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Expression analysis of nine small heat shock protein genes from *Tamarix hispida* in response to different abiotic stresses and abscisic acid treatment

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Abstract Heat shock proteins (HSPs) play important roles in protecting plants against environmental stresses. Furthermore, small heat shock proteins (sHSPs) are the most ubiquitous HSP subgroup with molecular weights ranging from 15 to 42 kDa. In this study, nine sHSP genes (designated as *ThsHSP*1–9) were cloned from *Tamarix hispida*. Their expression patterns in response to cold, heat shock, NaCl, PEG and abscisic acid (ABA) treatments were investigated in the roots and leaves of *T. hispida* by real-time RT-PCR analysis. The results showed that most of the nine *ThsHSP* genes were expressed at higher levels in roots than in leaves under normal growth condition. All of *ThsHSP* genes were highly induced under conditions of cold (4 °C) and different heat shocks (36, 40, 44, 48 and 52 °C). Under NaCl stress, all nine *ThsHSP*s genes were up-regulated at least one stress time-point in both roots and leaves. Under PEG and ABA treatments, the nine *ThsHSP*s showed various expression patterns, indicating a complex regulation pathway among these genes. This study represents an important basis for the elucidation of *ThsHSP* gene function and provides essential information that can be used for stress tolerance genetic engineering in future studies.

Keywords ABA · Abiotic stress · Expression pattern · Heat shock protein · *Tamarix hispida*

Abbreviations

ABA	Abscisic acid
sHSP	Small heat shock protein
PEG	Polyethylene glycol

Introduction

Heat shock proteins (HSPs) are known to be induced following exposure to increasing temperature, and were first discovered in Drosophila in 1962. These proteins have subsequently been reported in many organisms, including plants [1]. Plant HSPs are generally divided into five evolutionarily conserved groups according to molecular mass: small HSPs (sHSPs), HSP60, HSP70, HSP90 and HSP100 [2]. sHSPs are the most ubiquitous HSP subgroup with molecular weights ranging from 15 to 42 kDa [3], which includes an evolutionarily divergent N-terminal region, followed by a conserved α-crystallin domain (ACD) of approximately 90 amino acid residues homologous to the alpha-crystallin proteins of the vertebrate eye lens [4] and a short C-terminal tail [5].

sHSPs exist in many plants and play an important role in growth, defense and stress resistance [6]. In rice (*Oryza sativa* L. ssp. japonica), there are 23 *sHsp* genes (16 cytoplasmic/nuclear, 2 ER, 3 mitochondrial, 1 plastid and 1 peroxisomal), 19 of which have been shown by microarray and RT-PCR analyses to be upregulated by high temperature stress [7]. In *Arabidopsis thaliana*, 13 *sHSP* genes were classified as CI, CII and CIII [8, 9], three of which (*AtHsp17.4, AtHsp17.6* and *AtHsp17.7*) accumulated during the middle stage of seed maturation, with concentrations maintained at high levels during the late stage and in immature dry seeds [10].

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DcHsp17.7, a sHSP in carrot (*Daucus carota* L.), performs molecular chaperone activity in salt-stressed transgenic *E. coli*, and is involved in tolerance not only to thermal stresses but also to other abiotic stresses, such as salinity [11]. *JcHSP-1 and JcHSP-2,* identified and characterized from developing seeds of a promising biodiesel feed stock plant *Jatropha curcas*, play an important role in cell protection and seed development during seed maturation [12]. Overexpression of alfalfa mitochondrial *HSP23* in prokaryotic and eukaryotic model systems confers enhanced tolerance to salinity and arsenic stress [13].

Tamarix hispida is a shrub or small tree growing mainly in arid and semi-arid regions, which exhibits tolerance to high temperature, salt and drought. These characteristics make *T. hispida* an ideal model plant to study the physiological and molecular mechanisms of trees responses to various stresses and for cloning tolerance related genes.

In the present study, nine *ThsHSP* genes were cloned from *T. hispida*. To better understand the roles of *ThsHSP* genes in abiotic stress tolerance, the expression profiles of these nine *ThsHSP* genes were investigated by real-time RT-PCR in the root and leaf tissue of *T. hispida* in response to salt (NaCl), drought (PEG), salt and drought together (NaCl and PEG), hormone (abscisic acid, ABA), cold (4 °C) and heat (36, 40, 44, 48 and 52 °C) stresses. Our study represents a foundation for the elucidation of the roles of *ThsHSP* genes in stress tolerance in plants.

Materials and methods

Plant material, growth conditions and stress treatments

Tamarix hispida seedlings were planted in plastic pots ($16 \times 16 \times 16$ cm^3) containing a mixture of turf peat and sand (2:1 v/v) in a greenhouse at 24 °C and 70–75 % relative humidity with light/dark cycles of 14/10 h (lights on at 7.00 AM). After two months, these seedlings were used for the following experimental analyses. For NaCl, PEG and ABA treatments, the seedlings were well watered at the roots with 0.4 M NaCl, 20 % (w/v) polyethylene glycol 6000 (PEG_{6000}), 0.4 M NaCl and 20 % PEG_{6000}, or 100 μM ABA for 0, 3, 6, 9, 12 and 24 h, respectively. For cold stress, the seedlings were subjected to 4 °C for 24 h, for heat stress, the seedlings were independently exposed to 36, 40, 44, 48, 52 °C for 2 h. The seedlings under normal physiological conditions (watered with water and placed at 24 °C) were as control. After each treatment, three samples (the leaves or roots from at least 20 seedlings each sample) were independently harvested and prepared for real-time PCR. Supplementary Fig. 1 showed the growth state of *T. hispida* seedlings used in this assay.

