African Journal of Biotechnology Vol. 8 (12), pp. 2667-2676, 17 June, 2009
Available online at http://www.academicjournals.org/AJB
ISSN 1684–5315 © 2009 Academic Journals

*Full Length Research Paper*

# Molecular responses and expression analysis of genes in a xerophytic desert shrub *Haloxylon ammodendron* (Chenopodiaceae) to environmental stresses

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Accepted 6 January, 2009

***Haloxylon ammodendron* (C.A Mey.) Bunge is a xero-halophytic desert shrub with excellent drought resistance and salt tolerance. To decipher the molecular responses involved in its drought resistance, the cDNA-AFLP (amplified fragment length polymorphism) technique was employed to identify genes expressed differentially in the leaves following drought treatment in the seedlings of *H. ammodendron*. Eighty-six non-redundant TDFs (transcript-derived fragments) were identified as drought responsive after verified by reverse northern. Of these, 49 TDFs showed significant homology to genes with known or predicted function; 6 TDFs were homologous to unknown genes, while 31 TDFs did not show any significant matches. 10 TDFs were selected to further validate the cDNA-AFLP expression patterns by the semi-quantitative RT-PCR. 57% of TDFs corresponding to proteins of known or putative functions are likely to participate in signal transduction, transcription regulation, protein synthesis, senescence, transport, cell wall synthesis, stress and defense response, development and growth, photosynthesis, and so on. Moreover, not many functions of these genes have been reported in plants adaptation to unfavorable conditions. The spatial and temporal expression patterns of the tested genes displayed several distinct patterns in response to osmotic stress, desiccation stress and application of exogenous ABA. The results provided general insights into the molecular adaptation mechanisms involved in this desert shrub's response to desert conditions.**

**Key words:** ABA, cDNA-AFLP, Drought, Desert shrub, *Haloxylon ammodendron*.

## INTRODUCTION

Strategies for plants adaptation to abiotic stress have been extensively studied at the physiological, biochemical and molecular levels in the model plant, *Arabidopsis* (Shinozaki and Yamaguchi-Shinozaki, 1997; Zhu, 2001). But it is still difficult to find in this glycophyte for some novel processes or mechanisms unique to naturally stress-tolerant plants, halophytes and xerophytes (Zhu, 2001), resurrection plants (Mundree et al., 2002), under extreme conditions.

Deserts represent one of the harshest ecosystems on earth, combining drought and extreme temperatures, and trees, as dominant populations, play important roles in the sometimes also high salt (Brosché et al., 2005). Desert maintenance of the structure and function of the whole ecosystem of desert land (Sheng et al., 2005). Molecular mechanisms of adaptation to desert conditions have been studied in the desert shrub *Retama raetam* (Mittler et al., 2001; Pnueli et al., 2002) and desert arbor *Populus euphratica* (Brosché et al., 2005; Ottow et al., 2005; Wang et al., 2008), whereas the two desert trees are likely to use the different acclimation strategies under desert conditions (Brosché et al., 2005), which may be

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unique to these desert plants (Zhu, 2001). So it is necessary for the genetic improvement of tree to understand the molecular mechanisms involved in stress responses and stress adaptation in trees but not solely in crops and model plants (Dubos et al., 2003).

Today, desertification, a global problem, is still expanding at a rapid pace at present and affects 25% of the land surface (Sheng et al., 2005). To fight desertification and alleviate the effects of drought, efforts are undertaken to enhance the drought tolerance of important plants by traditional breeding as well as gene-engineering approaches (Ottow et al., 2005). So the use of native plants under extreme conditions in ecological restoration has attracted much attention recently.

*Haloxylon ammodendron* (C.A Mey.) Bunge, a stem-assimilating, xero-halophytic desert shrub, belongs to *Haloxylon* genus (Chenopodiaceae) distributing naturally in Asian and African deserts (Pyankov et al., 1999; Tobe et al., 2000). Due to its great drought resistance and salt tolerance, *H. ammodendron* is one of the main tree species used for ecological restoration in the northwest desert land of China (Sheng et al., 2005). Over the past decades, anatomical and physiological adaptations of this desert shrub have been extensively studied, such as the changes of sugar and proline (Jiang et al., 2001), betaine (Chen et al., 2001), $Na^+$ and $NO^{3-}$ (Song et al., 2005; Song et al., 2006), and protective enzymes, SOD (Zhang and Li, 1994), CAT and POD (Yao et al., 1997) under drought conditions. However, little is known about the molecular mechanism that enables this shrub to withstand harsh desert environments (Jiang et al., 2004).

The main objective of our study was to isolate drought-responsive genes and decipher the molecular responses involved in *H. ammodendron*'s response to drought. Drought treatment was established following 0 days, 10 days and 25 days without watering in eight-week old seedlings grown in pots. Due to the significant increase in the endogenous ABA content under drought stress for 25 days, the leaves between 0 days and 25 days were used to identify genes that exhibited differential expression at the transcriptional level by using the cDNA-AFLP (amplified fragment length polymorphism) fingerprinting technique (Bachem et al., 1996; Bachem et al., 1998), which has been successfully applied to identify the drought-responsive genes in wood plants, *Pinus pinaster* (Dubos et al., 2003) and *Phillyrea latifolia* (Paolacci et al., 2007).

