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Full Length Research Paper

cDNA- AFLP analysis of the response of tetraploid black locust (*Robinia pseudoacacia* L.) to salt stress

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Soil salinity is a major abiotic stress that limits plant growth. In this study, to understand the tolerance mechanism associated with salinity stress in tetraploid black locust (*Robinia pseudoacacia* L.), 2-year-old plants were treated by salinity. cDNA amplified fragment length polymorphism (cDNA-AFLP) and quantitative real-time polymerase chain reaction (qRT-PCR) were used to study the gene expression of tetraploid black locust in response to 5, 10 and 15 days after salinity stress (300 mM NaCl) treatment. One hundred and ten (110) transcript derived fragments (TDFs) with up-regulation expression in leaves of tetraploid black locust in response to salinity stress were characterized and classified into seven groups. The putative functions of these TDFs were related to energy metabolism, material metabolism, signal transduction, transcription factor, stress and defense-related proteins and transport facilitation. The expression patterns of six genes having direct or indirect relation with salt stress response were analyzed through qRT-PCR. The possible roles of these genes are discussed. These data may enhance the understanding of the salinity tolerance mechanisms in polyploid plants.

Keywords: cDNA-AFLP, salt stress, gene expression, qRT-PCR; tetraploid black locust.

INTRODUCTION

The black locust tree (*Robinia pseudoacacia* L.), is native to the southeastern USA, although widely cultivated in temperate regions elsewhere as an ornamental, particularly of street and parkland areas (Lavin and Sousa, 1995). Its fragrant white blossoms are a nectar source for bees, yielding a high quality monofloral honey that is produced mainly in Europe and the eastern USA (Iannuzzi, 1990). The tetraploid black locust tree (*R. pseudoacacia* L.) induced from the black locust tree is not only a promising plant for reforestation due to its fast growth but also can be use as fine feed of domestic fowl and livestock for its fleshy leaves rich in vitamins and minerals (Ren, 2000; Shu, 2003; Ewald et al., 2009). Importantly, tetraploid black locust is more resistant to salt stress than the diploid (Meng et al., 2009). Accordingly, it may have evolved a highly developed mechanism to adapt itself to salt stress. Thus, tetraploid black locust may serve as a suitable system for salt tolerance studies due to its polyploid nature, which would conduct some studies in other polyploid plants. However, the molecular tolerance mechanism associated with salt stress in tetraploid lack locust is still not clear, though significant efforts have been invested into the studies of physiological mechanisms and evaluating salinity resistance in this species (Meng et al., 2009).

Soil salinity is one of the major abiotic factors limiting plant growth. Increasing salinization is predicted to 50%

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Abbreviations: **AFLP**, Amplified fragment length polymorphism; **qRT-PCR**, quantitative real-time polymerase chain reaction;**TDFs**, transcript derived fragments.

of arable land in the next 25 years and up to 50% by 2050 (Wang et al., 2003). Therefore, it is essential to understand the mechanism underlying salt stress to strengthen and improve salt stress tolerance in plants. Indeed, the plant response to salt stress has been the focus of many researchers for decades at physiological, genetic and molecular aspects (Gong et al., 2001; Zhu et al., 2001; Xiong et al., 2006). Nevertheless, most advancements in understanding the salt tolerance mechanism are obtained from studies on the model plants, especially diploids, such as Arabidopsis (Kreps et al., 2002; Boudsocq et al., 2004; Ndimba et al., 2005), *Nicotiana tabacum* (Lin et al., 2006; Cao et al., 2007; Zhang et al., 2011) and *Oryza sativa* (Kawasaki et al., 2001; He et al., 2005; Chitteti and Peng, 2007). But these studies will not be sufficient to explain the adaptation of polyploid plants, which may survive under severe salt stress. In practice, many polyploid plants exhibited higher tolerance to salt stress as compared with their diploid relatives (Saleh et al., 2008; Yıldız and Terzi, 2008; Mouhaya et al., 2010; Meng et al. 2011). Therefore, polyploidy has been regarded as an efficient way to improve salt stress tolerance in plants and has played important roles in the practices of agriculture and forestry (Xiong et al., 2006). However, the molecular genetic basis of salt tolerance in polyploid plants has not been clearly elucidated.

cDNA amplified fragment length polymorphism (cDNA-AFLP) is an efficient technique to isolate genes in response to various abiotic stresses (including salinity) in many plant species due to its an efficiency, sensitivity and repetition (Bachem et al., 1996; Ditt et al., 2001; Baisakh et al., 2006; Jayaraman et al., 2008; Wang et al., 2011). Furthermore, it is a robust and high-throughput genome-wide expression analysis, where prior sequences information is not required (Durrant, 2000; Yao et al., 2007; Dal Cin et al., 2009). Here, the objective of this study was to identify key genes expressed in leaves of tetraploid black locust in response to salinity stress using cDNA-AFLP method and to validate their expression patterns through a quantitative real-time polymerase chain reaction (qRT-PCR) method, and then further discuss their functional relevance.

MATERIALS AND METHODS

Plant materials and salt stress treatments

Plants (two-year-old) of tetraploid black locust were grown in plastic pots (21 cm in diameter and 21 cm in depth) containing a 2:1 (v/v) mixture of nutrient soil and sand. The plants were kept in a greenhouse (day/night air temperature, 28/22°C; photoperiod, 12 h; relative humidity, 65 to 85%) during the entire growth period. Plants were subjected to salt stress by soil watering with 1 L of 0 (control), and 300 mM NaCl in full-strength Hoagland's nutrient solution on 1, 3, 8 and 13 days. The salinity concentration 300 mM was chosen on the basis of our earlier results (Meng et al., 2009). The leaf tissues were harvested after 0, 5, 10 and 15 days of exposure to the saline treatments and frozen immediately in liquid nitrogen, then stored at -80°C until RNA extraction.