Cloning and identification of nine *ThsHSP* genes

Using Solexa technology, seven transcriptomes were constructed comprising four transcriptomes from root tissues of *T. hispida* treated with 0.3 M $NaHCO_3$ for 0, 12, 24 and 48 h and three from leaves treated with 0.3 M $NaHCO_3$ for 0, 12 and 24 h. A total of 94,361 non-redundant unigenes (NRUs) were assembled using TGI Clustering tools [14], and all NRUs were subjected to BLASTX analysis against protein databases, NR and Swiss-Prot, to search for similarities. Unigenes with BLASTX E-values exceeding 10^{-5} were discarded during functional annotation. The *sHSP* genes were searched and identified according to the functional annotations of NRUs.

Sequence alignment and phylogenetic analysis

Nine unique *sHSP* genes with the completed ORFs from 33 putative *ThsHSP* unigenes were obtained (designated as *ThsHSP*1–*ThsHSP9*). The Compute pI/MW tool (http://www.expasy.org/tools/protparam.html) was used to analysis the molecular weight (MW) and isoelectric point (pI) predictions for every deduced *ThsHSP*. All nine *ThsHSP* genes were aligned by ClustalX. For phylogenetic analysis, the nine ThsHSP proteins from *T. hispida*, a phylogenetic tree reconstruction was constructed employing the neighbor-joining (NJ) method in MEGA 4.0. Furthermore, the classification of the nine *ThsHSP* genes was carried out according to the phylogenetic tree using the classification and designation method of Bondino et al. [15].

RNA isolation and reverse-transcription (RT)

Total RNA was isolated from leaves or roots using the CTAB method [16] and digested with DNase I (TaKaRa, USA) to remove any DNA residue. The quality of all RNAs was confirmed by assessment of the purity of RNA samples by the 260/280 nm ratio and by a 1 % agarose gel electrophoresis of ethidium bromide (EB) stained samples. Samples were quantified by absorbance at 260 nm. Approximately 0.5 μg of DNaseI-treated total RNA was reverse-transcribed into cDNA using an oligodeoxythymidine primer and six random primers in a final reaction volume of 10 μL following the PrimeScript™ RT reagent Kit protocol (TaKaRa). The synthesized cDNAs were diluted to 100 μL with sterile water and used as templates for real-time RT-PCR analysis.

Real-time quantitative RT-PCR

Real-time RT-PCR was performed using a MJ Opticon™2 machine (Biorad, Hercules, CA, USA) with using a real-

Table 1 Primer sequences used for quantitative RT-PCR analysis

Gene	Forward Primers (5′–3′)	Reverse Primers (5′–3′)
ThsHSP1	GCCTCAAGAAGCCAAGGTGG	ACGGCGCATGGTTCGCATCG
ThsHSP2	ACAGCCTCTGCGCTCCCAAC	GGACGCTGCAGTTCGGGCT
ThsHSP3	AGCCGTCGAAACCCAAGGCTC	AACCTTCCACCACCATCACC
ThsHSP4	TTGAGTCAGCCACTGTTTCG	TAGTGGTAGTGTTAGCATCT
ThsHSP5	AAGCGCACATAATCAAGGCGGA	TCCATCGAAGCCTTGACATCCT
ThsHSP6	TCCGAAGACGCCAATTCTCC	ACGGAGGTGCCATTTCCCGC
ThsHSP7	AAGCACGCCTGCAGACATCAAA	ACGCCATCTTTCTCTTCATCCC
ThsHSP8	AGCTTCTTTGGTGGCCTGCG	GAGATCAGCCTTGAATATGTG
ThsHSP9	CGTGGCTTAGGCAGTGCGGT	AGATCGACTCTAGTCGAATAC
Actin	AAACAATGGCTGATGCTG	ACAATACCGTGCTCAATAGG
α-tubulin	CACCCACCGTTGTTCCAG	ACCGTCGTCATCTTCACC
β-tubulin	GGAAGCCATAGAAAGACC	CAACAAATGTGGGATGCT

Table 2 Characteristics of the nine ThsHSP from *T. hispida*

Gene	GenBank accession number	Type	Deduced number of amino acid	Isoelectric point	Molecular mass (kDa)
ThsHSP1	JX482105	CIII	231	5.83	25.69
ThsHSP2	JX482106	CIII	245	9.01	27.76
ThsHSP3	JX482107	CI SII	169	4.67	19.28
ThsHSP4	JX482108	CI SII	154	6.66	17.04
ThsHSP5	JX482109	CI	163	5.71	18.41
ThsHSP6	JX482110	CI SII	174	6.60	19.33
ThsHSP7	JX482111	CII	157	5.93	17.67
ThsHSP8	JX482112	CI	162	6.86	18.45
ThsHSP9	JX482113	CIII	127	5.33	14.41

time PCR MIX Kit (SYBR Green as the fluorescent dye, Toyobo). The gene and internal control primers chosen for real-time RT-PCR are shown in Table 1, in which the *alpha tubulin* (FJ618518), *beta tubulin* (FJ618519), and *Actin* (FJ618517) genes were used as internal controls (reference genes) to normalize the total RNA amount present in each reaction. The 20 μL reaction mixture contained 10 μL of SYBR Green Real-time PCR Master Mix (Toyobo), 2 μL cDNA template (equivalent to 100 ng of total RNA), 0.5 μM of each forward and reverse primer (Table 1). The following cycling parameters were applied for amplification: 94 °C for 30 s followed by 44 cycles at 94 °C for 12 s, 60 °C for 30 s, 72 °C for 40 s, and 1 s at 81 °C for plate reading. To ensure the reproducibility of the real-time PCR results, three independent experiments were carried out. The relative expression levels of the nine *ThsHSP* genes was calculated according to the $2^{-\Delta\Delta Ct}$ formula [17]. The SPSS software package (SPSS, Chicago, IL, USA) was used to analyze the data. The expression patterns of *ThsHSP* genes were clustered under various stress time points for each treatment using Cluster 3.0.

Results

Isolation and characterization of nine *ThsHSP* genes

Nine *ThsHSP* genes with complete open reading frames (ORFs) were identified from seven *T. hispida* transcriptomes. The ORFs encoded deduced polypeptides of 127–245 amino acids, with a predicted molecular mass of 14.4–27.7 kDa and pI 5.33–9.01 (Table 2).