## MATERIALS AND METHODS

### Plant material

Seeds of *H. ammodendron* were sterilized with 0.3% $KnMO_4$ for 30 min, and then washed cleanly with water. For the experiment of physiological determination and cDNA-AFLP analysis, three groups of seeds were sowed in pots with sand and vermiculite (6:1), respectively. The seedlings were grown for eight weeks in well-watered conditions, and then the drought treatment was applied. First, watering was stopped for one group of seedlings, and the other groups were watered every three days. Fifteen days later, watering of another group was stopped, while the last group was still watered every three days. After 25 days, the drought stress was established at 25 days, 10 days and 0 days. The samples were collected and quickly frozen in liquid nitrogen. For the experiment of spatial and temporal expression, the seeds were transferred to wetted gauze placed on top of the pots, which were filled with water in a growth chamber with a 14/10 h photoperiod and a day/night temperature of 23/18°C. The seedlings were grown for two weeks, and the water in the pots was refreshed every two days. Subsequently, osmotic stress was imposed by adding PEG6000 (polyethylene glycol 6000); desiccation stress was imposed by exposing the seedlings to the air, and exogenous ABA was applied at different concentrations.

### RNA isolation and mRNA purification

The total RNA was extracted with Trizol reagent (Invitrogen, USA). Poly $(A)^+$ RNA was isolated from the total RNA with polyATract mRNA isolation system kit (Promega, USA) according to the manufacturer's instructions.

### cDNA-AFLP analysis

The cDNA-AFLP procedure was performed according to the method described by Bachem et al. (1996) with some modifications. First-strand and second-strand cDNAs were synthesized according to the standard protocols (Sambrook and Russell, 2001). After purification, the cDNA was digested with EcoRI and MseI and ligated to respective adaptors according to an AFLP core reagent kit (Invitrogen, USA). Selective amplification products were separated on 5% polyacrylamide gels, and then the gels were stained by the silver staining method (Chalhoub et al., 1997).

### Isolation and sequencing of TDFs

Bands expressed differentially were cut and extracted. The eluted cDNA of the extracts was re-amplified and purified, and then cloned into the pGEM-T vector (Promega, USA) and sequenced with ABI3730 at the Shanghai Sangon (Sangon, China).

### Reverse northern dot-blotting

TDFs were amplified by PCR using T7 and SP6 promoter primer of pGEM-T vector. The PCR products were diluted to a final concentration of 0.1 g/µl, denatured with NaOH (0.2 M final concentration) at 100°C for 10 min, and spotted onto nylon membranes (Hybond-N, Amersham Pharmacia, USA) using an Eppendorf eight-well apparatus. Each selected TDF was spotted on 2 different membranes. Mem-branes were baked for 2 h at 80°C. Total RNAs used for the cDNA-AFLP analysis were used to prepare probes labeled with DIG-11-dUTP by reverse transcription using SuperScript III (Invitrogen, USA). Positive hybridisation was detected by colouration of sample spots on the membrane with NBT-BCIP according to the manufacturer's instructions (Roche, USA).

**Table 1.** Primers for semi-quantitative RT-PCR.

| cDNA | Upstream primers | Downstream primers | Size (bp) |
|---|---|---|---|
| HaDR2 | 5'-ACTAACTGTGGGCAAATCCG-3' | 5'-GAATGTTTTCACACACCCTGC-3' | 187 |
| HaDR5 | 5'-TCAGATTTTGGTTTGAGTGC-3' | 5'-TTTTGTACGATGACATGAGG-3' | 216 |
| HaDR6 | 5'-ACGGGTCTACACCTTCAA-3' | 5'-CGAGAAGAAGAAGAACACCC-3' | 232 |
| HaDR9 | 5'-CACTTGTAAAAGGCTCTGGA 3' | 5'-CTGAGGGCACAAATGTCTGA-3' | 195 |
| HaDR22 | 5'-AGGGGACCCACTTCCTAATC-3' | 5'-CTTGCTTTGCCTCAACTGCC-3' | 407 |
| HaDR24 | 5'-GTTAGTGTTGCCATTGAAGC-3' | 5'-TCATCAGTGCCATAACCAAC-3' | 120 |
| HaDR26 | 5'-CTGTTGCTGGTTCCCTTGA-3' | 5'-GGCCTCCTTCTCCATTCTG-3' | 158 |
| HaDR27 | 5'-AGGCAAACATGAGTGGAACC-3 | 5'-ATCGAACCAGTTTCCTTGTG-3' | 181 |
| HaDR29 | 5'-ATCGGTTCACTTCTGAGCAT-3' | 5'-TTGGAGTTGGAAGCCATATC-3' | 210 |
| HaDR35 | 5'-CCCACACATTCACAAACAAC-3' | 5'- TTCAGTGGGAAACAAGGGT-3' | 191 |
| HaDR43 | 5'-AATCACGCTTAGTAGCCTGG-3' | 5'-GAGTTGGAACACGCATTTG-3' | 248 |
| HaDR44 | 5'-AAAGTTGGTGGCAATGGAG-3' | 5'-AAGGTATGATGGGGTCTGC-3' | 129 |
| 18S rRNA | 5'-ACCATAAACGATGCCGG-3' | 5'-CACCACCCATAGAATCAAGA-3' | 350 |