RNA extraction and cDNA synthesis

Total RNA was extracted from 150 mg frozen leaf tissues of plants using TRIzol reagent (Promega, Mandson, USA) and then purified with RNA purification reagent (Invitrogen Life Technologies, Carlsbad, CA, USA). Double-stranded (ds) cDNA from total RNA was synthesized using M-MLV RTase cDNA Synthesis Kit (TaKaRa, Kyoto, Japan) according to its instructions.

cDNA-AFLP reaction

The cDNA-AFLP procedure was conducted as described by Bachem et al. (1996) with some modifications. Thirty nanograms of cDNA were digested with EcoR I (Invitrogen, USA) and Mse I (Invitrogen, USA) at 37°C for 2 h, and followed by heat inactivation of enzymes at 75°C for 20 min. The digested products were ligated to adapters with sequences as follows: *Eco*RI adaptor, 5′-CTCGTAGACTGCGTACC-3′, 3′-CATCTGACGCATGGTTAA-5′; *Mse*I adaptor, 5′-GACGATGA-GTCCTGAG-3′,3′-TACTCAGGACT CAT-5′.

The ligated products were pre-amplified with the corresponding pre-amplification primers (*Eco*RI: 5′-GACTG CGTACCAATTC-3′, *Mse*I: pre-amplification primer, 5′-GATGAGTCCTGAGTAA-3′). Selective nucleotides were added to the 3′ end of pre-amplified products (*Mse*I, 5′- GATGAGTCCTGAGTAA + NN-3′; *Eco*RI, 5′-GACTGCGTACCAATTC + NN-3′). Selective amplification products (15 µl) were denatured at 95°C for 5 min after adding 10 µl of 98% formamide loading buffer. The denatured PCR products (10 µl) were separated by a 6% polyacrylamide gel electrophoresis run at 80 W until the xylene cyanole reached the bottom. The gels were silver-staining according to the Silver Sequence^{TM} DNA Sequencing System Technical Manual (Promega, USA).

Sequence analysis of TDFs

The polymorphic TDFs based on presence or differential intensity were marked on the films, cut from the gel with a sharp razor blade, and eluted in 30 µl of sterile double distilled water at 100°C for 10 min and then centrifuged. The extracts were subjected to recover using the same set of corresponding selective primers under the same PCR conditions as for re-amplification. The DNA fragments were cloned into the plasmid pMD18-T vector (TaKaRa, Kyoto, Japan) and subjected to sequencing analysis.

The sequences of the TDFs were analyzed for homology to nucleotide or protein sequences by searching the National Center for Biotechnology Information (NCBI) databases using the BLAST program. Known unigene matches were classified into different functional groups via GO website (http:// www. geneontology.org/). All of known genes were chosen for Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis (http://www. genome.-jp./kegg/pathway.html) and classified into different metabolism pathways.

qRT-PCR analysis

The RNA samples from leaf tissues at four times (0, 5, 10 and 15 d) were used for qRT-PCR. SYBR Premix Ex Tag^{TM} Kit (TaKaRa, Kyoto, Japan) with SYBR GreenII as the fluorescent dye was used for qRT-PCR. The primers for qRT-PCR analysis of selected sequences of the TDFs were designed with the Primer 5.0. These primers are listed in Table 1.

Each sample was performed in a final volume of 20 µl containing

Table 1. Primers used for qRT-PCR.

TDFs clone #	Amplicon length (bp)	Forward primer (5′-3′)	Reverse primer (5′-3′)
143	120	GGCTTTTTCCACCGATTC	TCCGACCCATAGCATACG
166	190	AGATTTTATGCTGCTGAT	TAACACATTCTCTGGCTT
210	101	TTATCCAAGATTTCCAGTTCC	CTTATAGATGTCATCGAGGGC
230	103	AAACTTGATGAATACGAGCAG	GAACAAAACAATGATGAGGGT
275	110	TTGAGCAGCAGTGGGACG	TCGGAAAAGGTGAAACGC
288	119	TGCTCCAAATCTACAAAGG	GCAATGTGGCAAATGAAC
Actin	109	CCTCTCTTCCTCTCACCTTGC	TATCCTCAGCGTCTGCCATCT