Multiple alignments and phylogenetic relationships among the nine ThsHSP proteins were performed (Supplementary Figs. 2, 3). The results showed that these nine ThsHSP protein sequences shared homology from 14 to 82 %, with the *ThsHSP5* and *ThsHSP8* sharing the highest sequence homology (82 %) (Table 3). The results of BALASX with protein database in NCBI showed that 9 ThsHSP proteins were all ACD sHSP. ACD HSP proteins were classified into Monophyletic clade I which contains the cytosolic CI, CII and CIII sHSPs, even they maybe divided into smaller classes (such as CISI) based on the phylogenetic analysis by Bondino et al. [15]. Nine ThsHSP proteins were

Table 3 Sequence similarity among the 9 ThsHSP (%)

Gene	*ThsHSP1*	*ThsHSP2*	*ThsHSP3*	*ThsHSP4*	*ThsHSP5*	*ThsHSP6*	*ThsHSP7*	*ThsHSP8*
ThsHSP2	31							
ThsHSP3	14	20						
ThsHSP4	18	16	22					
ThsHSP5	21	25	33	26				
ThsHSP6	16	17	25	29	30			
ThsHSP7	19	22	15	26	29	25		
ThsHSP8	20	19	31	31	82	33	29	
ThsHSP9	29	33	27	22	28	24	22	30

phylogenetic analysed with the proteins classified in their groups, the results indicated that these nine *ThsHSPs* were belonged to different classes (Table 1). In particular, *ThsHSP1, 2* and *9* were belong to the CIII type, *ThsHSP3, 4* and *6* were to the CI SII type and *ThsHSP5* and *8* were the CI type; while *ThsHSP7* belonged to CII type.

Relative expression levels of *ThsHSP* genes in roots and leaves

Relative expression levels of nine *ThsHSP*s in roots and leaves under normal growth conditions were analyzed by real-time RT-PCR. To compare the expression levels of *ThsHSPs*, the transcription level of the *Actin* gene was arbitrarily assigned as 100 (Table 4). The results showed that all the *ThsHSP* genes (except *ThsHSP9*) were mainly expressed in roots rather than in leaves. In roots, the most abundant gene was *ThsHSP1,* with a transcription level of 239.8 relative to that of *Actin*, although the level was just 1.6 in leaves.

Table 4 Relative abundance of the nine *ThsHSP* genes in *T. hispida*

Gene	Relative abundance	
	Roots	Leaves
ThsHSP1	239.8	1.6
ThsHSP2	24.2	1.5
ThsHSP3	50.9	5.3
ThsHSP4	29.9	3.6
ThsHSP5	54.7	4.8
ThsHSP6	16.5	0.8
ThsHSP7	13.1	0.1
ThsHSP8	7.7	2.5
ThsHSP9	15.1	37.4
Actin	100	100

The transcription levels were plotted relative to the expression of *Actin* gene, and the transcription levels of *Actin* gene in root and leaf were all assigned as 100

Expression patterns of *ThsHSP* genes in response to various stresses

In order to investigate the expression profiles of *ThsHSPs* in *T. hispida* in response to various abiotic stresses, real-time RT-PCR analysis was carried out.

Temperature treatments

These nine *ThsHSP* genes were all upregulated by cold and high temperature treatments in roots and leaves. In roots, *ThsHSP1*, *ThsHSP2*, *ThsHSP3*, *ThsHSP4* and *ThsHSP9* shared similar expression patterns, reaching peak expression levels at 52 °C (Fig. 1). The highest expression levels of *ThsHSP5* and *ThsHSP6* occurred following treatment at 4 °C, followed by the levels detected in response to treatment for 52 °C. *ThsHSP7* and *ThsHSP8* exhibited highest expression levels at 44 °C (Fig. 1). In leaves, all nine *ThsHSPs* were induced at 4 °C, albeit at lower levels than those induced by other treatments. The transcription levels of *ThsHSP1*, *ThsHSP5* and *ThsHSP6* all reached a maximum following exposure to 44 °C, while *ThsHSP2* and *ThsHSP4* reached peak expression at 40 °C. *ThsHSP3*, *ThsHSP8* and *ThsHSP9* exhibited highest expression levels at 36 °C. *ThsHSP7* reached peak expression at 52 °C stress (Fig. 1).

NaCl stress

In roots, *ThsHSP3* and *ThsHSP*4 expression patterns were consistent. In generally, the expression levels were slowly increasing with the stress time. At the beginning stress time (3 h), the expressions were inhibited, but no significant difference compared with the control. At the other times they were upregulated. *ThsHSP8* and *ThsHSP*9 expressions were obviously induced at 3 h. However, they were highly downregulated at 12 h, when they reached their lowest expression level (Fig. 2). The other 5 *ThsHSP* genes were downregulated at the early stress period (12 h), with expression levels subsequently increased at a later stage.

