized from 5 µg of total RNA using the StrataScript Kit (Stratagen, USA). The congruent first strand cDNA was used for PCR amplification of *GbADF1* ORF with gene-specific primers, PR and PF. The parameters were: 95°C for 1 min, followed by 23 cycles of 94°C for 1 min, 58°C

Expression of the recombinant protein For expression of the *GbADF1*, the coding region was inserted into the *Hind*III and *Eco*RI sites of vector pET-41a(+) (Novagen, Germany) by intermediate plasmid pMD-GbADF1. The expression of the recombinant protein in *E. coli* BL21 (DE3) with the induction of 1 mM IPTG and 2% glucose after 4 hr at 28°C by SDS-PAGE electrophoresis and stained with Coomassie Brilliant Blue R-250.

Western blotting Protein samples, including 25 μ g of total induced protein with negative control *E. coli* cell harboring no any plasmid, *E. coli* cell harboring pET-41a(+) and *E. coli* cell harboring pET-GbADF1, were separated by SDS-PAGE, and then visualized by Coomassie blue staining. The protein gel was transferred to a PVDF membrane (Millipore) by using a Trans-Blot SD semi-dry electrophoretic transfer cell (BIO-RAD) for immunoblotting, with 1–2 mA/cm² invariable electric current for 1 hr. Membrane was blocked with western blot blocking buffer (Tiangen, China) for 1 hr at room temperature with constant agitation. The Anti-His Antibody (Tiangen, China) was diluted with 1:10000 in fresh blocking buffer. Then the membrane was incubated in the primary antibody solution for 1 hr at room temperature with constant agitation. Excess antibody was removed by washing in PBS containing 0.1% Tween-20. The horseradish peroxidase-conjugated anti-rat IgG antibody

Fig. 1. Nucleotide sequence of *GbADF1* from gDNA and its deduced amino acid sequence. The small letters were untranslated sequence, and the capital letters were coding sequence. The green and red letters indicated initiation and termination codons, respectively.

(Tiangen, China) was diluted with 1:4000 in fresh blocking buffer for 1–2 hr at room temperature with constant agitation. After washing the excess antibody, proteins were detected with an enhanced HRP-DAB chromogenic substrate kit (Tiangen, China). Experiments were repeated at least twice using independently prepared samples.

RESULTS

Sequence characterization and gene structure of *GbADF1* An 881 bp gDNA of *GbADF1* was obtained from *G. barbadense*. The nucleotide sequence and deduced amino-acid sequence of this gene were shown in Fig. 1. A 420 bp ORF encoded a protein of 139 amino acids, including 39.57% nonpolar amino acids, 17.27% acidic amino acids, 15.83% basic amino acids and 31.92% hydrophobic amino acids. The pI was 5.04. A 504 bp fragment was obtained with gDNA by using gene-specific primers. A comparison of the 504 bp fragment and the ORF indicated that the *GbADF1* contained an 84 bp intron near the 3' end from 287 bp to 371 bp.

The 420 bp ORF was also obtained from two cotton species, *G. hirsutum* and *G. arboreum*. But there was no

intron in the ORF from gDNA of *G. hirsutum* and *G. arboreum*. Therefore, the gene structure of *GbADF1* differed from those of the two cotton species and the other reported ADFs in higher plant (Fig. 2).

High homology of *GbADF1* The possible conserved domains were shown in Fig. 3, which indicated that the deduced amino acid sequence included a phosphorylation site, 6-Ser, and a PIP2 binding site. Analysis of CAM potential binding sites indicated that possible CAM combining region was at the N terminus. There were five homogeneous amino acids substitutions (Leu46/Ile/Val/Thr, Ile50/Thr/Gly/Lys/Glu/Asn, Asp77/Ala/Ser/Val/Lys/Asn/Thr, Asp130/Gly/Ser and Ile134/Phe/Val/Leu) in the 20 ADFs of higher plant, which might effect on their structures and functions. In fact, there were only three homogeneous replacements (Asp93/Ser/Thr, Thr94/Val/Ala and Ala99/Ser) in the PIP2/actin binding site in ADFs of cotton. Different ADFs from higher plant had 76.6% sequence homology (data not shown). *GbADF1* was highly homologous to *AtADF1* with 87.8% amino acids identity and shared the highest homology (99% identity) with *GhADF1*, and only one amino acid difference, Ile50/Phe. ADFs in cotton had high homology from 56% to

