盐胁迫下棉属野生种旱地棉(*Gossypium aridum*)差异表达基因的cDNA-AFLP分析

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摘要:以一个耐盐的二倍体野生种旱地棉和对盐敏感的陆地棉栽培种苏棉12号为材料,运用cDNA-AFLP技术,比较两个材料分别在盐胁迫前后的表达情况,获得了25个仅在旱地棉盐胁迫下特异表达的转录片段(TDF)。将这些片段进行电子克隆,延伸后的序列进行BLAST分析,结果显示23个转录片段推断的氨基酸序列与已知的蛋白同源,这些盐诱导表达的基因主要涉及离子转运、活性氧清除、细胞信号传导、细胞分裂、转录调节、膜保护、渗透调节等功能蛋白。从23个差异表达的转录片段中选择9个进行实时定量PCR(qRT-PCR)分析,结果表明这些基因在盐胁迫后表达显著增强,而且多数在12~24 h达到高峰。这些cDNA克隆是开展棉花耐盐性分子基础研究的重要资源。

关键词:棉属;盐胁迫;cDNA-AFLP;转录片段(TDFs);实时定量PCR (qRT-PCR)

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cDNA-AFLP Analysis of Differentially-Expressed Genes in Response to Salt Stress in *Gosspyium aridum*

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Abstract: In this study, we used a salt-tolerant diploid *G. aridum* species and the salt-sensitive *G. hirsutum* cultivar Sumian 12 to investigate differential expression in the presence of salt stress. Using cDNA-AFLP, 25 transcript-derived fragments(TDFs) were isolated and confirmed to be present only in salt tolerant species *G. aridum* under salt-stressed conditions. BLAST analysis with sequences assembled using an *in-silico* approach demonstrated that 23 of the cDNA fragments had homology to known proteins. The up-regulated genes were mainly involved in ion transport, ROS scavenging, cell signaling, cell division, transcription regulation, membrane protection, and penetration regulation. Quantitative real-time PCR(qRT-PCR) was used to analyze the expression patterns of nine of the 23 TDFs at different stages of salt stress in the tolerant species *G. aridum*. Their expression showed a significant increase and reached a peak between 12 – 24 h of stress. These TDFs may serve as useful resources for future research on molecular mechanisms of salt stress response in cotton.

Key word: *Gossypium*; salt stress; cDNA-AFLP; transcript-derived fragments (TDFs); quantitative real-time PCR (qRT-PCR)

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Salinity and drought are the main abiotic stresses severely limiting plant growth and development, and salt tolerance is consequently one of the important areas of research in plant science. Over time, plants have evolved unique salt tolerance mechanisms, which can be broadly classified as ion homeostasis, osmotic homeostasis, stress damage control and repair, and growth regulation[1]. Plant

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exposure to saline environments leads to many physiological, biochemical, and molecular alterations that involve massive changes in gene expression profiles. Transcript analysis allows simultaneous detection of multiple gene expression changes and enables the elucidation of salt tolerance mechanisms, with the ultimate goal of improving crop productivity in saline soil.

Cotton, the world's leading fiber source, is a very important global cash crop. Domestic cotton is an ideal plant for research on the molecular basis of plant responses to water deficit and salinity, as it is derived from wild perennials adapted to semi-arid sub-tropical environments associated with highly saline soils and experiencing periodic drought and temperature extremes[2]. The geographical distributions of wild amphidiploids are on or near the path of prevailing currents in both the Pacific and Atlantic oceans. They have floated to their current locations or been carried there, intentionally or accidentally, by human beings. This long-distance dispersal via oceanic drift has played an important role not only in diversification of major evolutionary lines, but also in speciation within *Gossypium* lineages. Salt water tolerance of wild species' seeds enabled this long-distance dispersal[3]. In contrast, modern cotton cultivars are the result of intensive selection often carried out under unstressed conditions to produce large amounts of specific fiber types. Selection has thus unintentionally narrowed the genetic variability for salt tolerance. In one study, more than 3000 upland cotton lines were screened for salt tolerance, but only 3 lines tolerant to 0.4% NaCl stress during the seedling stage were identified[4]. To improve salt tolerance in upland cotton, exploitation of valuable alleles from wild *Gossypium* species is therefore needed.