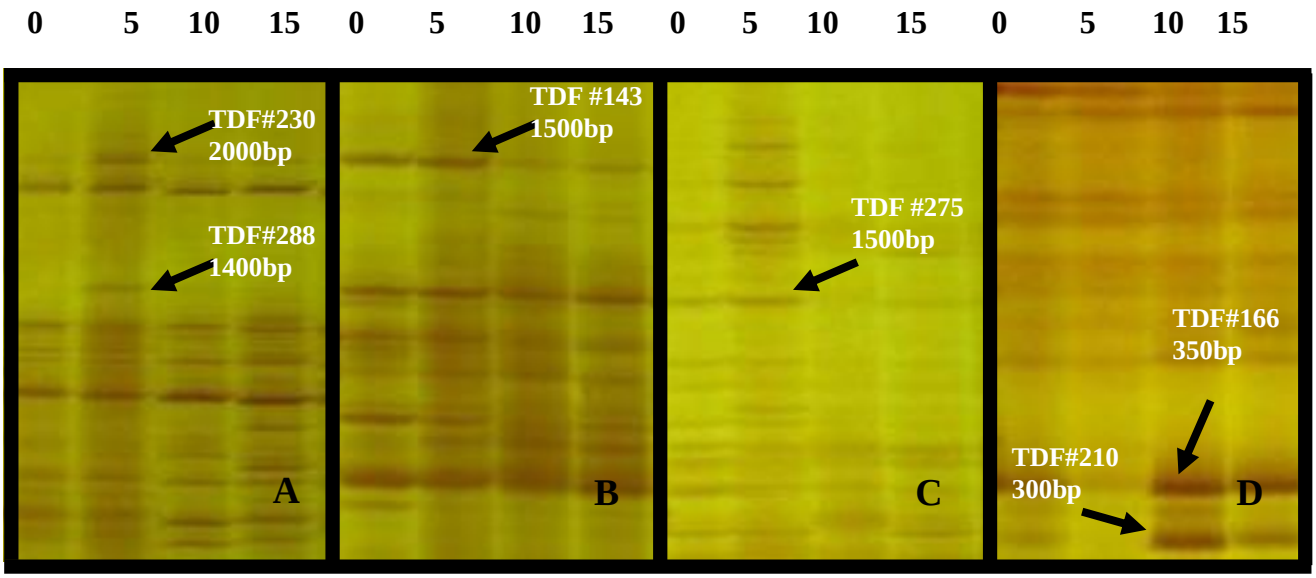

Figure 1. A cDNA-AFLP gel autoradiogram in the leaves of tetraploid black locust at four time of salt stress. The four figures (A, B, C and D) show cDNA-AFLP fingerprints of $NaCl_2$ treated 0 d (0), 5 d (5), 10 d (10) and 15 d (15) leaves of tetraploid black locust using same combination of primers. Some of the genes which we have selected for sequencing are indicated by arrows.

2 µl cDNA, 1 µl each of 4 µM forward and reverse primer, 0.2 µl SYBR Premix Ex Taq (5 U/µl, Takara, Dalian, China) and 6 µl sterile H_2O. The qRT-PCR amplification was performed as follows: 95°C for 1.5 min; 95°C for 30 s; 58°C for 30 s; 72°C for 30 s for 40 cycles, 79°C for 1 s. Melting curve analysis was performed to exclude the occurrence of primer dimers and unspecific PCR products of *R. pseudoacacia* actin, a housekeeping gene, was used for internal control. According to Applied BioSystems (Foster City, CA), the amount of the transcripts of each gene normalized to the internal control actin was analyzed using the $2^{-\Delta\Delta Ct}$ method (Livak and Schmittgen, 2001). For reproducing the results, the experiment was repeated three times independently.

RESULTS

Hundred (100) AFLP primer combinations were used for cDNA-AFLP analysis on cDNA samples from leaves at four different times. 60 primers pairs led to the identification of fragments with differentially expressed profiles. Three thousasnd eight hundred (3800) TDFs were generated with an average of 63 fragments per primer pair. One hundred and ten (110) TDFs were differentially expressed, which were up-regulated based on presence, absence or differential intensity. Therefore, 110 up-regulated TDFs were recovered from gels, cloned and sequenced (Figure 1).

One hundred and ten (110) TDFs were searched for homologous to known databases and were described based on previously reported gene function and information from the Gene Ontology. Their accession numbers and DNA sequence identities are shown in Table 2. Of 110 TDFs, 47.3% were highly homologous to known genes, whereas, 30.9% encoded unclassified proteins and unknown function and 21.8% showed no significant matches. The identification of unknown TDFs may represent new salt-induced genes in leaves that have not been previously characterized and could contribute to further understanding of salt stress tolerance in tetraploid black locust. Therefore, to assess the association of these DNA sequences to salt-tolerance in tetraploid black locus, we need characterize them in

Table 2. Similarity of up-regulated TDFs expressed during the salt stress given to leaves of tetraploid *R. pseudoacacia* with known gene sequences.