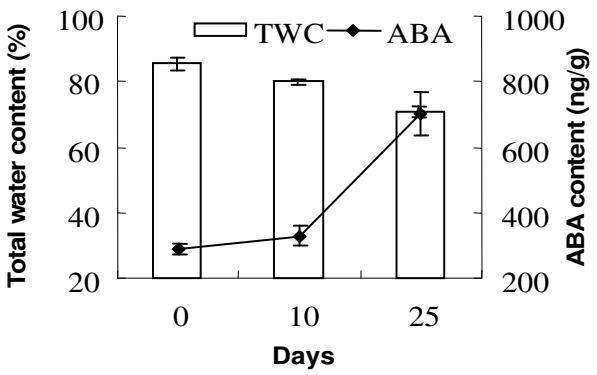


**Figure 1.** Changes of endogenous ABA in the leaves of *H. ammodendron* seedlings response to drought stress. The data are representative of three separate experiments.

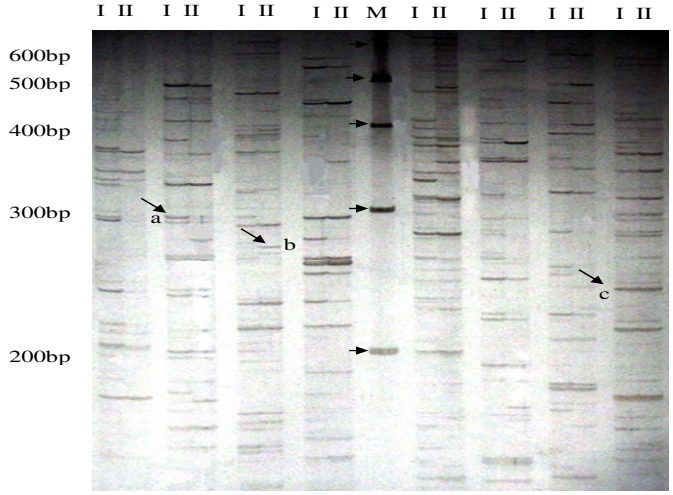


**Figure 2.** cDNA-AFLP finger-printing of cDNA of eight-week seedlings response to soil drought stress. I and II represented 0-day and 25-day treatment, respectively. M: 100 bp marker. a, b and c was bands of genes expression that were downregulated, upregulated and constitutive, respectively, under drought stress in the cDNA-AFLP profile.

#### Semi-quantitative RT-PCR

The total RNAs of young seedlings in various stress treatments were used as templates and the first-strand cDNAs were synthesized according to the SuperScript III First-Strand Synthesis System (Invitrogen, USA). The selected TDFs were amplified with different cycles. 18S rRNA was amplified as the internal control. The PCR primers were synthesized from Shanghai Sangon (Sangon, China; Table 1). All PCR product intensities were confirmed by separation on 1.5% agarose gel.

#### Determination of endogenous ABA

The water content of the leaves was measured with a drying method. The endogenous ABA content was measured by the HPLC method at the Chinese Academy of Forestry Analysis Center.

## RESULTS

### Analysis of drought-responsive genes using cDNA-AFLP

Drought stress induces the increase of endogenous ABA content, and elicits the expression of ABA- or stress-related genes (Finkelstein et al., 2002). In this experiment, endogenous ABA increased 13.3 and 141.9%, respectively, at the treatment of 10 and 25 days compared to 0 days in the leaves of *H. ammodendron* (Figure 1). So, 0- and 25 day treatment was used to cDNA-AFLP analysis to identify differentially expressed genes of the leaves of *H. ammodendron* response to drought stress.

Out of 426 isolated TDFs up- and down- regulated from cDNA-AFLP profiles (Figure 2), 256 were sequenced successfully and analysed for their sequences. In total, eighty-six non-redundant TDFs were obtained (Table 2) after the verification by the Reverse Northern (Figure 3).

**Table 2.** Homologies of TDFs to reported proteins in GenBank. The homologies of TDFs were analyzed using the BLAST program and E-values < 0.001 were considered to have significant similarities, TDFs of *Ha*DR56- *Ha*DR86 (E-values > 0.001) were not implicated.