Gossypium aridum is a D genome diploid native to the Pacific coast of Mexico. Although many wild *Gossypium* species are becoming extremely rare due to the disappearance of *in situ* area, a recent expedition report from American scientists indicates that *G. aridum* does not appear to be threat ened[5]. This favorable situation is most probably due to its high genetic diversity, which arises from its resistance to abiotic stresses, including drought and salinity. Research on seed viability of coastal and inland forms of *Gossypium* has shown that the seeds of most coastal ecotypes are remarkably toler ant to salt water immersion[6]. In seed germination experiments in our own lab, we have observed seed germination rates as high as 92% for *G. aridum* in 1.2% NaCl solution(data not shown). All this information suggests that *G. aridum* is a valuable species for understanding salt tolerance mechanisms in *Gossypium* and improving salinity resistance in upland cotton.

Although several techniques, such as suppressive subtractive hybridization(SSH), cDNA microarrays, and RNA sequencing, are currently available for transcriptome analysis, the PCR-based technique of cDNA-amplified fragment length polymorphism (cDNA-AFLP) is still a valuable mRNA fingerprint method for isolation of differentially-expressed genes due to its high sensitivity and low cost[7-8]. The objective of this study was to develop transcripts that were differentially expressed in *G. aridum* leaves in response to salinity stress using cDNA-AFLP, and to validate changes in their expression patterns at different stress stages using real time PCR(qRT-PCR).

1 Materials and methods

1.1 Plant materials and salinity treatments

The *Gossypium* wild species *G. aridum*(D4) and the upland cotton cultivar Sumian 12 (*G. hirsutum*) were used for this study. In addition, *G. davidsonii*(D3-k), which belongs to the same subgenome as *G. aridum* and has salt tolerance potential[9], was used as a positive control for cDNA-AFLP analysis. Young uniform plants that were 20 cm tall were selected and treated with 300 mmol·L^{-1} NaCl for 24 h.

For qRT-PCR analysis, young *G. aridum* plants were treated with 200 mmol·L^{-1} NaCl. Leaves were collected at different intervals(unstressed, 1, 3, 6, 12, 24, and 72 h after stress). All tissues were frozen immediately in liquid nitrogen and stored at -70 ℃.

1.2 cDNA synthesis and cDNA-AFLP analysis

Total RNA was isolated from frozen tissues using a cold-acidic phenol method with a modified extraction buffer (10 mmol·L^{-1} Tris-HCl, pH 8.0; 25 mmol·L^{-1}, EDTA pH 8.0; 2% CTAB; 2% PVP). The isolated RNA was precipitated with ethanol, dissolved in DEPC water, and stored at -70 ℃. Using a Takara M-MLV RTase cDNA synthesis kit (Dalian, China), first strand synthesis was carried out with 20 μg of total RNA, which was then followed by second strand synthesis. Second strand cDNAs were purified by chloroform extraction and dissolved in 30 μL TE solution to give an approximate concentration of 100 ng·$μL^{-1}$. The double-stranded cDNA was digested with restriction enzymes *Mse*I/*Pst*I for 3 h at 37 ℃, and then ligated to *Mse*I and *Pst*I adapters using T_4 DNA ligase (Takara) for 12 h at 16 ℃. The adaptor sequences were as follows: *Pst*I adapter, 5'-CTCGTAGACTGCGTACATGCA-3', 3'-CATCTGACTGT-5'; *Mse*I adapter, 5'-GACGATGAGTCCTGAG-3', 3'-TACTCAGGACTCAT-5'. Ligated products were preamplified with the appropriate preamplification primers (*Pst*I: 5'-GACTGCGTACATGCAG-3', *Mse*I: 5'-GATGAGTCCTGAGTAAT-3'). The pre-amplified products were then diluted 20-fold and selectively amplified with 200 primer combinations. About 8 μL of 95 ℃ heat-denatured AFLP product was resolved on a 6% denaturing PAGE gel containing 7 mol·L^{-1} urea, and the resulting bands were visualized with silver staining.