TDFs clone # [a]	Accession number [b]	Homology in the Genbank database [c]	E-Value [d]
Energy metabolism			
103	XM_002509493.1	*Ricinus communis* ribulose-5-phosphate-3-epimerase, putative, mRNA	1e-83
125	GQ893027.1	*Vigna radiata* cultivar kampangsan1 chloroplast, complete genome	7e-70
126	AY147669.1	*Lanaria lanata* photosystem II D2 protein (psbD) and photosystem II CP43 protein (psbC) genes, partial cds	8e-69
129	AF314923.1	*P. vulgaris* phosphoenolpyruvate carboxylase housekeeping isozyme pepc2 mRNA, partial cds	2e-13
135	JF433943.1	*Asclepias syriaca* voucher Fishbein 4885 (OKLA) chloroplast, partial genome	3e-22
154	HM029371.1	*Lathyrus sativus* cultivar Cicerchia Marchigiana chloroplast, complete genome	3e-176
160	NM_001125805.1	*A. thaliana* ATP binding / protein kinase (AT5G26110) mRNA, complete cds	3e-07
180	EU912419.1	*Glycine max* serine hydroxymethyltransferase 1 mRNA, complete cds	3e-58
210	EU236700.1	*Pisum sativum* developmentally regulated GTP binding protein 1 (DRG1) mRNA, complete cds	5e-80
274	XM_002516400.1	*R. communis* glycine dehydrogenase, putative, mRNA	4e-16
298	NM_122512.3	*A. thaliana* ATP binding / catalytic/ proteinkinase (AT5G26110) mRNA, complete cds	0.007
299	XM_002524697.1	*R. communis* o-sialoglycoprotein endopeptidase, putative, mRNA	9e-08
Material metabolism			
86	GU433011.1	*Salix miyabeana* clone pMS105 glycosyltransferase family 8B mRNA	7e-24
95	DQ205523.1	*Vigna unguiculata* digalactosyldiacylglycerol synthase 1 mRNA, complete	8e-52
97	L01660.1	*M. sativa* NADH-glutamate synthase mRNA, comlete cds	6e-39
110	NM_117602.8	*A. thaliana* catalytic / choline-phosphate cytidylyltransferase/ nucleotidy transferase (CCT2) mRNA, complete cds	9e-67
120	NM_105289.2	*A. thaliana* aspartyl protease family protein mRNA, complete cds	3e-16
128	EU716314.1	*Camellia sinensis* histone H1-like protein (H1) mRNA, complete cds	2e-15
143	DQ335798.1	*M. truncatula* cytochrome P450 monooxygenase mRNA, complete cds	2e-44
150	EU340886.2	Black locust cysteine protease-like protein mRNA, partial cds	3e-70
152	AK176881.1	*A. thaliana* mRNA for putative esterase, complete cds	1e-07
156	NM_105289.2	*A. thaliana* aspartyl protease family protein (AT1G66180) mRNA, complete cds	1e-14
159	DQ097733.1	*Arachis hypogaea* isomerase-like protein mRNA, complete cds	7e-58
169	D31700.1	*G. max* mRNA for cysteine proteinase inhibitor, complete cds	5e-147
182	AY040033.1	*A. thaliana* putative histidine decarboxylase (At1g43710) mRNA, complete cds	2e-53
197	XM_002530845.1	*R. communis* cysteine-type peptidase, putative, mRNA	9e-40
209	AY062625.1	*A. thaliana* nucleotide sugar epimerase-like protein (F14O13.1) mRNA, complete cds	1e-26
224	NM_111956.2	*A. thaliana* GDSL-motif lipase/hydrolase family protein (AT3G11210) mRNA, complete cds	3e-30
225	L25042.1	*M. sativa* acetyl-CoA carboxylase (ACCase) mRNA, complete cds	2e-78

Table 2. Contd.

TDFs clone # [a]	Accession number [b]	Homology in the Genbank database [c]	E-Value [d]
Signal transduction			
85	AB204873.1	*P. vulgaris* Pvphot1b mRNA for phototropin, complete cds	9e-38
93	XM_002514207	*R. communis* protein phosphatase 2c, putative, mRNA	5e-102
132	HM369805.1	*Vitis vinifera* cultivar PN40024 chloride channel ClC7 mRNA, complete cds	1e-74
166	AB204872.1	*P. vulgaris* Pvphot1a mRNA for phototropin, complete cds	3e-95
172	NM_111006.2	*A. thaliana* armadillo/beta-catenin repeat family protein (AT3G01400) mRNA, complete cds	6e-07
265	XM_002532610.1	*R. communis* mitochondrial carrier protein, putative, mRNA	8e-10
275	AJ575100.1	*M. sativa* mRNA for putative mitogen-activated proteinkinase 1 (mekk1 gene)	1e-76
Transcription factor			
23	DQ792718.1	*G. max* transcription factor bZIP38 (bZIP38) mRNA, partial cds	2e-14
84	XM_002525609.1	*R. communis* transcription factor, putative, mRNA	3e-52
122	XM_002517723.1	*R. communis* RNA polymerase sigma factor rpoD, putative, mRNA	1e-04
185	XM_002511377.1	*R. communis* Spotted leaf protein, putative, mRNA	9e-06
230	XM_002531222.1	*R. communis* calmodulin-binding transcription activator (camta), plants, mRNA	8e-82
231	XM_002519309.1	*R. communis* calmodulin-binding transcription activator (camta), plants, putative, mRNA	4e-48
241	U77939.1	*P. vulgaris* ubiquitin-like protein mRNA, complete cds	8e-49
288	AF160977.1	*P. sativum* zinc finger protein mRNA, complete cds	1e-48
Stress-and defense-related proteins			
189	DQ868542.1	*Ammopiptanthus mongolicus* cold-induced wall associated kinase mRNA, partial cds	4e-10
233	AB185947.1	*Clitoria ternatea* CtGT4 mRNA for putative glycosyltransferase, complete cds	2e-73
289	XM_002523747.1	*R. communis* catalytic, putative, mRNA	2e-20
Transport facilitation			
146	NM_102326.1	*A. thaliana* UDP-3-0-acyl N-acetylglucosamine deacetylase family protein / F-box protein-related (AT1G25141) mRNA, complete cds	2e-07
179	XM_002312768.1	*Populus trichocarpa* cholesterol transport protein, mRNA	5e-47
228	HM064455.1	*Astragalus sinicus* DnaJ-like protein 1 (DJL1) mRNA, complete cds	2e-35
278	XM_002531115.1	*R. communis* vacuolar sorting protein, putative, mRNA	2e-17
292	XM_002323323.1	*P. trichocarpa* aromatic and neutral amino acid transporter, mRNA	3e-26

[a]The codes of the fragments identified from tetraploid *R. pseudoacacia* by cDNA-AFLP; bGenBank accession number; [C]homology analyses of TDF sequence using BLASTX and TBLASTX searches; [D]the E-value was used to indicate the significance of sequence similarity.

detail. In addition, 47.3% TDFs were grouped into functional categories: energy metabolism (10.9%), material metabolism (15.5%), signal transduction (6.4%), transcription factor (7.3%), stress-and defense-related proteins (2.7%) and transport facilitation (4.5%) (Figure 2).

