Plant Growth Regul (2015) 75:549–556
DOI 10.1007/s10725-014-0020-z

ORIGINAL PAPER

Expression of rice gene *OsMSR4* confers decreased ABA sensitivity and improved drought tolerance in *Arabidopsis thaliana*

Xuming Yin · Lifang Huang · Xin Zhang · Manling Wang · Guoyun Xu · Xinjie Xia

Received: 11 May 2014 / Accepted: 21 December 2014 / Published online: 30 December 2014

Abstract The small heat shock proteins (sHSPs) are most prevalent in plants and are believed to play an important role in stress tolerance. Our microarray and qRT-PCR analyses of rice plants showed that the gene *Oryza sativa Multi-Stress-Responsive 4* (*OsMSR-4*) is induced by heat, drought, and cold in different tissues at various developmental stages. *OsMSR-4* encodes a Class III sHSP. Its expression in *Arabidopsis thaliana* conferred enhanced tolerance to drought accompanied by altered expression of other stress-related genes. Under drought conditions, levels of free proline were higher in transgenic plants than in the wild-type. The transgenics also showed decreased sensitivity to abscisic acid (ABA) during the seed germination and post-germination stages. Our study provides evidence that *OsMSR4* has a key role in regulating plant responses to ABA and drought.

Keywords Small heat shock protein gene · Drought · ABA · *Arabidopsis* · *OsMSR4*

Abbreviations

ABA	Abscisic acid
ACD	"a-crystallin" domain
MS	Murashige and Skoog
NLS	Nuclear localization signal
ORF	Open reading frame
OsMSR-4	*Oryza sativa Multi-Stress-Responsive Gene 4*
qRT-PCR	Quantitative real-time PCR
RT-PCR	Reverse-transcription PCR
sHSPs	Small heat shock proteins
WT	Wild type

Introduction

Abiotic stress usually leads to the dysfunction of proteins. Thus, maintaining their appropriate conformation and preventing the aggregation of non-native proteins are particularly important for cell survival in plants growing under stress conditions. Small heat shock proteins (sHSPs) act as molecular chaperones, facilitating the synthesis and folding of proteins in many normal cellular processes. Thus, they may play a crucial role in protecting plants against stress by re-establishing normal protein conformation (Wang et al. 2003, 2004). Based on their approximate molecular masses, HSPs are divided into five families: HSP100s (105 ± 5 kDa), HSP90s (approximately 90 kDa), HSP70s (73 ± 5 kDa), HSP60s (60 ± 5 kDa), and sHSPs (≤40 kDa) (Trent 1996). The sHSPs are perhaps the most widespread members, with monomers ranging in size from 12 to 40 kDa. Bioinformatics analysis has revealed that an average sHSP has 161 amino acids, and is characterized by a C-terminal "a-crystallin" domain (ACD) that consists of 90 amino acids (Basha et al. 2012). Plant sHSPs are divided into six classes. Classes I, II,

Electronic supplementary material The online version of this article (doi:10.1007/s10725-014-0020-z) contains supplementary material, which is available to authorized users.

X. Yin · L. Huang · M. Wang · G. Xu · X. Xia (✉)
Laboratory for Agro-ecological Process in Subtropical Region, Institute of Subtropical Agriculture, Chinese Academy of Sciences, Hunan 410125, People's Republic of China
e-mail: jxxia@isa.ac.cn

X. Yin
e-mail: yingxuming304@163.com

X. Zhang
Graduate School of the Chinese Academy of Sciences, Beijing 100049, People's Republic of China

and III are localized in the cytosol or nucleus while CIV through CVI occur in the plastids, endoplasmic reticulum, and mitochondria (Mahmood et al. 2010).

The sHSPs are synthesized ubiquitously in prokaryotic and eukaryotic cells in response to heat and other abiotic stresses. In tolerant plants, sHSPs can act as molecular chaperones and bind target proteins that have become damaged (Horwitz 1992). Expression patterns for some sHSPs can vary according to tissue type or stage of development (Waters et al. 1996). This includes most sHSPs from rice (Sarkar et al. 2009). Zou et al. (2009) have reported that rice sHSPs display different levels of expression in response to NaCl or mannitol treatment. Furthermore, transgenic rice plants over-expressing OsHSP17.0 and OsHSP23.7 show greater tolerance to drought and salt stresses when compared with the WT (Zou et al. 2012).

The phytohormone abscisic acid (ABA) plays a crucial role in regulating plant responses to abiotic stress and in controlling seed germination, plant growth, and stomatal behavior (Verslues and Zhu 2007). Expression of some sHSP genes from rice differs in response to ABA treatment. For example, transcripts of OsHSP18.03 are more abundant in the presence of ABA while levels of those for OsHSP24.1 are decreased (Zou et al. 2009; Ye et al. 2012).