1.3 Isolation of transcript-derived fragment (TDF)

Polymorphic TDFs, which were verified based on presence and absence of bands in the two tolerant wild species(*G. aridum* and *G. davidsonii*) compared against the sensitive upland cotton cultivar Sumian 12, were cut from the gel with a sharp razor blade, avoiding any contaminating fragment(s). The excised bands were eluted in 50 μL of sterile double distilled water at 95 ℃ for 15 min and hydrated overnight at 4 ℃. About 5 μL of the eluent was used as a template for re-amplification in a total volume of 25 μL PCR reaction mixture using the same set of corresponding selective primers and PCR conditions as follows: 95 ℃ denaturation for 3 min, followed by 36 cycles at 94 ℃ for 30 s, 56 ℃ for 30 s, and 72 ℃ for 1 min. After the last cycle, the amplification was extended for 10 min at 72 ℃. PCR products were resolved on a 2% TAE-agarose gel; each single band was isolated and eluted using an AxyGEN gel extraction kit(Axygen Scientific, USA).

1.4 Cloning, sequencing and sequence analysis of TDFs

Eluted TDFs were cloned into a plasmid pTG19-T vector(GENEray, Shanghai) following the manufacturer's protocol. Monoclones were sequenced by Beijing Genomics Institute(BGI) (Shanghai, China). TDF sequences were analyzed using NCBI BLAST (http://www.ncbi.nlm.nih.gov/BLAST). For some smaller TDFs ranging from 50 bp to 500 bp long, BLAST results were unsuccesful. We therefore assembled these sequences from related *Gossypium* ESTs using an *in silico* cloning strategy and CAP3 software; The assembled cDNA sequences were then analyzed and sequence-verified with *G. aridum* cDNA. The verified sequences were re-submitted for BLAST analysis.

1.5 Quantitative reverse transcriptase PCR (qRT-PCR) analysis

Total RNA was isolated from leaves at various time intervals up to 72 h of salt stress using the method described above. Gene-specific primers were designed using PRIMER3 (http://frodo.wi.mit.edu/primer3/input.htm)(Table 1). For qRT-PCR, assays were performed in triplicate on 1 μL aliquots

of each cDNA dilution using SYBR Green Master Mix(Applied Biosystems, Dalian) on an ABI 7500 sequence detection system(Applied Biosystems). PCR was performed for each sample in triplicate. UBQ, a constitutive protein, was used as an internal control to normalize all data (Forward primer: 5'-GAAGGCATTCCACCTGACCAAC-3'; Reverse primer: 5'-CTTGACCTTCTTCTTCTTGTGCTTG-3'). The relative quantification method (ΔΔCT) was used for quantitative evaluation of variation between replicates.