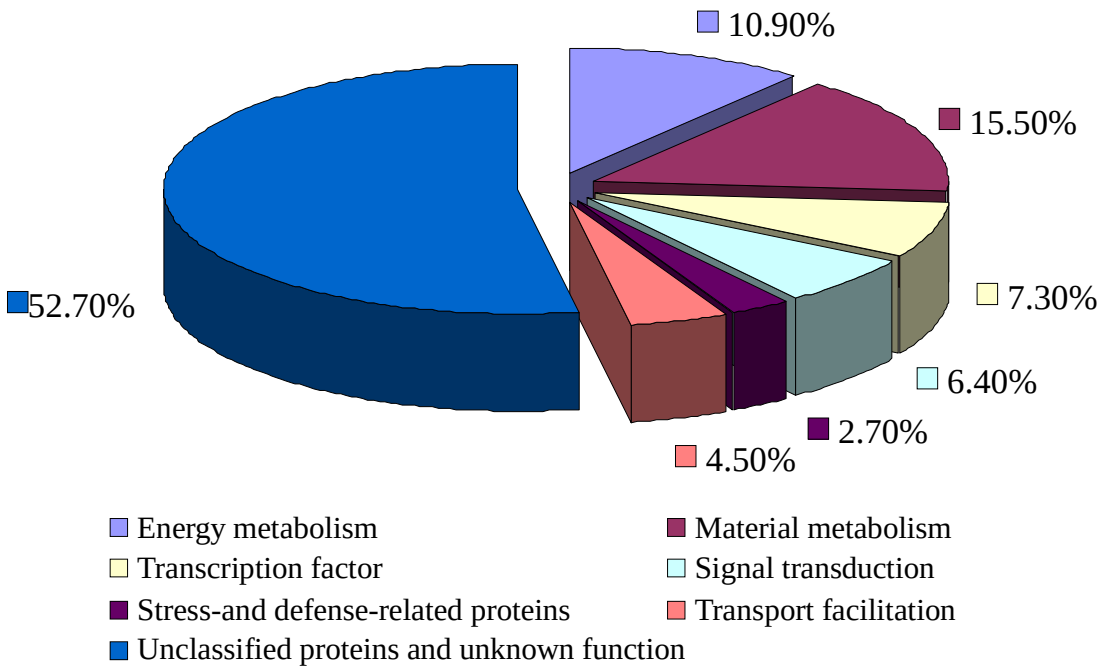

Figure 2. Functional categories of the transcriptomes from leaf tissues of tetraploid *R. pseudoacacia* derived from the cDNA-AFLP based on gene homology.

To verify the data obtained with the cDNA-AFLP analysis, 6 clones that have been reported to have some relationship with environmental tolerance mechanism were selected for qRT-PCR. The results indicate that the expression profiles were in agreement with the results obtained from cDNA-AFLP experiment (data not shown). Therefore, the results indicated that cDNA-AFLP is an effective technique for identifying differentially expressed genes in tetraploid black locust under salt stress. However, one clone (TDF #166) was not similar in both procedures based on different expression levels (quantitative variants).

The expression levels of TDF #143 increased greatly in response to salt stress, while a 3.2 fold increase was detected at 10 days after salt stress (Figure 3a). The basal expression of TDF #166 was the highest at 15 days of salt treatment and increased to 5.3-folds compared to the control (Figure 3b). TDF #210 showed 3.4 fold higher expression after 15 d of salt stress compared to the control (Figure 3c). The expression level of TDF #230 was very high at 5 days under salt stress (Figure 3d). TDF #275 was over-expressed by about 3-fold increase at 10 days of salt stress compared to the control (Figure 3e). The expression of TDF #288 after treatment was 1.9 fold higher than the control at 5 days (Figure 3f).

DISCUSSION

Many genes transcript in plants were confirmed with the environmental stress including to drought, salt and low temperature (Baisakh et al., 2006; Jayaraman et al., 2008; Wang et al., 2011). cDNA-AFLP was used to identify some of the inducible TDFs, which have been reported in a number of plant species (Bachem et al., 1996; Ditt et al., 2001). These results indicated that our analysis system functioned properly to search for stress-inducible genes. However, in this study, the different expression level of TDF #166 was not similar with the result of cDNA-AFLP. The reason for this difference was due to the high sensitivity of qRT-PCR compared with cDNA-AFLP. Another reason could be due to the polyploidy nature of tetraploid black locust because some genes might be amplified by qRT-PCR and could not be detected by cDNA-AFLP for the lack of the sites for the primers, which have also been reported in maritime pine and *Spartina alterniflora* (Dubos and Plomion, 2003; Baisakh et al., 2006). Moreover, more up-regulated TDFs were observed under salt stress, which is in agreement with previous reports (Jayaraman et al., 2008; Wang et al., 2011). The reason could be due to down-regulated TDFs becoming of rare abundance which make them at selective disadvantage from being amplified.