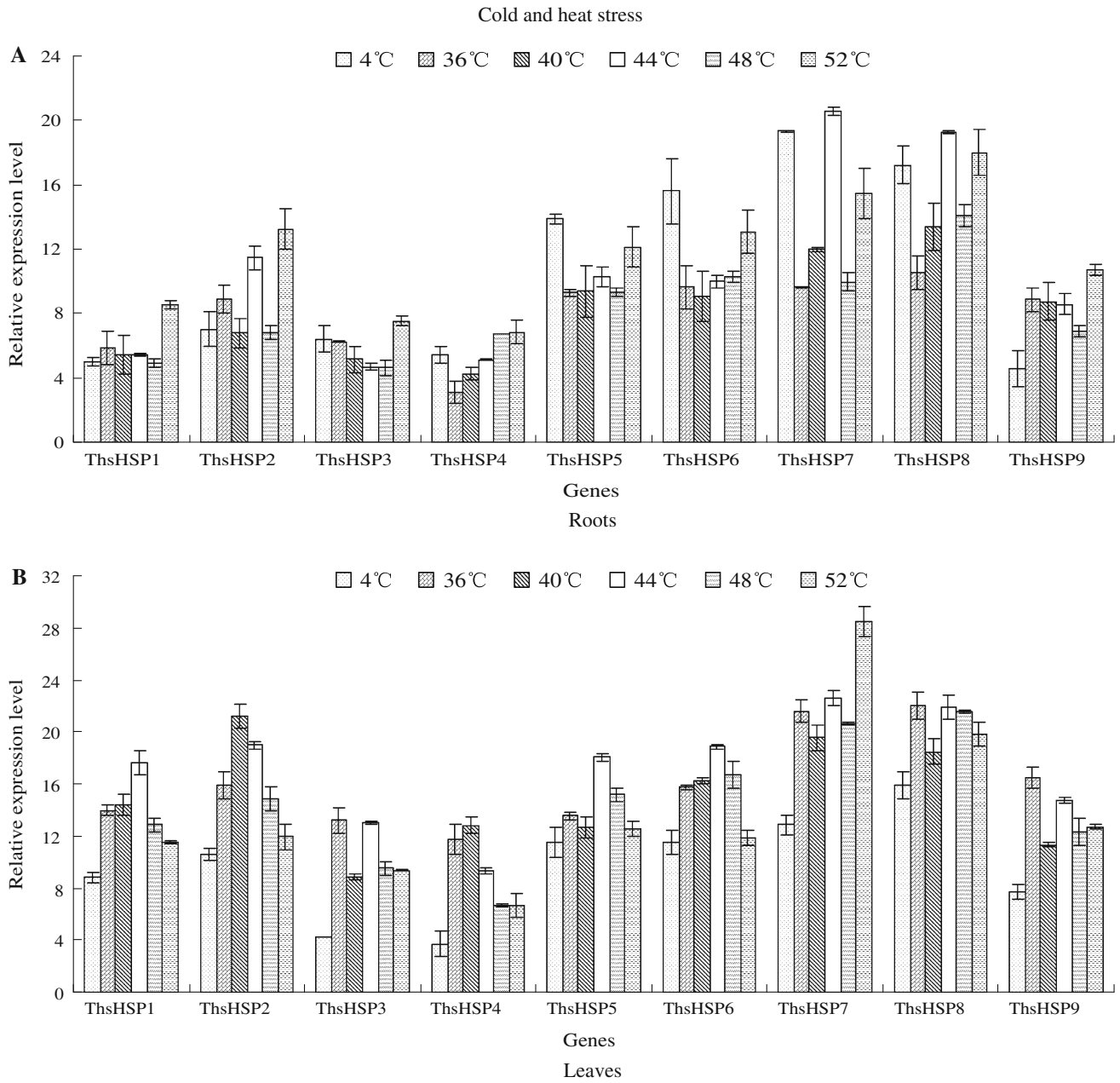

Fig. 1 Transcription analysis of the nine *ThsHSPs* responding to cold and heat shock in roots and leaves. The relative transcription level = transcription level under stress treatment/transcription level under control condition (0 h). All relative transcription levels were log2-transformed. **a** Roots; **b** Leaves

Following NaCl stress for 24 h, all *ThsHSP* genes were upregulated, with 6 *ThsHSP* genes reaching peak expression level at this time-point. In leaves, *ThsHSP8* and *ThsHSP*9 were downregulated after NaCl stress for 0–12 h, and were upregulated at 24 h. The remaining 7 *ThsHSP* genes were predominantly upregulated during the stress period. Interestingly, all nine genes reached the highest transcription levels at 24 h. The most highly induced gene was *ThsHSP3* (337.8-fold at 24 h) (Fig. 2).

PEG stress

In roots, all *ThsHSP* genes (except *ThsHSP6*) were mainly downregulated under PEG stress, especially *ThsHSP1*, *ThsHSP7*, *ThsHSP8* and *ThsHSP9*, for which highly downregulated expression was detected during the PEG stress period, with the lowest expression levels at 9 h. Compared with the expression levels at 0 h, *ThsHSP1*, *ThsHSP7*, *ThsHSP8* and *ThsHSP9* expression was