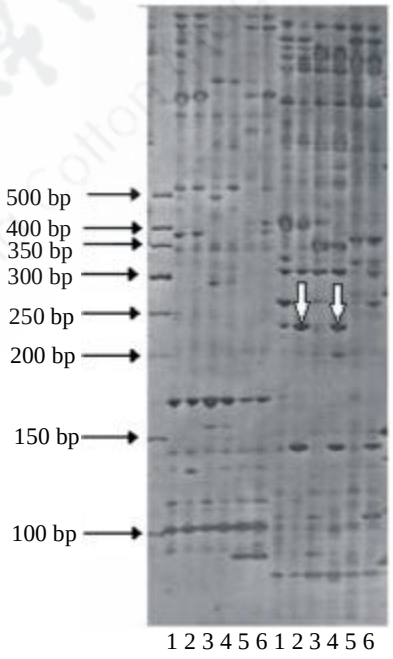
Table 1 Primers used for qRT-PCR

TDF	Forward primer (5'-3')	Reverse Primer (5'-3')	Size of amplicon /bp
TDF2	CGCTTACAAGACCAGTGGTGAGGAT	CGGGACTAGATGTTTCTGCAGCATT	296
TDF3	GCAGACTCGGTCTCGGTATGCTTAT	CCACTGCAGCAACATTGACTCTTAG	208
TDF8	GATTGTTCGAGCATCCGAGGACGAT	ACTGCTGCTGCAGCTGAATGTTACG	257
TDF10	CCAGACCAGCAGAGGCTTATCTTTG	AGCTGGTTGGTGTGTCCACACTTCT	245
TDF11	CCTTGCTGAGCTTGGCCTTATGCAA	CCAGGGCATTACGATCTGGACTTGA	263
TDF13	GGATCTCAGTACCTATACTAGATCG	ATCGAGTGGGAGACCATCCATGTAT	167
TDF18	GCTGTTGTGTCCATGCCTGCAGTAA	TGTTTCCATTGGAGGAGGAGGCTTT	223
TDF21	GAAGACAATGGAGACCGCAGAAGCA	GGCATCCGCCATTCCAAAGGTATGT	251
TDF24	GCTTAGCAATAGAATCGGCCTCACT	GCTGCACGGTTCAACATGCCAGAAA	243

2 Results

2.1 Identification of salinity-regulated TDFs

A total of 200 primer combinations were used for cDNA-AFLP analysis. Expression profiles were compared among the two salt-tolerant wild species and a sensitive variety under both control conditions and after 24 h of salt stress based on presence or absence of bands. Fragments greater than 50 bp long were considered for analysis. On average, about 40 clear bands(TDFs) were generated with each primer combination, yielding a total of about 8000 bands. A total of 25 TDFs were isolated from the silver-stained gels based on their presence in *G. aridum* and *G. davidsonii* under salt stressed conditions and absence under unstressed conditions, or, for *G. hirstrum* Sumian 12, their absence under both stressed and unstressed conditions(Fig. 1). The experiment for these 25 TDFs was repeated twice for reproducibility and verification of the data.



1=*G. aridum* before stress; 2=*G. aridum* after 12 h stress; 3=*G. davidsonii* before stress; 4=*G. davidsonii* after 12 h stress; 5=Sumian 12 before stress; 6=Sumian 12 after 12 h stress.

Fig. 1 A silver-stained cDNA-AFLP gel showing the differential expression of genes induced after 12 h of salt stress in two wild species(*G. aridum* and *G. davidsonii*) and a *G. hirsutum* variety(Sumian 12)

2.2 Sequence extension and analysis of TDF clones

All 25 identified TDFs were sequenced. The BLASTN program failed to locate homologous EST sequences or unigenes of some of the small TDFs in GenBank. An *in silico* strategy was thus used to assemble original TDFs with homologous *Gossypium* ESTs. Most were greatly extended, and some full-length genes were identified (TDF3, TDF4, TDF18, TDF22, TDF23, TDF24, and TDF25).

To verify consistency between assembled sequences and known *G. aridum* sequences, primers

from TDFs 2, 3, 8, 10, 11, 13, 18, 21, and 24 were designed based on assembled sequences and used to amplify corresponding sequences with *G. aridum* cDNA. Amplified products were sequenced as described above. The resulting match rates between assembled and verified sequences ranged from 90.3% to 100% (TDF2 95.58%, TDF3 99.26%, TDF8 97.16%, TDF10 95.24%, TDF11 97.74%, TDF13 90.3%, TDF18 98.37%, TDF21 100%, and TDF24 100%), indicating the *in silico* cloning strategy was an effective way to extend target sequences in *Gossypium*.