In this study, 6 clones TDFs which were most likely to represent candidate genes with potential functions to improve the salt stress tolerance of plants were discussed based on some earlier reports. They were TDF #143 (Cytochrome P450), TDF #166 (Pvphot for phototropin), TDF #210 (GTP binding protein), TDF #230 (calmodulin-binding transcription activator), TDF #275

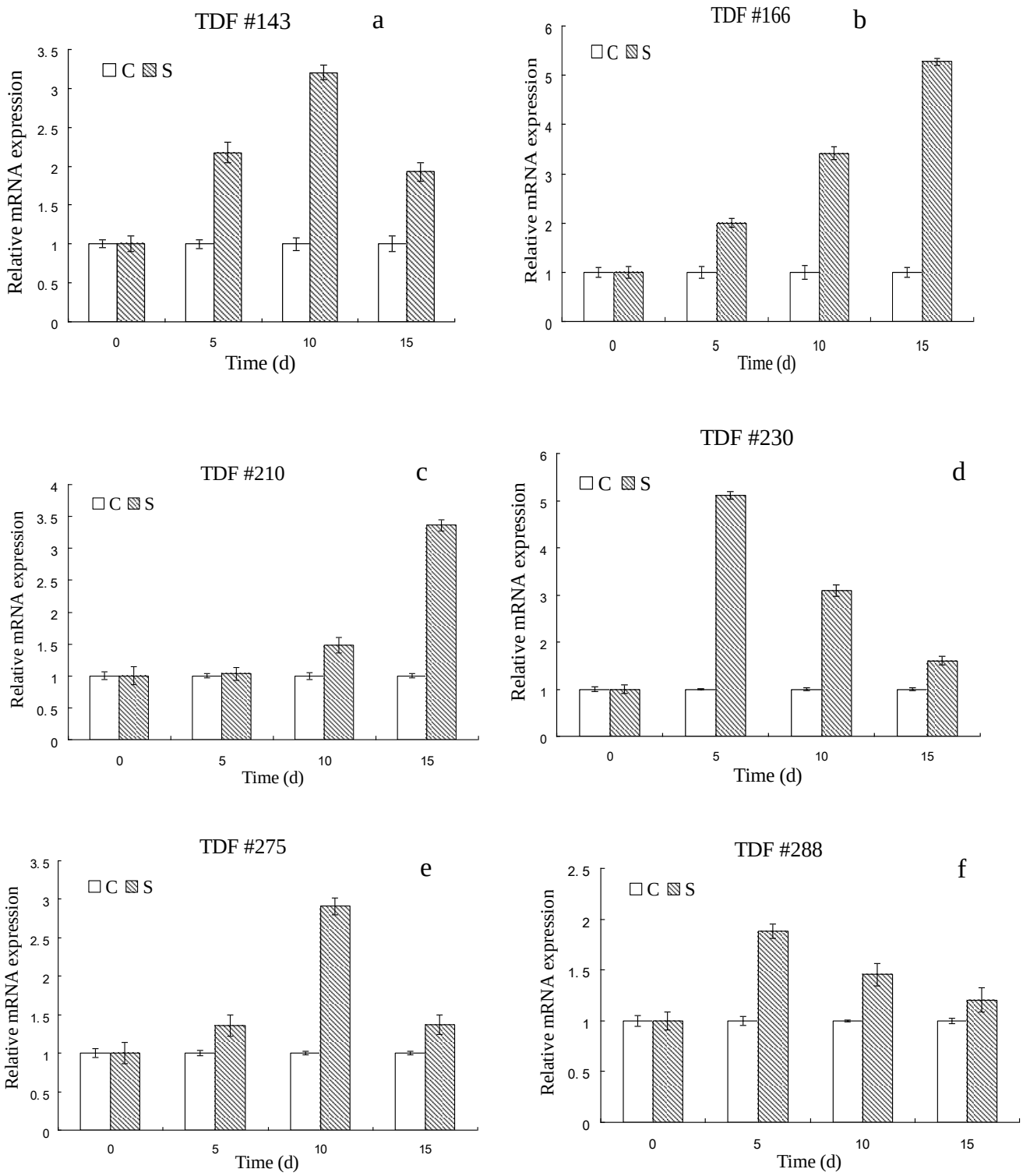

Figure 3. Expression analysis of TDF #143; a, TDF #166; b, TDF #210; c, TDF #230; d, TDF #275; e, TDF #288; f, in leaves of tetraploid R. pseudoacacia seedlings under salt stress using qRT-PCR. Bars indicate the standard error (± SE) calculated from three independent experiments.

(mitogen-activated protein) and TDF #288 (zinc finger protein). TDF #143 showed similarity to cytochrome P450 of *Medicago truncatula*, which plays important roles in some processes such as plant defense, viability and development (Chandhry et al., 2002). In addition, P450 genes were detected in many plants under slat stress

(Ueda et al., 2002).

TDF #166 had similarity to pulvinar blue-light receptor of *Phaseolus vulgaris* L. phototropin (Pvphot) from the *Phaseolus vulgaris* L. pulvinus, which caused dephosphorylation of the plasma membrane H^+-ATPase (Ishikawa et al., 2009). Ma et al. (2002) provided the first direct evidence for the existence of H^+-ATPase from cell suspensions of *Populus euphratica,* which plays an important role in its salt tolerance. The basal expression of this clone increased to 5.3-folds compared to the control (Figure 3b). Therefore, tetraploid black locust may have similar signaling pathway between blue light stimulation and salt stress, which will be study further.