Fig. 2. Position and size of introns in ADF genes. *GbADF1*, *GhADF1* and *GaADF1* were described in this study. The sequence of the *GhADF7* was described before (Zhang et al., 2006). The sequences of the *AtADF1*, *AtADF5*, *AtADF6* encoded *Arabidopsis* ADF (Dong et al., 2001b). The blue box indicated the exon, and the black line indicated the intron. The number of amino acid on the box meant the intron cleavage site. The red number under the black line meant the size of intron. The ATG meant the initiation codon and the TAA, TGA, TAG meant the termination codon. The total number of amino acids of each ORF was in the right of the box.

GhADF1	MANAAS*GMVHDDCKLKFL [#] ELKAKRTYRFIVFK	33
GhADF8	MANAASGMVHDDCKLKFL [#] ELKAKRTYRFIVFK	33
GhADF1	MANAASGMVHDDCKLKFL [#] ELKAKRTYRFIVFK	33
GhADF2	MANAASGMVHDDCKLKFL [#] ELKAKRThRFIVFK	33
GhADF3	MANAASGMVHDDCKLKFL [#] ELKtKRThRFIVFK	33
GhADF4	...msfrggNAS [*] SGMgVaehsKstyleLELqrKkvfRyviFK	37
GhADF5	mafkmAttGMwVaDeCKnsFmEmKwKkvhRyIVFK	35
GhADF6	MANAASGMVHDDCKLKFL [#] ELKAKRThRFIVFK	33
GhADF7	MANsASGMVnDeCKtKFL [#] ELKAKRnYRFIVFK	33
LiADF	MANssSGMAVdDeCKLKFL [#] ELKAKRnFRFIVFK	33
AtADF2	MANAASGMVHDDCKLKFL [#] ELKAKRTfRtIVyK	33
ZmADF1	MANssSGlAvnDeCKvKFR [#] ELKsRtRtRtRtIVFr	33
PeADF1	MANAASGMVHDDCKLrFL [#] ELKAKRThRFIVyK	33
NtADF1	MANAvSGMAVqDeCKLKFL [#] ELKtKRnYRFIIFK	33
NtADF2	MANAASGMVIdDeCKLKFL [#] ELKAKRnYRFIVFK	33
AtADF6	msfrglserpNAiSGMgVaDesKttFL [#] ELqrKkThRyvVFK	40
Consensus	g v k e K r	
GhADF1	IEE..KQKQVVVEKVGEPIDSYEAFTASLPAD [*] ECRYAVYD	71
GhADF8	IEE..KQKQVVVEKVGEPtD [*] SYEAFTASLPAD [*] ECRYAVYD	71
GhADF1	IEE..KQKQVVVEKVGEPtD [*] SYEAFTASLPAD [*] ECRYAVYD	71
GhADF2	IEE..KQKQViVEKlGEpteSYEdFTkclPAD [*] ECRYAVYD	71
GhADF3	IEE..KQKQViVEKlGEpteSYEdFTkclPAD [*] ECRYAVYD	71
GhADF4	IdEkkKeviVekiggptesyddfaaslpesdcryavvdfD	77
GhADF5	IdEksKlvtVdkvggagessyddftaslpdddcryavvdfD	75
GhADF6	IEE..KQKQViVEKlGEpteSYEdFTkclPAD [*] ECRYAVYD	71
GhADF7	IEE..nlqQiVVEKVGaPKDSYEklcsSLPsDEC [*] RYAVYD	71