An NCBI database search revealed that 23 cDNA fragments had homology to known proteins, with their corresponding genes involved in responses to various stresses, such as drought, cold, and salinity(Table 2). Most of the TDFs and assembled fragments of the homologous genes were found to be involved in functions such as ion transport (TDF2), ROS scavenging(TDF23), cell signaling (TDF1, 7, 15, 25), cell division(TDF11, 17), transcription regulation(TDF19), protein metabolism

Table 2 Nucleotide-homology of TDFs with known gene sequences in GenBank using the BLASTN algorithm

TDF	Original size/bp	Assembled size/bp	Homologous gene	GenBank Accession Number	E-value	Similarity
TDF1*	158	164	Phospholipase D (PLD) (*Gossypium hirsutum*)	GU569955.1	9E-14	100%
TDF2	133	1475	MscS 2 like protein (*Arabidopsis thaliana*)	XP_002533682	1.7E-151	69.5%
TDF3 #	153	1311	Threonine aldolase (*Populus trichocarpa*)	XP_002325334	2.7E-161	88.1%
TDF4 #	131	1863	3-chloroallyl aldehyde dehydrogenase/ oxidoreductase (*Corylus heterophylla*)	ADW80331	0	88.8%
TDF5	91	1869	4-hydroxy-3-methylbut-2-en-1-yl diphosphate synthase (*Vitis vinifera*)	XP_002285130	0	95.15%
TDF6	95	95	No significant similarity			
TDF7*	175	660	WD-repeat protein GhTTG3 gene (*Gossypium hirsutum*)	AF530911.1	3E-05	41%
TDF8	131	1035	Beta-fructofuranosidase/hydrolase (*Citrus sinensis*)	BAF34362	7.8E-147	85.75%
TDF9	79	900	No significant similarity			
TDF10	111	258	Ubiquitin fusion protein (*Populus trichocarpa*)	ABK93568	1.0E-40	95.6%
TDF11	110	931	Cyclin B1 (*Populus trichocarpa*)	XP_002314016	3.6EE-81	80.75%
TDF12	106	1708	Serine-type endopeptidase/ serine-type peptidase (*Ricinus communis*)	XP_002515283	0	86.4%
TDF13	160	1050	ABC transporter B family member 26 (TAP1) (*Arabidopsis thaliana*)	NM_105729.5	1E-96	73%
TDF14	131	992	Carbonic anhydrase (*Gossypium hirsutum*)	AAM22683	8.0E-154	82.4%
TDF15	64	1423	Stress-induced receptor-like kinase (*Vitis vinifera*)	XP_002268775	9.2E-133	79.05%
TDF16	89	204	Early fruit mRNA (*Populus trichocarpa*)	XP_002324260	1.8E-5	77.75%
TDF17	54	192	Transitional endoplasmic reticulum ATPase (*Vitis vinifera*)	XP_002282146	2.6E-42	90.25%
TDF18#	272	1757	Cytochrome P450 (*Populus trichocarpa*)	XP_002308860	0	92.15%
TDF19	202	1860	Transcription factor LHY(*Populus nigra*)	BAH09382.1	3E-134	65%
TDF20	301	613	Alcohol oxidase (Populus trichocarpa)	XP_002314488	6.0E-81	80.35%
TDF21	139	411	Late embryogenesis abundant protein D-7 (*Gossypium hirsutum*)	CAA31589	2.4E-15	74.22%
TDF22#	125	684	Copper binding protein 6 (*Gossypium hirsutum*)	ADV57641	1E-89	100%
TDF23#	169	1159	Beta carotene hydroxylase (*Vitis vinifera*)	XP_002273581	2.8E-87	80.65%
TDF24#	121	765	RNA recognition motif-containing protein (*Populus trichocarpa*)	XP_002323849	1.3E-78	71.8%
TDF25#	139	1950	Armadillo/beta-catenin-like repeat-containing protein (*Arabidopsis thaliana*)	NP_001190236.1	1E-122	77.5%

Note: All data are BLASTN scores except those marked with * which are tBLASTx scores, full-length ORF obtained through assembling marked by #.

(TDF3, 5, 7, 10, 12), protein and membrane protection(TDF16, 21, 22, 24), and penetration regulation (TDF4, 8, 13, 18, 20). Only two TDFs(TDF6 and TDF9) had no significant similarity with known sequences or mapped to unclassified proteins with unknown function(Table 2).