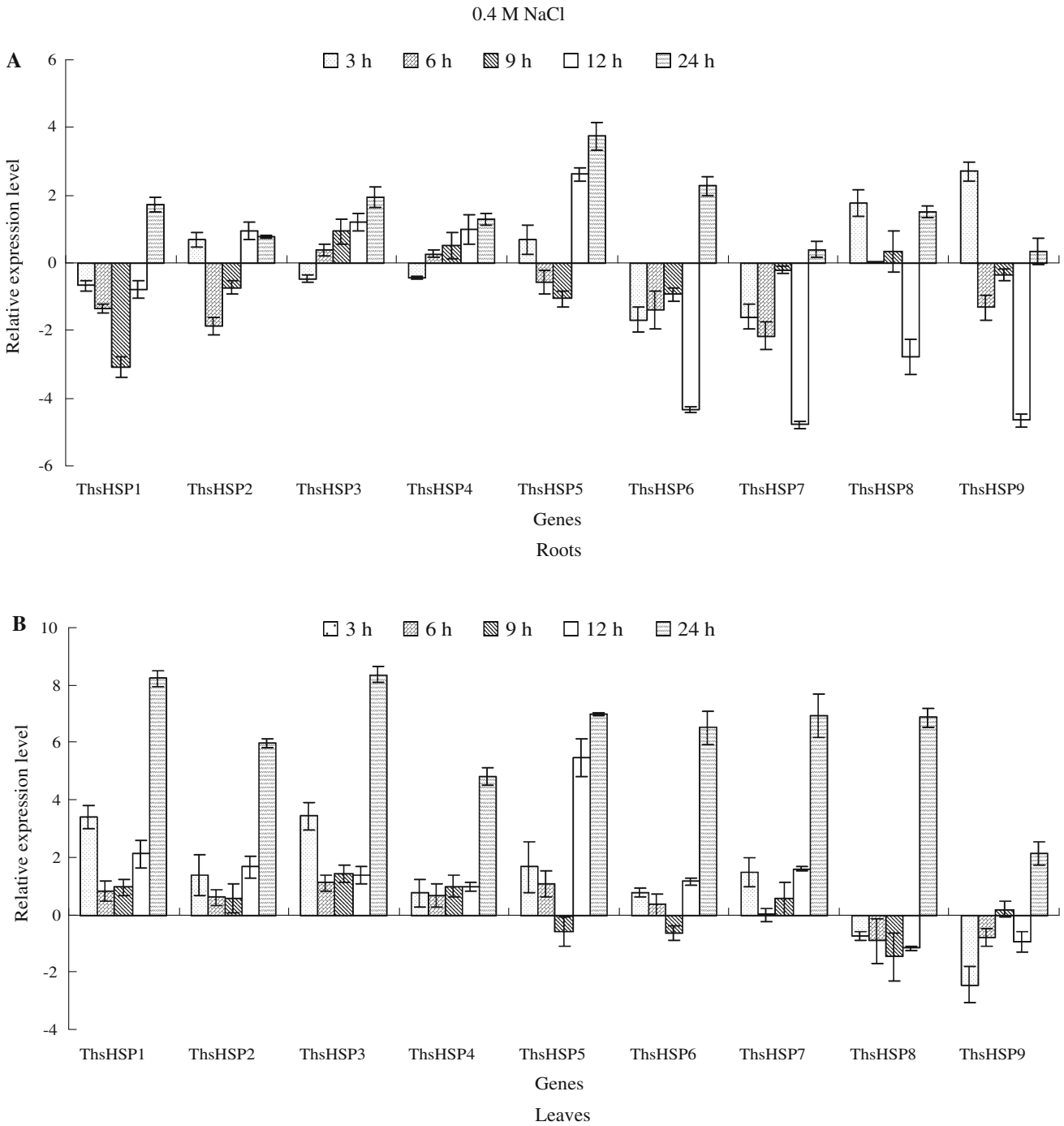

Fig. 2 Transcription analysis of the nine *ThsHSPs* responding to NaCl in roots and leaves. The relative transcription level = transcription level under stress treatment/transcription level under control condition (0 h). All relative transcription levels were log2-transformed. **a** Roots; **b** Leaves

decreased by 10.2, 1.4, 18.9 and 2.1 % at 9 h, respectively (Fig. 3). In leaves, the expression levels of the nine *ThsHSP* genes were divided into two main groups (Supplementary Fig. 4). One group comprised *ThsHSP1*, *ThsHSP2*, *ThsHSP3*, *ThsHSP5*, *ThsHSP6* and *ThsHSP7*, which were induced at most stress time-points. The other group comprised *ThsHSP4*, *ThsHSP8* and *ThsHSP9*, which were mainly downregulated (Fig. 3).

NaCl and PEG stress

In roots, all *ThsHSP* genes were highly up-regulated at all treated time points. However, their induced expression patterns and levels were diversity. *ThsHSP2* induced expression level was highest (15.6–133.6-fold of control). And the maximum expression level was at the 12 h. At the 9 h, the expression also was more than 102-fold. *ThsHSP1*,