LiADF	IEEkvqQv..tVerlGqPneSYddFTecLPpneCR [*] YAVfD	71
AtADF2	IEd...KQViVEKlGEPeqSYddFaASLPAD [*] dCRYciYD	69
ZmADF1	Idd...tdmeikVdrLGEpnnqYGdFTdSLPAnECRYAiYD	71
PeADF1	IEE..KQKQVVVEKlGEpteSYEdFaASLPenECRYAVYD	71
NtADF1	Idg...qev..VVEKlGsPeesSYEdFanSLPAD [*] ECRYAVfD	69
NtADF2	IEg...qQv..VVEKlGnPeenYddFTnSLPAD [*] ECRYAVfD	69
AtADF6	IdEskKevvVektgnptesyddflasl [*] lpdndcryavvdfD	80
Consensus	i d	
GhADF1	FDFVTDENCQKSRIFFIAWSPDTSKVRSKMIYASSKDRFK	111
GhADF8	FDFVTDENCQKSRIFFIAWSPDTSKVRSKMIYASSKDRFK	111
GhADF1	FDFVTDENCQKSRIFFIAWSPDTSKVRSKMIYASSKDRFK	111
GhADF2	FDFlTaENvpKSRIFFIAWSPDTsriRSKMIYASSKDRFK	111
GhADF3	FDFlTaENvpKSRIFFIAWSPDTsriRSKMIYASSKDRFK	111
GhADF4	FvtsencqksKiffiawspSvsrirskmlyatskdrfRre	117
GhADF5	FvtVdnckrksKiffiawsp [*] tasrirakmlyatskdglRrv	115
GhADF6	FDFlTaENvpKSRIFFIAWSPDTsriRSKMIYASSKDRFK	111
GhADF7	FDFtTDENCQKSkIFFIAWSPDTsrVRSKMIYASSKDRFr	111
LiADF	FDFVTDENCQKSkIFFIsWSPDTsrVRSKMIYASStKDRFK	111
AtADF2	FDFVtAENCQKSkIFFIAWSPDTaKVRdKMIYASSKDRFK	109
ZmADF1	lDfTtTiENCQKSkIFFfsWSPDtArtRSKMIYASSKDRFr	111
PeADF1	FDFVtAENCQKSRIFFIAWcPDtArVRSKMIYASSKDRFK	111
NtADF1	lDfiThENCQKSkIFFIAWSPeTsrvRmKMvYASSKDRFK	109
NtADF2	FDFiTTENCQKSkIFFIAWSPDTSKVRmKMvYASSKDRFK	109
AtADF6	FvtsencqksKiffFswspStspvrakvlystskdqlske	120
Consensus	k	
GhADF1	RELDGIQVE LQATD PSEMDLDVIRSRAN	139
GhADF8	RELDGIQVE LQATD PSEMDLDVIRSRAN	139
GhADF1	RELDGIQVE LQATD PSEMDLDVIRSRAN	139
GhADF2	RELDGIQVE LQATD PTEMgLDVfksRAN	139
GhADF3	RELDGIQVE LQATD PTEMgLDVfksRAN	139
GhADF4	lEgihyeiqatdp [*] temdlevireRah	143
GhADF5	ldgihyeVqatdp [*] temgMDvikhkay	141
GhADF6	RELDGIQVE LQATD PTEMgLDVfksRAN	139
GhADF7	RELDGvQVE LQATD PSEMSfDivkeRaf	139
LiADF	RELDGIQVELQATDPSEMSmDiIkARaf	139
AtADF2	RELDGIQVE LQATD PTEMgLDVfksRtN	137
ZmADF1	RELDGIQcEiQATD PSEMSLdivRSRtN	139
PeADF1	RELDGIQVE LQAcD PTEMgLDVIqSRAN	139
NtADF1	RELDGIQVE LQATD PSEMSfDivkaRAY	137
NtADF2	RELDGIQVE LQATD PSEMSfDiIkSRAl	137
AtADF6	lqgihyeiqatdp [*] tevdlevlreRan	146
Consensus		