| TDF[a] | Expression | Size (bp) | Function of prediction | GenBank Hit |
|---|---|---|---|---|
| **Signal Transduction** | | | | |
| *Ha*DR1 | U | 210 | Putative calcium-dependent protein kinase | AT1G12680 |
| *Ha*DR2 | U | 271 | Small Ran-related GTP-binding protein | AT5G55190 |
| *Ha*DR3 | U | 243 | Polyglutamine tract-binding protein | AT2G41020 |
| *Ha*DR4 | D | 94 | Calcineurin B-like protein | AT3G18430 |
| *Ha*DR5 | D | 287 | CBL-interacting protein kinase 23 | AT1G30270 |
| *Ha*DR6 | D | 299 | Putative lung seven transmembrane receptor 1 | XP_493716 |
| *Ha*DR7 | D | 297 | Putative lung seven transmembrane receptor 1 | XP_493716 |
| **Transcription regulation** | | | | |
| *Ha*DR8 | U | 128 | HMG transcription factor | AT1G20696 |
| *Ha*DR9 | U | 264 | bZIP transcription factor ATB2 | AT4G34590 |
| *Ha*DR10 | U | 173 | bZIP transcription factor | AT2G18160 |
| *Ha*DR11 | U | 128 | Chromomethylase | AT4G19020 |
| *Ha*DR12 | U | 289 | DNA-dependent ATPase | AT5G18620 |
| *Ha*DR13 | U | 135 | ATP-dependent RNA helicase A, putative | AT2G01130 |
| *Ha*DR14 | U | 171 | Histone deacetylase (HDAC) | AT5G61060 |
| *Ha*DR15 | U | 273 | RNA helicase/RNAseIII | AT3G20420 |
| *Ha*DR16 | U | 250 | RNA helicase SDE3 | AT1G05460 |
| *Ha*DR17 | U | 177 | F-box family protein / SKIP3 | AT3G53000 |
| **Protein synthesis** | | | | |
| *Ha*DR18 | U | 113 | TIF3B1 | AT5G25780 |
| *Ha*DR19 | U | 315 | Chloroplast-encoded 23S ribosomal RNA | ATCG01180 |
| *Ha*DR20 | D | 195 | Arginyl-tRNA synthetase | AT4G26300 |
| *Ha*DR21 | D | 181 | Nucleotidyltransferase family protein | AT1G17980 |
| **Senescence** | | | | |
| *Ha*DR22 | U | 144 | Prohibitin, putative | AT5G40770 |
| *Ha*DR23 | U | 209 | Putative ribosomal S29 protein | AT3G43980 |
| *Ha*DR24 | D | 309 | Cysteine proteinase | AT5G43060 |
| *Ha*DR25 | D | 331 | Serine protease-like protein | BAD36430 |
| *Ha*DR26 | D | 394 | Senescence-associated protein | AT1G32400 |
| **Transporter** | | | | |
| *Ha*DR27 | U | 234 | PDR-like ABC transporter | AT1G59870 |
| *Ha*DR28 | U | 135 | ABC1 family protein | AT1G71810 |
| *Ha*DR29 | U | 289 | Amino acid transporter | AT2G41190 |
| *Ha*DR30 | U | 185 | Sugar transporter | AT4G35300 |
| **Cell wall synthesis** | | | | |
| *Ha*DR31 | U | 164 | Putative (1-4)-beta-mannan endohydrolase | AT4G28320 |
| *Ha*DR32 | U | 225 | UDP-D-apiose/UDP-D-xylose synthase | AT2G27860 |
| *Ha*DR33 | D | 198 | Proline-rich family protein | AT5G26080 |
| **Stress and defense responses** | | | | |
| *Ha*DR34 | U | 278 | UDP-glycosyltransferase | AT1G22340 |
| *Ha*DR35 | U | 297 | Cytochrome P450 family protein | AT3G26330 |
| *Ha*DR36 | U | 262 | GDSL-motif lipase | AT5G37690 |
| *Ha*DR37 | D | 301 | Aldo/keto reductase | AT1G60680 |
| *Ha*DR38 | D | 340 | Oxidoreductase | AT1G16720 |
| *Ha*DR39 | D | 206 | Dehydration-responsive protein | AT4G14360 |

**Table 2.** Contd.

| | | | | |
|---|---|---|---|---|
| **Development and growth** | | | | |
| *Ha*DR40 | U | 130 | Dem protein | AT4G33400 |
| *Ha*DR41 | U | 118 | Profilin (pro1 gene) | AT2G19760 |
| *Ha*DR42 | D | 223 | Tubulin folding cofactor D | AT3G60740 |
| **Photosynthesis** | | | | |
| *Ha*DR43 | D | 372 | RbcS small subunit 1A | AT1G67090 |
| *Ha*DR44 | D | 157 | Chlorophyll a/b-binding protein type II | AT2G05070 |
| *Ha*DR45 | D | 148 | Chlorophyll a/b-binding protein type II | AT2G05070 |
| *Ha*DR46 | D | 437 | Chloroplast NAD-dependent malate dehydrogenase | AT3G47520 |
| **Other metabolism** | | | | |
| *Ha*DR47 | U | 199 | Alpha-glucosidase | AT5G11720 |
| *Ha*DR48 | U | 184 | Acyl-CoA thioesterase/catalytic/ hydrolase | AT2G29590 |
| *Ha*DR49 | D | 153 | Transferase | AT4G15390 |
| **Function-unknown protein** | | | | |
| *Ha*DR50 | U | 166 | Unknown protein | AT3G24080 |
| *Ha*DR51 | U | 230 | Unknown protein | AT5G39590 |
| *Ha*DR52 | U | 184 | Unknown protein | AT3G19190 |
| *Ha*DR53 | D | 235 | Unknown protein | AT3G09980 |
| *Ha*DR54 | D | 202 | Unknown protein | AT3G15840 |
| *Ha*DR55 | D | 226 | Unknown protein | AT1G21760 |