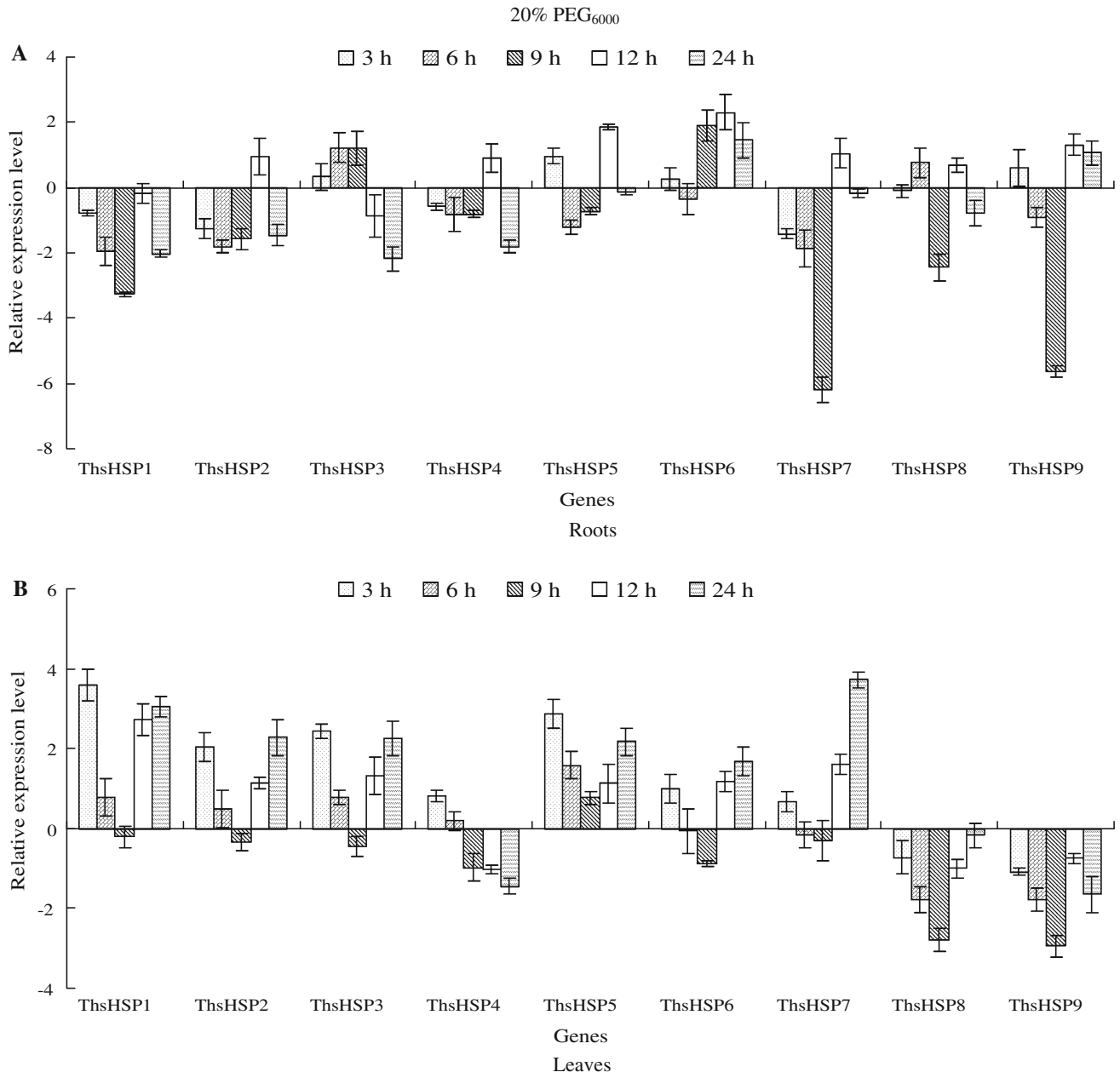

Fig. 3 Transcription analysis of the nine ThsHSPs responding to PEG in roots and leaves. The relative transcription level = transcription level under stress treatment/transcription level under control condition (0 h). All relative transcription levels were log2-transformed. **a** Roots; **b** leaves

ThsHSP6, *ThsHSP7* and *ThsHSP9* reached peak at 9 h, while *ThsHSP3*, *ThsHSP4*, *ThsHSP5* and *ThsHSP8* displayed their peak at 3 h or 24 h. In leaves, all the *ThsHSP* genes (except the *ThsHSP4*) also were highly upregulated at all stress time points. And other 7 *ThsHSP* genes reached their highest induced expression levels at 3 h. The most highly induced folds were 24.4–236.3. The *ThsHSP4* expression was downregulated at the beginning stress period (3 h). At the other stress times, the expression levels of *ThsHSP* we higher than control and the highest expression level was 33.8-fold of the control (12 h) (Fig. 5).

ABA treatment

In roots, all nine *ThsHSPs* were notably downregulated at most time-points. In particular, *ThsHSP* 1, 4, and 7 were downregulated during the stress period. Furthermore, the expression levels of *ThsHSP* 7 and 9 at 12 h were decreased by 2.7 and 2.5 % of the levels detected at 0 h. In leaves, the expression patterns of the *ThsHSP* genes were generally divided into three groups. One group contained *ThsHSP* 8 and 9, for which downregulated expression was detected at most time-points. The second group consisted

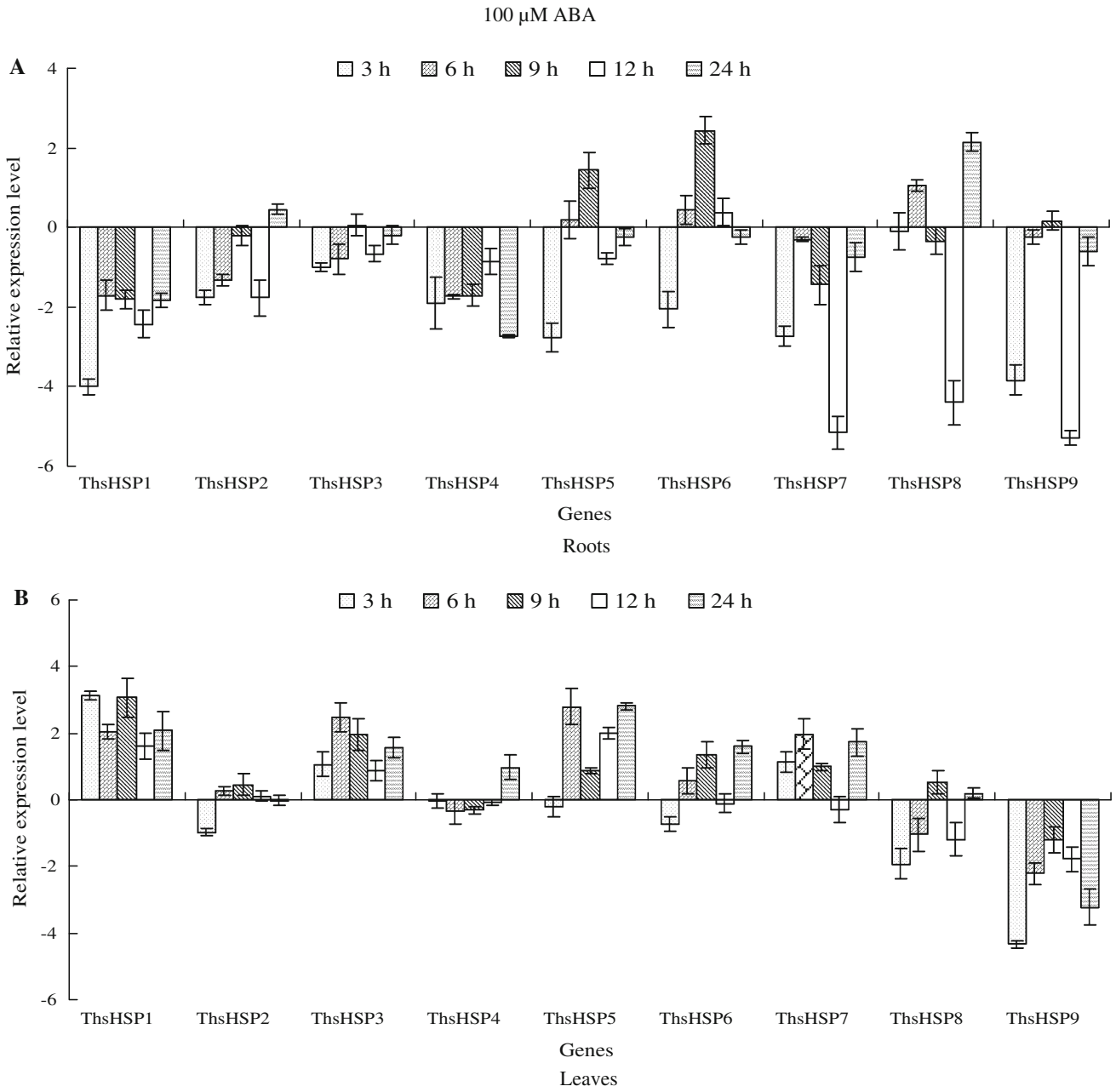

Fig. 4 Transcription analysis of the nine ThsHSPs responding to abscisic acid (ABA) in roots and leaves. The relative transcription level = transcription level under stress treatment/transcription level under control condition (0 h). All relative transcription levels were log2-transformed. **a** Roots; **b** leaves