Fig. 3. Amino acid sequence alignment of the GhADF1. The asterisk indicated the position of the putative phosphorylation site at the N-terminus. The well symbol indicated the position of the putative nuclear binding site. The point indicated the position of the putative special F-actin binding site. The red rectangle and blue line showed the PIP2/actin binding site and the possible CAM combining region, respectively. Accession numbers: GhADF1 (AAV88048), GhADF2 (ABD63906), GhADF3 (ABD66505), GhADF4 (ABD66506), GhADF5 (ABD66507), GhADF6 (ABD66508), GhADF7 (ABD66503), GhADF8 (ABD66504), AtADF1 (NP_190187), AtADF2 (NP_566882), AtADF3 (NP_851227), AtADF4 (NP_851228), AtADF5 (NP_565390), AtADF6 (AAM63510), PeADF1 (AAK72617), PeADF2 (AAK72616), NtADF1 (AAL91666), NtADF2 (AAL91667), ZmADF1 (P46251).

100% (data not shown).

To study the phylogeny of the ADFs, the complete protein sequences were aligned, and the phylogenetic tree was generated by the neighbor-joining method using 25 ADFs including GbADF1 (Fig. 4). The tree indicated that the 25 ADFs in plants, including all 11 *Arabidopsis* ADFs, subclass-representative rice (*Oryza sativa*) and all 9 *Gossypium* ADFs. They were assembled using clustralW, and the protein destrin from human (DEST1_human, replaced by DEST_Pig in 2005) was a outgroup. The plant ADF proteins were grouped into four subclasses according to the output tree. The dicot cotton and the monocot rice were ranged in each subclass. The data suggested that the three plant spe-

cies had evolved with a large pre-existing repertoire of ADFs. The GbADF1 and AtADF1,-2,-3,-4 were clustered in the subclass I, which expressed constitutively in all vegetative and reproductive tissues except pollen (Ruzicka et al., 2007).

Expression profiles of *GbADF1* in different fiber developmental stage and multi-tissues To determine the expression pattern of *GbADF1*, semi-quantitative RT-PCR analysis was performed. The results showed that all tissues contained *GbADF1* mRNA. Higher level was detected in fiber tissues than in trophic tissues (Fig. 5). This revealed that *GbADF1* was a constitutive expression gene in cotton, and the expression levels might

Fig. 4. ADF phylogeny. Neighbor-joining protein sequence phylogeny showed all 11 AtADFs, all nine ADF proteins from *Gossypium* and representatives from *Oryza*. Some accession numbers were the same as Fig. 3, and other accession numbers were as follows: AtADF7(AAQ65136), AtADF8(NP_567182), AtADF9(NP_195223), AtADF10(NP_568769), AtADF11(NP_171680), OsADF1(Q6EUH7), OsADF2(Q9AY76), OsADF5(Q10P87), OsADF6(Q7SXN9), OsADF7(Q0DLA3), DEST1_human(P60982). The Arab numbers meant the bootstrap values. Subclasses I to IV were indicated by vertical bars on the right.

Fig. 5. Semi-quantitative RT-PCR analysis of *GbADF1* expressed in different tissues and fiber developing stages. 20: DPA20, 15: DPA15, 10: DPA10, R: root, H: hypocotyls, C: cotyledon. Ubiquitin: the internal control for RT-PCR.

Fig. 6. Expression of the recombinant *GbADF1* in *E. coli*. M: ladder marker; 1 and 4: control *E. coli* cell harboring no plasmid; 2 and 5: *E. coli* cell harboring pET-*GbADF1*. The white arrow indicated the position of the *GbADF1* recombinant protein. 3 and 6: *E. coli* cell harboring pET-41a(+). The white arrow indicated the position of the pET-41 tag protein. Proteins were separated by SDS-PAGE and stained with Coomassie Brilliant Blue. 0 hr meant the non-induced cell. 4 hr meant that the cell was produced with IPTG for 4 hr.

vary in trophic tissues and fiber tissues.

Prokaryotic expression the *GbADF1* protein So far, no cotton ADFs was expressed in *E. coli*. Compared with the control of non plasmid *E. coli* cell and harboring pET-41a(+), the *E. coli* cell harboring pET-*GbADF1* could be induced by IPTG, and the recombinant His-tagged and GST-tagged *GbADF1* was expressed (Fig. 6). The recombinant *GbADF1* was approximately 50 kDa. The protein before the multiple cloning sites, including His and GST was about 35.6 kDa. Thus, *GbADF1* was a ~15 kDa peptide.