TDF #210 was similar to a GTP binding protein (G-protein), which is a highly conserved family and plays important roles in fundamental pathways (O′Connell et al., 2009). TDF #230 is identified calmodulin-binding transcription activator (CAMTA) involved in the defense against stresses from heat, cold, drought and salinity (Yang and Poovaiah, 2002). TDF #275 showed similarity to a putative mitogen-activated protein kinase (MAPK) from *Medicago sativa* which responded to osmotic stress or heat shock and regulated cellular activities (Pearson et al., 2001). An increase in the expression of mitogen-activated protein kinase (MAPK) gene was reported in *Arabidopsis* under salt stress (Boudsocq et al., 2004). TDF #288 showed similarity with the zinc finger protein (ZFP) from *Arabidopsis thaliana,* which belong to CCCH-type ZFP. ZFP is important in enhancing tolerance of plants to salt stress (Sun et al., 2007).

Polyploidy has occurred throughout the evolutionary history of eukaryote. In plants, genetic and physiological changes occur rapidly after formation of polyploidy. Abiotic stresses can cause the differentially gene expression in diploid plants, while little is known about the genetic changes of polyploidy plants imposed by salt stress (Mouhaya et al., 2010; Meng et al., 2011). In tetraploid black locust under salt stress, significant gene expression alteration occurred; interestingly most genes were up-regulated. These changes could be driven by expression of a duplicated gene, which caused disruption of genome and transcriptome (Leitch and Leitch, 2008).

In conclusion, the usefulness of the cDNA-AFLP approach to identify genes related to salt tolerance of tetrapolid black locust was demonstrated in this study. However, it is important to mention that our analysis was restricted to up-regulated genes, thus limiting our understanding of the mechanisms of salt tolerance in tetrapolid black locust. Therefore, firstly, further study should be done to isolate other genes. Secondly, the roles of differentially expressed genes in tetrapolid black locust by transgenic approaches needs to be determine.

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REFERENCES

Bachem CWB, van der Hoeven RS, de Bruijn SM, Vreugdenhil D, Zabeau M, Visser RGF (1996). Visualization of differential gene expression using novel method of RNA fingerprinting based on AFLP: analysis of gene expression uring potato tuber development. Plant J. 9: 745-753.

Baisakh N, Subudhi PK, Parami NP (2006). cDNA-AFLP analysis reveals differential gene expression in response to salt stress in a halophyte *Spartina alterniflora* Loisel. Plant Sci. 170: 1141-1149.

Boudsocq M, Barbier-Brygoo H, Laurière C (2004). Identification of nine sucrose nonfermenting 1-related protein kinases 2 activated by hyperosmotic and saline stresses in *Arabidopsis Thaliana*. J. Biol. Chem. 279: 41758-41766.

Chitteti BR, Peng Z (2007). Proteome and phosphoproteome differential expression under salinity stress in rice (*Oryza sativa*) roots. J. Proteome Res. 6: 1718-1727.

Cao WH, Liu J, He X J, Mu RL, Zhou HL, Chen SY, Zhang JS (2007). Modulation of ethylene responses affects plant salt-stress responses. Plant Physiol. 143: 707-719.

Dal Cin V, Barbaro E, Danesin M, Murayama H, Velasco R, Ramina A (2009). Fruitlet abscission: A cDNA-AFLP approach to study genes differentially expressed during shedding of immature fruits reveals the involvement of a putative auxin hydrogen symporter in apple (*Malus domestica* L. Borkh). Gene. 442: 26-36.

Ditt RF, Nester EW, Comai L (2001). Plant gene expression response to *Agrobacterium tumefaciens*. Proc. Natl. Acad. Sci. USA. 98: 10954-10959.

Dubos C, Plomion C (2003). Identification of water-deficit responsive genes in maritime pin (*Pinus pinaster* Ait.) roots. Plant Mol. Biol. 51: 3389-3402.

Durrant WE, Rowland O, Piedras P, Hammond K, Jones JDG (2000). cDNA-AFLP reveals a striking overlap in race specific resistance and wound response gene expression profiles. Plant Cell. 12: 963-977.

Ewald D, Ulrich K, Naujoks G, Schröder MB (2009). Induction of tetraploid poplar and black locust plants using colchicine: chloroplast number as an early marker for selecting polyploids in vitro. Plant Cell Tissue Org. 99: 353-357.

Gong Z, Koiwa H, Ann cushman M, Ray A, Bufford D, Kore-eda S, Matsumoto TK, Zhu J, Cushman JC, Bressan RA, Hasegawa PM (2001). Genes that uniquely stress regulated in salt overly sensitive (*sos*) mutants, Plant Physiol. 126: 363-375.

He XJ, Mu RL, Cao WH, Zhang ZG, Zhang JS, Chen SY (2005). AtNAC2, a transcription factor downstream of ethylene and auxin signaling pathways, is involved in salt stress response and lateral root development. Plant J. 44: 903-916.

Iannuzzi J (1990). Black locust as a honey plant. Am. Bee J. 315-317.

Ishikawa M, Takahashi F, Nozaki H, Nagasato C, Motomura T, Kataoka H (2009). Distribution and phylogeny of the blue light receptors aureochromes in eukaryotes. Planta, 230: 543-552.

Jayaraman A, Puranik S, Rai NK, Vidapu S, Sahu PP, Lata C, Prasa M (2008). cDNA-AFLP Analysis Reveals differential gene expression in response to salt stress in Foxtail Millet (*Setaria italica* L.). Mol. Biotechnol. 40: 241-251.