To investigate the expression of several abiotic stress-related genes, we used leaves and panicles sampled from 'Pei'ai 64S' rice at various stages of development and under different stress conditions. In particular, *OsMSR4* (*Oryza sativa Multi-Stress-Responsive 4*) encodes a Class III sHSP and is highly induced under cold, drought, and heat treatments. We also examined the role that *OsMSR4* might have in regulating the response of transgenic *Arabidopsis* to exogenous ABA and drought stress.

Materials and methods

Plant materials, growing conditions, and stress treatments

Seeds of rice (*Oryza sativa* L. ssp. *indica*) cultivar Pei'ai 64S were treated with 0.1 % $HgCl_2$ (w/v) for 10 min and washed three times with sterile water. The sterilized seeds were kept in distilled water for 72 h at 25 °C in the dark, then germinated at 37 °C under darkness for 48–72 h. The germinants were transferred to plastic pots (20 cm tall, 10 cm in diameter) containing soil and placed in a growth chamber at 28 °C/22 °C (day/night) under long-day conditions (16-h photoperiod). For microarray analysis, stress treatments were applied at the five-leaf, booting, and heading stages. Drought stress was imposed by spilling excess water out of the pots and then withholding further irrigation. The leaves of rice at the booting stage and panicles at the booting and heading stages were harvested 16 h after their leaves began to curl. For the cold-stress experiments, rice plants at the five-leaf, booting, and heading were placed in a PCG15.5 Percival growth chamber (USA) for 12 h where conditions included 4 °C and darkness. For heat stress treatments, rice plants at the booting and heading stages were placed in the same type of chamber for 2 h with conditions of 45 °C and darkness. The unstressed control plants remained in the standard growing environment described above.

In a separate trial, seeds of *Arabidopsis thaliana* (Columbia 0) were surface-sterilized with 10 % bleach (v/v) for 25 min and washed five times with distilled water. The sterilized seeds were sown on a ½-strength Murashige and Skoog (MS) medium supplemented with 1 % (w/v) sucrose and 0.8 % (w/v) agar (pH 5.8). When seedlings achieved the four-leaf stage, they were transplanted into soil and placed in a growth room at 26 °C/22 °C (day/night), under a 16-h photoperiod.

Three previously recognized stress-related genes *RD29A*, *P5CS1*, and *NCED3* were monitored in our qRT-PCR analysis. Three-week-old *Arabidopsis* seedlings were immersed in ½-strength MS solutions containing standard MS components (unstressed control), 10 % polyethylene glycol (PEG) for drought treatment, or 100 μmol L^{-1} ABA. Samples were collected after 2 h of exposure.

Microarray analysis

Total RNAs were isolated from rice tissues using TRIzol reagent (Invitrogen). RNA samples were processed according to instructions from the technical manual for Affymetrix GeneChip expression analysis (Affymetrix, Inc.). CRNA was synthesized and labeled using an Affymetrix GeneChip IVT Labeling Kit, and was hybridized to the probe sets (Affymetrix Rice Genome Array; http://www:Affymetrix.com//rice.affx). After the GeneChip array was washed and stained, it was scanned using an Affymetrix GeneChip Scanner 30007G. The microarray data were first analyzed via GeneChip Operating Software (GCOS1.2). Further analyses were performed with the R/Bioconductor (http://www.bioconductor.org/). In particular, Robust Multi-Array Average and probe sequence information, known as gcRNA, was used for correcting and normalizing the microarray background (Irrizary et al. 2003; Cope et al. 2004). Each experiment was repeated three times.

Quantitative real-time PCR (qRT-PCR) analysis

The DNase-treated RNAs were employed for first-strand cDNA synthesis, using Superscript II reverse transcriptase (Invitrogen) according to the manufacturer's instructions. Our qRT-PCR analysis of *OsMSR4*, *NCED3*, *RD29A*, and *P5CS1*

expression was performed with a Platinum® SYBR® Green qPCR SuperMix-UDG (Invitrogen). The gene for 18S rRNA was used as the endogenous control. All primer pairs are listed in Table 1 of electronic supplementary material. Each qRT-PCR was conducted according to the manufacturer's instructions in an ABI 7900HT (Applied Biosystems, Foster City, CA, USA) under the following conditions: 95 °C for 10 min, then 40 cycles of 95 °C for 15 s and 58 °C for 40 s, with a final 20 s at 72 °C. The data were analyzed via the comparative *Ct* method. Amplification experiments were conducted in triplicate, and dissociation curve analysis (60–95 °C) was done to verify the fidelity of the amplification. PlantCARE software (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) was used to identify *cis*-elements related to stress responses in the putative promoter region of *OsMSR4*.