Western blotting analysis No bands were discovered in the negative control *E. coli* cell harboring no plasmid by using western blotting. About 36 kDa protein from *E. coli* cell harboring pET-41a(+) expressed tag had been tested by His-tag antibody. The recombinant protein of about 50 kDa was present at substantial levels in *E. coli* cell harboring pET-*GbADF1* (Fig. 7). These data con-

Fig. 7. Western blotting analysis of *GbADF1* expression in *E. coli*. BL21: negative control *E. coli* cell harboring no plasmid; pET-*GbADF1*: *E. coli* cell harboring pET-*GbADF1*; pET41a(+): *E. coli* cell harboring pET-41a(+); M: protein molecular marker.

firmed the existence of a protein corresponding to GbADF1.

DISCUSSION

In this study, *GbADF1* with a 420 bp ORF was cloned and characterized. The gene encoded a protein of 139 amino acids with two conserved domains, 6-Ser and the PIP2/actin binding site. Its amino acid sequence was similar to that of the ADF/cofilin family in other plants. The gene structure of *GbADF1* differed from that of the other reported ADFs in higher plant. An 84 bp intron existed at the beginning of the PIP2/actin binding site from 287 bp to 371 bp in gDNA. Semi-quantitative RT-PCR showed higher levels *GbADF1* in fibers than in trophic tissues, suggesting that it might be involved in regulating the development of cotton fiber.

None of cotton ADFs was expressed in *E. coli* in previous researches. In this study, the results from western blotting and the expression in prokaryote confirmed the existence of a protein corresponding to GbADF1, and the GbADF1 protein would be ~15 kDa. The real molecular weight of GbADF1 was just in the range of theoretically deduced molecular weight, 15–22 kDa, in ADF family (Staiger et al., 1997).

The structure of *GhADF7* showed two introns existed as found in the most *AtADF*s (Zhang et al., 2006). But no more structure reports of *GhADF*s were found. In this study, the ORFs of ADF were cloned from other two cotton species, *G. hirsutum* and *G. arboreum*, respectively. There were no introns in the ADFs of the two cotton species. As far as can currently be determined, plant ADF/cofilin genes are organized in a similar manner, with an intron following the exon encoding the amino terminus and a conserved intron further 3'. This pattern held for *Arabidopsis* and *Oryza sativa* ADF/cofilin genes (Maciver and Hussey, 2002). The structure of *GbADF1* was different from this manner. *GbADF1* had only one intron near the 3' end just before the α -helix 3 which had been researched in comparative structural analysis of the ADF/cofilin family (Bowman et al., 2000). And the intron of *GbADF1* was at the beginning of the PIP2/actin binding site, which indicated that there were some differences on the ontogeny and evolution.

As we know, actin is a major, dynamic component of the plant cytoskeleton. The plant actin gene family could be divided into two major classes, a vegetative class and a reproductive class (Mun et al., 2000). A clear specialization between vegetative and reproductive isovariants had also been observed in profilin (Staiger et al., 1997). The thorough analysis of the ADF gene family in *Arabidopsis* was done by tissue-specific expression patterns and sub-cellular localization (Ruzicka et al., 2007). The phylogenetic tree indicated that the two ancestral ADF genes, vegetative-expressed and pollen-expressed genes, had

acquired their tissue specificity before monocot-dicot divergence, which was also supported by this research. *Gossypium*, *Arabidopsis* and *Oryza sativa* evolved with a large pre-existing repertoire of ADFs. And the data also indicated that the ADFs had important functions in flowering plants. Just as the phylogenetic tree described, *GbADF1* was constitutive expression gene in cotton, and the GbADF1 belonged to subclass I, which was classified into the predominant vegetative group of ADF isovariants in the plant in the four subclasses (Ruzicka et al., 2007). The promoter analysis would be done to discuss expression profile of *GbADF1*. Otherwise, ADF was more potent in depolymerization than cofilin (Giuliano et al., 1988). The function in cotton fiber development would require more detailed research.

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