Blast analysis showed that 55 of them had significant homology to the sequences in GenBank (E-value <0.001), whereas 49 corresponded to known or predicted proteins that had been reported in various species, 6 correspon-ded to unknown or hypothetical proteins, and 31 the others did not match any sequences (E-value > 0.001). Ten TDFs from various groups were further conformed by semi-quantitative RT-PCR and showed the same expression profile as shown in the cDNA-AFLP analysis (Figure 4). In total, 52 genes were upregulated, and 34 genes were downregulated when the seedlings were subjected to drought stress for 25 days (Table 2). TDFs had been categorized as belong-ing to two general groups: regulatory genes and functional genes, based on their predicted functions. The former included signal transduction and transcription regulation (*Ha*DR1-17); the latter included protein synthesis, sene-scence, transporters, cell wall, stress and defense res-ponses, development and growth, photosynthesis, and so on (*Ha*DR18- 49; Table 2). The large group of regula-tory genes (35% of the 49 known or predicted genes) suggested that *H. ammodendron* can rapidly initiate the regulatory system and control the expression of drought resistance-related genes. Also, many unknown genes (*Ha*DR50-*Ha*DR86), 43% of the total, had not been reported, and these genes may play important roles in the response of *H. ammodendron* to desert environments.

## Osmotic stress, desiccation and hormonal regulation of gene expression

Many studies have explored plant responses to stresses and ABA in an attempt to understand stress signaling and stress tolerance mechanisms. To determine temporal expression patterns of drought-responsive genes of *H. ammodendron* under different stress factors, osmotic stress and desiccation stress were applied on two-week seedlings for an extended period of time in order to indicate the role of each gene during the successive stages of stress. Several groups (regulatory, transporter, senescence and photosynthesis) of them were considered in the present study (Figure 5a and b). The first five (*Ha*DR5, *Ha*DR9, *Ha*DR22, *Ha*DR27 and *Ha*DR29) of the tested genes behaved similarly and upregulated in the two stress conditions, but the desiccation treatment obviously hastened the expression of genes at the transcriptional levels prior to the osmotic treatment. However, the expression of the last three genes (*Ha*DR26, *Ha*DR43, and *Ha*DR44) decreased compared to the above, except for *Ha*DR44 under osmotic stress. The result showed that *H. ammodendron* may start different regulatory mechanism when exposed to a combination of environmental stress conditions.

ABA is produced under water stress conditions and plays pivotal roles from water stress perception to activetion of genes in response to drought stress (Finkelstein et al., 2002; Boudsocq and Lauriere, 2005). Drought stress prompted the significant increase of endogenous ABA of *H. ammodendron* in our determination (Figure 1). Thus, we carried out the study of gene expression by the application of exogenous ABA on two-week seedlings. The expression patterns of the selected genes except for *Ha*DR29 were affected significantly at the transcription level (Figure 5). Of these genes, *Ha*DR5, *Ha*DR9,

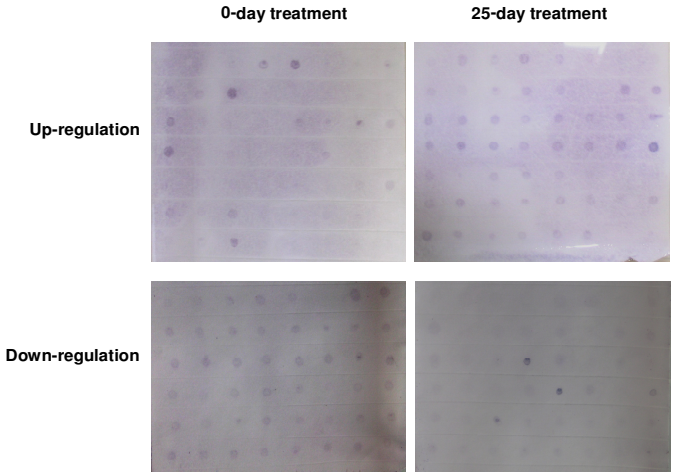


**Figure 3.** Verification of expression patterns of TDFs from cDNA-AFLP profile by Rerverse Northern Dot-Blotting. TDFs were spotted on 2 different nylon membranes and probes were labeled with DIG-11-dUTP. Left probe: Reverse transcription labeling total RNA of 0-day treatment; Right probe: Reverse transcription labeling total RNA of 25-day treatment.