2.3 TDF expression analysis

Of the 25 sequences, 9 assembled TDFs that had been confirmed with *G. aridum* cDNA were further analyzed for their expression patterns and their relative abundance in *G. aridum* leaves at different stress stages(0, 1, 3, 6, 12, 24, 48, and 72 h) was quantitatively assessed. The quantitative expression patterns for all 9 TDFs were similar in both cDNA-AFLP and qRT-PCR experiments (up-regulated in leaves under salt stress). The expression of most TDFs reached a peak at 12-24 h(Fig. 2).

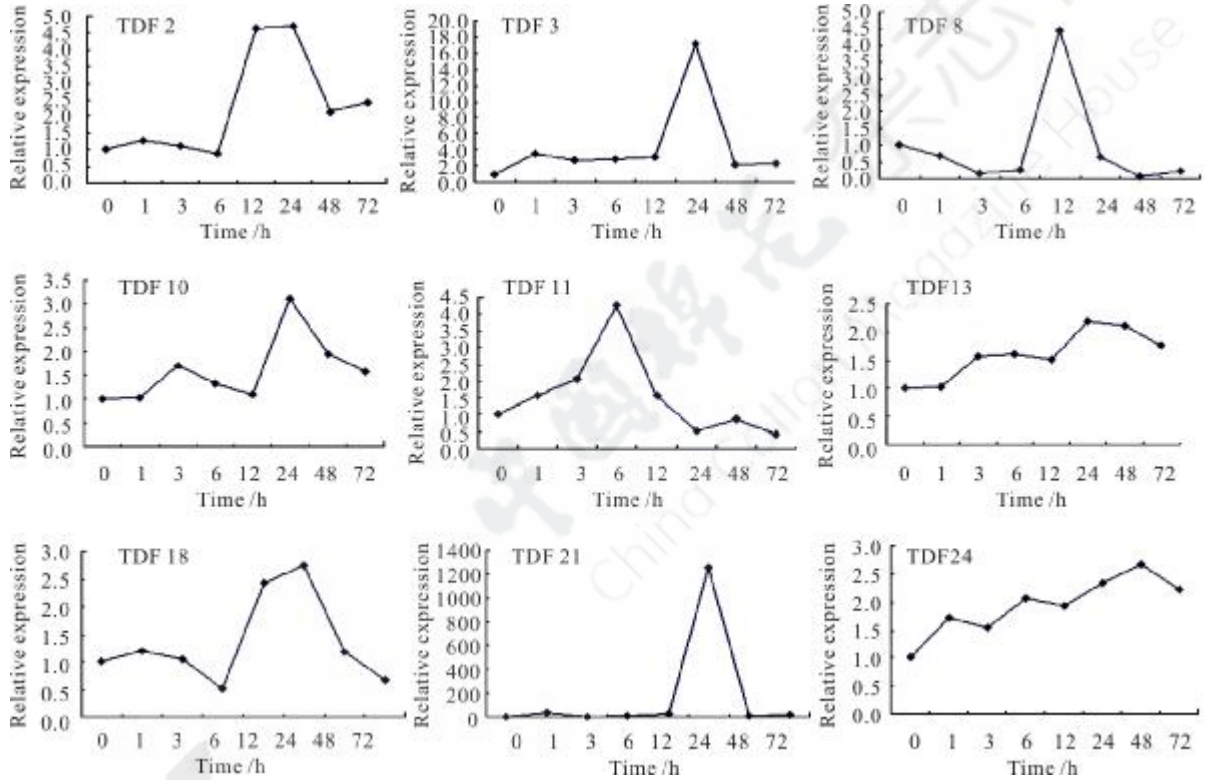


Fig. 2 qRT-PCR analysis of the expression patterns of 9 TDFs at different stress stages in *G. aridum*

TDF2 was homologous to *Arabidopsis* MscS-like genes *AtMSL*2 and *AtMSL*3. MscS-like genes are responsible for perceiving mechanical stimuli such as touch and osmotic pressure. In one study, *Arabidopsis* insertion mutants *MSL*3 and *MSL*2 showed abnormalities in plastid size and shape, and MSL2-GFP and MSL3-GFP, along with the plastid division protein AtMinE, were found to be localized to discrete foci on the plastid envelope[10]. Our qRT-PCR results showed that TDF2 expression increased significantly, peaking at 12-24 h before declining.