of *ThsHSP*2 and *ThsHSP4*, for which expression levels were not noticeably altered during the stress period. The remaining *ThsHSP* genes constituted the third group, which were mainly upregulated after ABA treatment (Fig. 4).

Discussion

Most sHSPs cannot be detected in the vegetative tissues under normal growth conditions, but are rapidly produced exposed to heat [6]. However, it is interesting that, in this study, nine *ThsHSP* genes were showed varied transcript abundance in roots and leaves under normal conditions. Eight *ThsHSP* genes were highly expressed in roots compared with leaves, while the expression level of *ThsHSP9* was higher in leaves than in roots (Table 4), suggesting different *ThsHSPs* genes play different role in roots and leaves.

The accumulation extent of sHSP under heat stress depends on the temperature and the duration of the stress

period [18]. And sHsps may be important for plant recovery after the heat stress has been released [19]. Definitely, when *T. hispida* seedlings were treated with various temperatures, all *ThsHSP* genes were induced remarkably. Especially when temperature higher than 44 °C, they were induced more notable. The results are consistent with reports that elevated temperature enhances *HSP* genes expression levels in many other plant species [20–22]. Guan et al. [23]. showed that eight *sHSP-CI* genes in rice (*Oryza sativa* Tainung No. 67) were induced strongly after a 2-h heat shock treatment. However, the sHSP genes on chromosome 3 were induced rapidly at 32 and 41 °C, whereas those on chromosome 1 were induced slowly by similar conditions. With the onset of treatment at 41 °C some *sHSP-CI* genes were induced within only 5 min, although expression of all nine *sHSP-CI* genes was detected after 15 min. In Arabidopsis, heat stress treatment at 40 °C, induced expression of some *HSP70s* by 2–20-fold [21]. Zou et al. [24] also showed that transcripts of all nine *OsHSP* genes investigated in rice increased under heat shock treatment. These results demonstrate that heat shock is a basic stimulus for the response of sHSP.

However, heat shock is not the only stimulus to trigger the expression and protein synthesis *sHSP*. Some *sHsps* are in response to osmotic and salt stress. The expression of *OsHSP23.7* in rice (*O. sativa* L.) was increased during salt stress treatment, and *OsHSP24.1* gene was enhanced following treatment with 10 %PEG [24]. Upon exposure to 0.3 M NaCl, the *DcHsp17.7* protein level of carrot (*Daucus carota* L.), increased dramatically in leaf tissue (14-fold) [11]. *At-HSP17.6A* and *At-HSP17.6-II* were induced by 0.2 M NaCl and 20 % PEG with similar kinetics in Arabidopsis [25]. In *Rosa chinensis*, *RcHSP17.8* was induced by 0.3 M NaCl, 10 % polyethylene, glycol and 0.4 M mannitol [26], *OsHsfC1b* was induced in *O. sativa* roots after 30 min of salt stress, while expression was downregulated in roots by mannitol treatment [27]. In current study, under NaCl treatment, *ThsHSP* genes showed various expression patterns at the treat points in roots, while in leaves, the expression of these nine *ThsHSPs* were mainly up-regulated and showed their