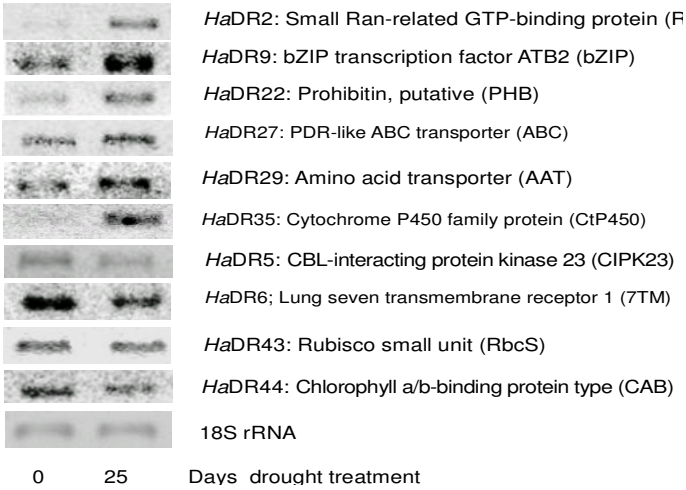


**Figure 4.** Semi-quantitative RT-PCR analysis of selected TDFs in response to soil drought stress. The selected TDFs included: *Ha*DR2, *Ha*DR5, *Ha*DR6, *Ha*DR9, *Ha*DR22, *Ha*DR27, *Ha*DR29, *Ha*DR35, *Ha*DR43 and *Ha*DR44. 18S rRNA is shown as an internal control. The data are representative of two separate experiments and at least four repeats with similar final results.

*Ha*DR22, *Ha*DR24, *Ha*DR27, *Ha*DR43 and *Ha*DR44 were upregulated, and only *Ha*DR26 was downregulated. Among of these selected genes, most were regulated significantly by ABA. Taken together, it is evident that ABA, as a stress signal hormone, has great influence in resisting unfavorable conditions in *H. ammodendron*.

### Spatial expression patterns of regulatory gene during osmotic stress

Two important regulatory genes, *Ha*DR5 (CIPK23) and *Ha*DR9 (bZIP), whose expression increased significantly in PEG-treated leaves of *H. ammodendron* (Figure 5a), were selected to be analyzed the spatial patterns of gene expression. Indeed, the expression of the two genes showed different spatial patterns in the different organs (Figure 6). In normal conditions, the *Ha*DR5 mRNA levels had some degree of basal expression in all organs. In contrast to this, the *Ha*DR9 mRNA levels were markedly higher in the stems and roots than in the leaves. With the increase in the intensity of osmotic stress, the expression patterns of the two selected genes also showed completely distinct patterns from each other. The expression of *Ha*DR5 was induced at mild stress (5% PEG concentration) in all organs, but their transcription levels stayed higher in the leaves than in the stems and roots, throughout all the stress stages, whereas the expression of *Ha*DR9 was still contrary to that of *Ha*DR5 (Figure 6). This result suggested that the two regulatory genes may play different roles in the leaves and roots under osmotic stress.

## DISCUSSION

To survive in the extreme deserts with little precipitation, *H. ammodendron* must have a set of unique strategy for stress response and stress adaptation: perceiving rapidly a stress stimulus, switching on signal transduction pathways and resulting in physiological changes in the plant cell (Luan et al., 2002; Chinnusamy et al., 2004). Thus TDFs of several groups implicated in this process were further discussed.

### Genes potentially involved in signaling in stress response of *H. ammodendron*

In our study, 35% of the known or predicted genes were regulatory genes including signal transduction and transcription regulation, and this maybe ensure *H. ammedendron* to promptly adapt to the changing conditions by initiating the regulatory system. We isolated three TDFs of $Ca^{2+}$-related proteins, which are the main component of $Ca^{2+}$ signaling pathways (Luan et al., 2002). Of them, one TDF (*Ha*DR1) encoding a calcium-dependent protein kinase (CDPK), was prompted at mRNA level in the drought-treated 25 days, and the homologs of this gene have been characterized in

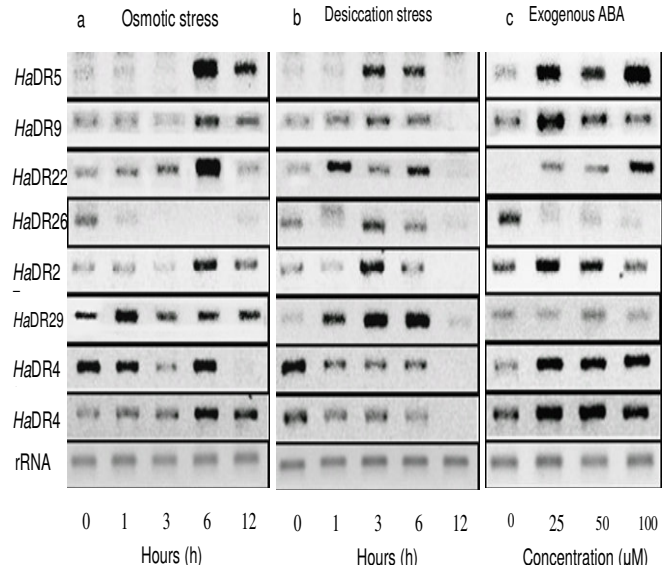


**Figure 5.** Effects of osmotic stress (10% PEG; a), desiccation stress (b) and exogenous application of abscisic acid (c) on the expression profiles of various drought-responsive genes in the leaves of two-week seedlings, which were analysed by semi-quantitative RT-PCR. The selected TDFs included four groups: regulatory genes (*Ha*DR5, *Ha*DR9); senescence-associated genes (*Ha*DR22, *Ha*DR26); transporters (*Ha*DR27, *Ha*DR29) and photosynthesis-associated genes (*Ha*DR43, *Ha*DR44). 18S rRNA was shown as an internal control. The data are representative of two separate experiments and at least six repeats with similar final results.