TDF3 showed similarity with threonine aldolase, which is an important enzyme splitting threonine into glycine and acetaldehyde. An investigation in *Arabidopsis* revealed that N-methyltransferase genes catalyze the transformation of glycine to betaine via a three-step methylation process, and demonstrated that glycine accumulation may play a role in plant salt stress response[11]. The qRT-PCR results in our study revealed that TDF3 expression was greatly induced after 12 h salt stress, with 17-fold overexpression at 24 h salt stress compared with the untreated control.

TDF8 was identified as soluble invertase, which can convert sucrose into glucose and fructose, used not only for plant growth and metabolism, but also for maintenance of cell osmotic pressure. Under drought stress during early devel

opment, repression of the soluble invertase gene leads to ovary abortion in *Zea mays*[12]. In our study, this gene was up-regulated 4.4-fold at 12 h of salt stress.

TDF10 was homologous to the *Arabidopsis* ubiquitin extension protein gene. Ubiquitination takes place in diverse cellular processes in higher plants, including differentiation, cell division, hormonal responses, and biotic and abiotic stress responses. In one study, *pub*22 and *pub*23 knockout mutants were more drought-tolerant than wild-type plants, with a *pub*22 *pub*23 double mutant display ing even greater drought tolerance[13]. These results indicated that PUB22 and PUB23 function as negative regulators in water stress response. Using differential hybridization technology, the *Arabidopsis* ubiquitin extension protein was one of three ERD (early-responsive to dehydration) proteins strongly induced by dehydration stress, but it was not significantly affected by ABA[14]. Plant ubiquitin extension protein thus appears to play an important role in dehydration stress through an ABA-independent pathway. According to our qRT-PCR results, it was 3-fold overexpressed at 24 h of salt stress.

TDF11 was homologous to the cyclin B gene, which is involved in cell mitosis. Overexpression of the *OsMYB3R*-2 gene, which specifically promotes the *OsCycB*1 gene, has been shown to increase proline content and cold tolerance in rice[15]. The CycB protein is thus seen to be important in plant stress resistance. We found that TDF11 expression was rapidly altered in response to salt stress, with 4-fold overexpression at 6 h.

TDF13 corresponded to the ATP-binding cas sette transporter protein gene, and, according to BLAST results, had highest homology with the *Arabidopsis thaliana* ABC transporter B family member 26 gene. Related research has shown that this type of plant protein is transcriptionally active and encodes forward-orientation half-molecule proteins that localize to the membranes bounding small intra-cellular compartments. The precise functions of this type of protein remain unclear[16]. The *AtABCB*27 (TAP2) gene is involved in plant aluminum tolerance, suggesting the protein may be associated with plant abiotic stress[17]. In our study, TDF-13 expression increased significantly under salt stress. The qRT-PCR results revealed an increase after 3 h, which reached its highest level at 24 h of salt stress.

TDF18 was homologous to the *CYP*98A3 subclass of cytochrome P450. Plant P450s are involved in a variety of metabolic pathways, including plant hormone and defensive secondary metabolite (e.g., phytoalexin) biosynthesis and detoxification of exogenous chemicals, such as herbicides. Although functional information during stress is unavailable for this protein, a previous study indicated that cytochrome P450 mono-oxygenases were up-regulated under salt stress in *Arabidopsis*, rice, and *Mesem bryanthemum*(ice plant)[18]. Our qRT-PCR results showed that TDF18 expression in *G. aridum* was significantly increased at 12-24 h of salt stress.

TDF21 was related to LEA(late embryogenesis abundant) protein, which is well known for helping plants resist salt stress. Genetically-engineered rice plants constitutively overexpressing the barley LEA gene(*HVA*1) driven by a rice actin-1 promoter generally showed better salt tolerance (at 200 mmol·L^{-1} NaCl) compared with wild-type plants[19]. Four *LEA* genes, *Td* 29b, *Td*11, *Td*16, and *Td* 25a , were strongly induced (1000- to 9000-fold) under salt stress[20]. In our study, TDF21 was strongly expressed at 12 h of salt stress, but dramatically declined thereafter.