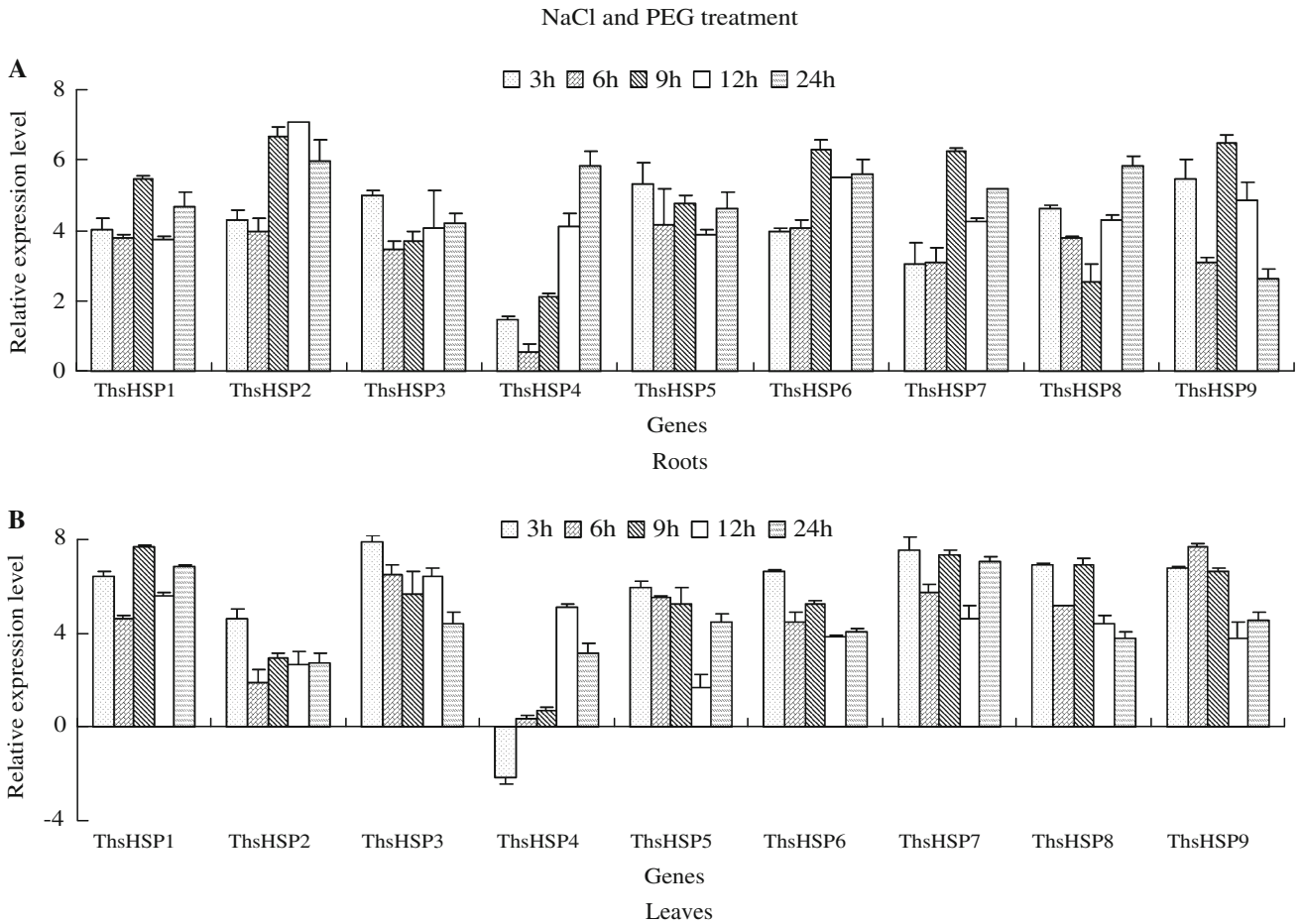

Fig. 5 Transcription analysis of the nine *ThsHSPs* responding to co-NaCl and PEG treatment in roots and leaves. The relative transcription level = transcription level under stress treatment/transcription level under control condition (0 h). All relative transcription levels were log2-transformed. **a** Roots; **b** Leaves

maximum expression level at 24 h. Under PEG stress, the expression of *ThsHSPs* were showed similar patters to NaCl (Figs. 2, 3). Furthermore, when treated with NaCl and PEG together, *T. hispida* showed higher and more stable inducement in roots and leaves (Fig. 5), suggesting that salt and/or PEG may share the same induce pathway for *ThsHSPs*.

Exogenous ABA is an important stimulus for sHSP. Previous reports have shown that some HSPs are ABA responsive genes, which occur in many species including tobacco [28], sunflower [29, 30], bean [31], wheat [32] and maize [33]. In the current study, all *ThsHSPs* were down-regulated in roots under ABA treatment. In leaves, the expression patterns of these genes could be divided into distinct three types, including induced, decreased and no obvious change. These results suggest that the expression of *ThsHSP* genes is involved in ABA-dependent stress signal transduction pathways. However, these genes may play different roles in the ABA signaling pathway in stress responses in root and leaves.

The expression patterns of the *ThsHSP* genes under various abiotic stresses indicated that *ThsHSP* genes were induced by heat shock, NaCl, PEG and ABA, suggesting the *ThsHSP* genes maybe involve in the *T. hispida* responding to these stresses. But the tolerance mechanisms of *ThsHSPs* response to these stresses were still unknown. The previous study showed that the half-life of HSP70 mRNA after transcription was only 15 to 30 min, but under heat shock conditions it is able to keep for 4 h, and inferred that HSP70 was aimed to keep the organism from harm by enhancing the stability and preferential translation of mRNA [34]. Given the fact that sHSP and HSP70 were all belong to HSP superfamily, *ThsHSPs* may be concern in such mechanism as HSP70.

Some sHSPs have been suggested to act as molecular chaperone in vitro and in vivo [35, 36], and sHSP chaperone activities are ATP independent [37], i.e., sHSPs can keep them in a state competent, selectively bind nonnative proteins and avoid aggregation for ATP dependent refolding by other chaperones [36]. Experiments have demonstrated that sHSPs from diverse organisms are particularly effective in avoiding thermal aggregation of other proteins by an ATP-independent mechanism [37]. Furthermore, abiotic stress including heat, osmotic and salt are always accompanied to generate with oxidative stress, while an increasing proper level of the reactive oxygen species (ROS) at least in part in favour of mediating various environmental stresses [38]. HSP70 acts as chaperone by effectively controlling the release of protein binding and correctly folding of nascent polypeptide chains, selectively participating in degradation of some damage protein and membrane transport protein [39]. Overexpression of rice mitochondrial HSP70 inhibits heat and oxidation induced apoptosis, and this inhibition is achieved by maintaining the stability of the mitochondrial membrane potential and inhibiting of reactive oxygen diffusion [40]. Under combined drought and high temperature stresses, HSP101 can increase corn leaves antioxidant defense capacity [41]. In the current study, nine *ThsHSP* genes were induced under NaCl, PEG, ABA, heat shock treatments, however, whether these nine *ThsHSP* genes are involved with ROS scavenging and how they interact, whether they are ATP-dependent regulation genes remain to be answered, and a better understanding of the mechanism of *ThsHSP* might provide further powerful information to modify their stress tolerance.

In conclusion, nine *ThsHSP* genes with complete ORFs were cloned from *T. hispida*. Expression analysis showed that these *ThsHSP* genes were expressed more highly in roots than in the leaves, suggesting that they may play more major roles in roots than in leaves. Furthermore, these nine *ThsHSP*s are all associated with heat shock, PEG and NaCl stresses and are involved in the ABA signaling pathway, and displayed a complex regulation pathway, indicating that some of these genes have potential roles in the genetic improvement of abiotic stress tolerance in plants. Our study may lay an important basis for revealing the function of *ThsHSP* in response to abiotic stress and provides essential information for selection of candidate genes used for stress tolerance genetic engineering in future studies.

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