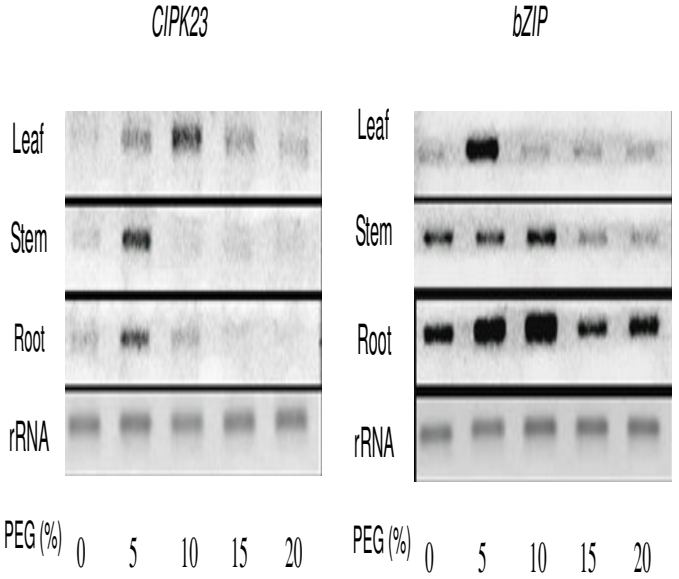


**Figure 6.** Spatial expression of drought-responsive regulatory genes, *CIPK23* (*Ha*DR5) and *bZIP* (*Ha*DR9), which were analysed by semi-quantitative RT-PCR, in the leaf, stem and root when two-week seedlings were subjected to osmotic stress at different PEG6000 concentrations for 9 h. 18S rRNA was shown as an internal control. The data are representative of two separate experiments and at least six repeats with similar final results.

*Arabidopsis* and ice plant, in which they were correlated with ABA- or stress-induced gene expression (Urao et al., 1994; Patharkar and Cushman, 2000). The genes, however, encoding calci-neurin B-like protein (CBL; *Ha*DR4) and CBL-interacting protein kinase (CIPK23; *Ha*DR5), respectively, were found to be downregulated under the same condition. The latter can be activated by CBL binding $Ca^{2+}$, and then regulate the responses of downstream genes (Luan et al., 2002; Boudsocq and Lauriere, 2005; Xu et al., 2006). However, the pattern of *Ha*DR5 in drought-treated 25 days was contrary to that of the test of osmotic and desiccation stress, where showed the expression of *Ha*DR5 (*Ha*DR1 and *Ha*DR4 not amplified successfully) could be initiated rapidly at the early treatment (Figure 5a and b), and also increased significantly by exogenous ABA (Figure 5c). The results suggested that *Ha*DR5, as a component of CBL-CIPK23 pathways, functions in the early response of genes. In addition to three $Ca^{2+}$-related genes, we found one TDF (*Ha*DR3) encoding polyglutamine tract-binding protein (PQBP) that includes WW and $C_2$ domains known to function in signal transduction by interacting with $Ca^{2+}$ in mammals (Waragai et al., 1999). It appeared to prove that $Ca^{2+}$ and $Ca^{2+}$-regulated genes may play an important role in *H. ammodendron* response to adverse conditions. Additionally, Two TDFs (*Ha*DR6, *Ha*DR7) encoded unknown proteins containing the lung_7-TM_R conserved domain in *Arabidopsis*, which had lower homology to the G protein-coupled receptor 107 in *Homo sapiens*. In our opinion, the two TDFs were considered to be necessary components of signal transduction in stress response of *H. ammodendron*.

## Genes potentially involved in transcription regulation in stress response of *H. ammodendron*

Transcription activation and gene silencing play critical roles in controlling the states of gene activity in most eukaryotic organisms (Cao et al., 2000). In this study, all the genes of transcription regulation (*Ha*DR8-*Ha*DR17) were upregulated in drought-treated 25 days. Our experiment demonstrated that drought stress induced the expression of two TDFs (*Ha*DR8 and *Ha*DR9) encoding bZIP transcription factors which regulate the processes of stress response (Jakoby et al., 2002). Semi-quantitative RT-PCR demonstrated that *Ha*DR9 increased drastically when exogenous ABA was applied (Figure 5c). In *Arabidopsis*, only one bZIP subfamily has been linked