TDF24 was homologous to a nuclear acid binding protein. Its counterpart in *Arabidopsis* had the highest coexpression potential with the *SOS*1 gene (http://atted.jp/data/locus/At1g14340.shtml), an important gene in *Arabidopsis* salt tolerance[21]. We observed that TDF24 expression increased steadily after stress, but declined at 48 h of salt stress.

3 Discussion

Unlike animals, which can move to avoid different stresses, plants must respond and adapt to stresses in place to survive severe environmental conditions. During the long course of evolution, plants have developed a number of mechanisms to resist these stresses. Cotton is a moderately salt tolerant species. Previous studies have revealed some mechanisms of salt tolerance in cotton. For example, the cotton DRE-binding transcription factor gene (*GhDREB*) conferred enhanced tolerance to drought, high salt, and freezing in transgenic wheat[22]. Three ethylene-responsive factors (*GhERF*2, *GhERF*3, and *GhERF*6) were induced by ethylene, abscisic acid, salt, cold, and drought[23]. A stress responsive group C MAPK gene (*GhMPK*2) in cotton (*G. hirsutum*) was induced by abscisic acid (ABA) and abiotic stresses such as NaCl, PEG, and dehydration[24]. Expression of an *Arabidopsis* vacuolar H^+-pyrophosphatase gene (*AVP*1) in cotton improved drought and salt tolerance and increased fiber yield[25]. A cDNA clone designated *Gossypium hirsutum* zinc finger protein 1 (GhZFP1), which was isolated from a salt-induced *G. hirsutum* cDNA library using differential hybridization screening, encodes a novel CCCH-type zinc finger protein. GhZFP1-overexpressing transgenic tobacco plants showed enhanced salt stress tolerance and resistance to *Rhizoctonia solani*[26]. GhCyp1, an immunophilin protein cloned from *Gossypium hirsutum* using rapid amplification of cDNA ends, conferred higher tolerance to salt stress and *Pseudomonas syringae* pv. *tabaci* infection in overexpressed transgenic tobacco compared with control plants[27]. All of these results indicate that many kinds of cotton salt stress genes have important regulatory roles in responses to both abiotic and biotic stress.

Gossypium wild species that often experienced periodic drought and extreme temperature and dispersed their seeds by seawater flow have evolved a unique stress tolerance system. Exploiting differentially expressed genes derived from salt-tolerant *Gossypium* species should further enhance our understanding of salt tolerance mechanisms in cotton. In this study, we investigated the salt and drought tolerant wild species *G. aridum*. Using cDNA-AFLP, we obtained an expression profile of early response transcripts following salinity stress. Up-regulated genes were mainly involved in ion transport, ROS scavenging, cell signaling, cell division, transcription regulation, protein metabolism, protein and membrane protection, and penetration regulation. These newly-identified TDFs should be useful resources for future research on saline stress in cotton.

This work resulted in the identification of different cDNA fragments induced by salt stress in *G. aridum* leaves. Some of the cDNAs showed homology with known proteins involved in various stress responses, including LEA (TDF-21), β-OHASE (TDF-23), and BADH (TDF-4). For homologous genes of some of the other TDFs, however, no such salt tolerance function has been discovered in other plants. In *Arabidopsis thaliana*, for example, the TDF2 homologous gene regulates perception of physical force, response to osmotic pressure changes, and control of plastid size and shape. The induction of the gene at an early stage of salt stress, however, suggests a possible regulatory function in salinity adaptation, probably by preventing cellular damage and establishing a homeostatic environment. TDF13 corresponded to the ATP-binding cassette transporter protein TAP gene. The *AtABCB*27 (TAP2) gene is involved in plant aluminum tolerance, suggesting this type of protein may play a role in plant abiotic stress response[17]. Further research is necessary to reveal the function of these genes in cotton.

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