Kawasaki S, Borchert C, Deyholos M, Wang H, Brazille S, Kawai K, Galbraith D, Bohnert HJ (2001). Gene expression profiles during the initial phase of salt stress in rice. Plant Cell, 13: 889-906.

Kreps JA, Wu Y, Wang X, Zhu T, Harpe JF (2002). Transcriptome changes for *Arabidopsis* in response to salt, osmotic and cold stress. Plant Physiol. 130: 2129-2141.

Lavin M, Sousa M (1995). Phylogenetic systematics and biogeography of the tribe Robinieae (Leguminosae). Syst. Bot. Monogr. 45: 1-165.

Leitch AR, Leitch IJ (2008). Genomic plasticity and the diversity of polyploid plants. Science, 320: 481-483.

Lin J, Wang Y, Wang G (2006). Salt stress-induced programmed cell death in tobacco protoplasts is mediated by reactive. J. Plant Physiol. 163: 731-739.
Livak KJ, Schmittgen TD (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2−ΔΔCt method. Methods, 25: 402-408.
Ma T, Liu Q, Zhuo L, Zhang X (2002). Tonoplast H+-ATPase in response to salt stress in *Populus euphratica* cell suspensions. Plant Sci. 163: 499-505.
Meng H, Jiang H, Hua S, Lin X, Li Y, Guo W, Li J (2011). Comparison between a tetraploid turnip and its diploid. Agric. Sci. China, 10: 363-375.
Meng F, Wang Q, Yang C, Liu J (2009). Investigation of anti-salt stress on tetraploid *Robinia pseudoacacia*. Front. For. China, 4(2): 227-235.
Mouhaya W, Allario T, Brumos J, Andrés F, Froelicher Y, Luro F, Talon M, Ollitrault P, Morillon R (2010). Sensitivity to high salinity in tetraploid citrus seedlings increases with water availability and correlates with expression of candidate genes. Funct. Plant Biol. 37: 674-685.
Ndimba BK, Chivasa S, Simon WJ, Slabas AR (2005). Identification of Arabidopsis salt and osmotic stress responsive proteins using two-dimensional difference gel electrophoresis and mass spectrometry. Proteomics, 5: 4185-4196.
O'Connell A, Robin G, Kobe B, Botella JR (2009). Biochemical characterization of *Arabidopsis* developmentally regulated G-proteins (DRGs). Protein Expres. Purif. 67: 88-95.
Pearson G, Robinson F, Beers GT, Xu BE, Karandikar M, Berman K, Cobb MH (2001). Mitogen-activated protein (MAP) kinase pathways: regulation and physiological functions. Endocr. Rev. 22: 153-183.
Ren MT (2000). Tetraploid black locust tree (in Chinese). J. Shanxi For. 3: p. 20.
Saleh B, Allario T, Dambier D, Ollitrault P, Morillon R (2008). Tetraploid citrus rootstocks are more tolerant to salt stress than diploid. C. R. Biologies. 331: 703-710.
Shu QY, Liu GS, Qi DM, Chu CC, Liu J, Li HJ (2003). An effective method for axillary bud culture and RAPD analysis of cloned plants in tetraploid black locust. Plant Cell Rep. 22: 175-180.
Sun J, Jiang H, Xu Y, Li H, Wu X, Xie Q, Li C (2007). The CCCH-Type zinc finger proteins AtSZF1 and AtSZF2 regulate salt stress responses in Arabidopsis. Plant Cell Physiol. 48: 1148-1158.
Ueda A, Shi W, Nakamura T, Takabe T (2002). Analysis of salt inducible genes in barley roots by differential display. J. Plant Res. 115: 119-130.
Wang W, Vinocur B, Altman A (2003). Plant responsed to drought, salinity and extreme temperatures: Towards genetic engineering for stress tolerance. Planta, 218: 1-14.
Wang L, Zhou B, Wu L, Guo B, Jiang T (2011). Differentially expressed genes in *Populus simonii×Populus nigra* in response to NaCl stress using cDNA-AFLP. Plant Sci. 6: 796-801.
Xiong YC, Li FM, Zhang T (2006). Performance of wheat crops with different chromosome ploidy: root-sourced signals, drought tolerance, and yield performance. Planta, 224: 710-718.
Yang T, Poovaiah BW (2002). A calmodulin-binding/CGCG box DNA-binding protein family involved in multiple signalling pathways in plants. J. Biol. Chem. 277: 45049-45058.
Yao YX, Li M, Liu Z, Hao YJ, Zhai H (2007). A novel gene, screened by cDNA-AFLP approach, contributes to lowering the acidity of fruit in apple. Plant Physiol. Bioch. 45: 139-145.
Yıldız M, Terzi H (2008). Effects of NaCl on protein profiles of tetraploid and hexaploid wheat species and their diploid wild progenitors. Plant Soil Environ. 54: 227-233.
ZhangJhttp://pubs.acs.org/doi/abs/10.1021/pr101140n,ZhangYhttp://pubs.acs.org/doi/abs/10.1021/pr101140n, Du Y, Chen S, Tan H (2011). Dynamic Metabonomic Responses of Tobacco *(Nicotiana tabacum)* Plants to Salt Stress. J. Proteome Res. 10: 1904-1914.
Zhu JK (2001). Cell signaling under salt, water and cold stresses. Curr. Opin. Plant Biol. 4: p. 401.