genetically to the ABA response in 81 predicted bZIP factors (Finkelstein et al., 2002), which suggested that *Ha*DR9 may act as an ABA-responsive gene in *H. ammodendron* response to stress conditions. Another TDF (*Ha*DR10) encoded the HMG transcription factor regulating transcription and cell differentiation by modulating DNA structures (Grosschedl et al., 1994). However, its function in the stress response had not been reported. In addition to the above transcription factors, *Ha*DR11 encoding DNA-dependent ATPase (Björklund et al., 1999) and *Ha*DR12 encoding ATP-dependent RNA helicase A (Aratani et al., 2001) could also prompt the transcription of genomic DNA by transcription activation. Yet their functions in stress responses had not been reported either. On the other hand, we obtained some genes that can trigger gene silencing. Two TDFs of *Ha*DR13 (chromomethylase, CMT) and *Ha*DR14 (histone deacetylase, HDAC) can lead to gene silencing at the transcriptional level (Cao et al., 2000; Tian and Chen, 2001). Whereas *Ha*DR15 (RNA helicase/RNAseIII) and *Ha*DR16 (RNA helicase SDS3) are required for post-transcriptional gene silencing (Harfe et al., 2005; Dalmay et al., 2001). RNA helicase/RNAseIII, constituting the RNaseIII-containing enzyme Dicer, is considered to be required for the processing of microRNAs and small interfering RNA (Harfe et al., 2005). Similarly, RNA helicase (SDS3) is also required for post-transcriptional gene silencing in *Arabidopsis* (Dalmay et al., 2001). But only HDAC has been reported to be involved in ABA and abiotic stress responses, and enhances the tolerance of salt and drought (Sridha and Wu, 2006). The results suggested that TDFs involved in gene silencing may benefit *H. ammodendron* to adapt to desert climes.

**Senescence-associated genes may delay senescence of *H. ammodendron***

Many genes expressed in senescing leaves are also found in stressed leaves (Gepstein et al., 2003). Some of them can delay the process of senescence and maintain cell viability (Buchanan-Wollaston, 1997). *Ha*DR22 encoded a Prohibitin (PHB) protein which has been shown to play an important role in senescence and maintenance of mitochondrial integrity in animals and plants (Piper et al., 2002; Chen et al., 2005). In *Petunia phb* mutants, smaller and distorted leaves are observed and the lifespan of flowers are shorter than that of controls (Chen et al., 2005). In *H. ammodendron*, drought stress induced the upregulation of *PBH*. Besides, the mRNA level of *PBH* increased rapidly at the early treatment of PEG and dehydration (Figure 5a and b). Especially, one-hour dehydration treatment promptly triggered *PBH* expression, which suggested *PHB* may be an early dehydration responsive gene, and also an ABA-responsive gene (Figure 5c). The result indicated the *PHB* gene of *H. ammodendron* may have similar functions as mentioned above, because the leaves did not show significant wilt syndrome in our observation under 25 day drought treat-ment (data not shown). Another unknown senescence-associated gene (SAG; *Ha*DR26), which downregulated in drought-treated 25 days, also inhibited significantly by PEG- and ABA- treatment (Figure 5a and c). One rea-sonable explanation may be that 'turnoff' of *Ha*DR26 gene can reduce unnecessary dissipation of *H. ammodendron* under the extreme desert conditions.

**Transportation of useful or toxic metabolites**

Transporters are used to transport useful or toxic metabolites when plants are subjected to abiotic stresses. In this study, the expression of all transporter TDFs was elicited under drought stress. Of them, two TDFs (*Ha*DR27, *Ha*DR28) encoded ABC transporters. The former, the highest homology to PDR-ABC, contributes to transport and eliminate damaged matter during the stress process (Crouzet et al., 2006). Whereas the func-tion of *Ha*DR28 encoding the ABC1 transporter, which is required for anions transportation in animals (Becq et al., 1997), had not been reported in plants, including its functions in various stress processes. The rest two transporters, amino acid transporter encoded by *Ha*DR29 and sugar transporter encoded by *Ha*DR30, benefits to the accumulation of amino acids, quaternary amino compounds or sugars when subjected to stress conditions (Bohnert and Cushman, 2002; Mundree et al., 2002; Wang et al., 2004). In our study, the upregulation of *Ha*DR29 and *Ha*DR30 may increase transmembrane movements of these osmotic matters under drought stress. But amino acid transporter *Ha*DR29 was not affected by the application of ABA (Figure 5c).

**Conclusion**

In conclusion, we have conducted a relatively comprehensive study of gene identification related to a dominant desert shrub, *H. ammodendron*. The functions of many genes have not been previously shown in relation to the stress response in plants, such as *Ha*DR3, *Ha*DR6 and *Ha*DR7 (signal transduction), *Ha*DR10 (transcription factor), *Ha*DR15 and *Ha*DR16 (gene silencing), *Ha*DR26 (senescence), *Ha*DR28 (ABC transporter1). The results demonstrated that *H. ammodendron*, a desert shrub, may hold the key to specific drought-resistance pathways in nature. Our study also indicated that this shrub is well suited for the investigation of drought resistance, and useful genes can be further studied by comparing to genome of the model plant *Arabidopsis*.

## ACKNOWLEDGEMENTS

We thank the National Program for Key Basic Research Projects in China (G1999016000; 2009CB119100) and China Postdoctoral Science Foundation (20070410578) for financial supports, Dr Janice Willson for proofreading this manuscript.

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