Vector construction and *Arabidopsis* transformation

To construct the plant transformation vector, we utilized reverse-transcription PCR (RT-PCR) to amplify a cDNA fragment containing the entire open reading frame (ORF) of *OsMSR4* from 'Pei'ai 64S' rice. Forward primer 5′-CCATGGAAATGGCGGACCAGCTCTCC-3′ and reverse primer 5′-GGATCCTTACAGGATCACGCACTTCTGGC-3′ were used (the underlined bases indicate restriction sites Nco *I* and BamH *I*, respectively). After the PCR fragment was obtained, it was sub-cloned into the pMD18-T (Takara) vector and sequenced. Following sequence verification, the pMD18-T plasmid harboring the cloned cDNA was digested by Nco *I* and BamH *I* and ligated into vector pJIT163, which carries the double 35S promoter. The resulting plasmid containing the cloned cDNA was digested with Kpn *I* and Xho *I*, and the purified DNA fragment was ligated into the pCAMBIA1300 plasmid, downstream of the 35S promoter between the Kpn *I* and Sal *I* sites.

The construct was introduced into *Agrobacterium tumefaciens* strain GV3101. *Agrobacterium*-mediated transformation of *Arabidopsis* plants was performed via the floral dip method (Clough and Bent 1998), with some modifications. Transgenic *Arabidopsis* seedlings were verified by plating seeds on plant nutrient agar plates supplemented with 25 mg L^{-1} hygromycin. Transgenic lines displaying a segregation ratio of 3:1 (resistant:sensitive) were selected to produce T3 seeds. Those that exhibited 100 % resistance to hygromycin were used for further experiments.

Stress tolerance assays for *Arabidopsis*

For the germination assays, seeds from WT *Arabidopsis* and two transgenic lines were sown in triplicate on Petri dishes containing 1/2 MS media and different concentrations of either mannitol (0, 200, 250, 300, or 325 mM) or ABA (0–0.6 μM). The seeds were stratified by incubating them at 4 °C for 2 days to synchronize germination before the dishes were transferred to a growth room. To test drought tolerance, germination was recorded after 6 days based on radicle emergence. For determining ABA sensitivity, we recorded the presence of seedlings with leaves as a percentage of total seed sown at 10 days after transfer.

Root growth was monitored in response to drought treatment. Briefly, *Arabidopsis* seeds were germinated on 1/2 MS agar media in Petri dishes containing different concentrations (0, 225, 275, or 300 mM) of mannitol. Dishes were placed on shelves in a vertical orientation to facilitate comparisons of their root development.

To induce drought stress, 3-week-old plants were transferred to a growth chamber under normal illumination. Standard irrigation was withheld for 12 days before watering resumed for 7 days.

Proline measurements

Free proline concentrations in leaf extracts from drought-stressed transgenic and WT *Arabidopsis* were determined as described by Bates et al. (1973). For each sample, the measurement was repeated three times.

Statistical analyses

All data were examined by ANOVA, using the SAS statistics program. Differences in values were considered statistically significant at $P = 0.05$.

Results

Expression analysis of *OsMSR4*

To identify the genes related to abiotic stresses, we used Affymetrix rice genome arrays for 'Pei'ai 64S' rice. Our data from this microarray analysis showed that imposition of drought increased the expression of *OsMSR4* by 17.4- and 20.0-fold in leaves and panicles, respectively, at the booting stage. Heat stress (45 °C) caused expression in the leaves to rise by 45.6- and 18.0-fold at the booting and heading stages, respectively. Results from qRT-PCR analysis revealed patterns of expression similar to those found in our microarray analysis (Supplemental Fig. S1). This suggested that *OsMSR4* is a multiple stress-responsive gene in rice.

OsMSR4 encodes a Class III sHSP

To study the possible roles of *OsMSR4*, we used RT-PCR to clone a corresponding cDNA containing its ORF from 'Pei'ai 64S' rice. Sequence analysis showed that this cDNA was 829 bp long. Comparison of the cDNA and its corresponding

genomic DNA sequence from GenBank showed that *OsMSR4* contains one intron and is located on Chromosome 2. Several putative *cis*-elements related to stress responses were identified in the putative promoter region of *OsMSR4*, approximately 1 kb upstream of the putative TATA box. They included MBS, involved in drought-inducibility, and an RY-element that functions in seed-specific regulation (Chen et al. 2012).

The *OsMSR4* cDNA sequence encodes a protein of 172 amino acid residues with a calculated molecular mass of 18.6 kDa and a pI of 8.448. No putative functional domain was found in the protein sequence except for the ACD predicted by InterProScan (http://www.ebi.ac.uk/InterProScan/). Alignment of the deduced amino acid sequence from *OsMSR4* with other representative CIII sHSPs showed that identities ranged from 75 to 92 % (Fig. 1). The nuclear localization signal (NLS) sequence was highly conserved in the proteins from all of those plant species. Consistent with this alignment, cluster analysis indicated that the protein encoded by *OsMSR4* is more closely related to sHSPs from CIII than from any other class (Fig. 2). Subcellular localization of the encoded protein was predicted to be within the nucleus, based on an online investigation with a web server (http://chemdata.shu.edu.cn/subcell/).

Drought tolerance is enhanced in transgenic *Arabidopsis*

To elucidate the biological functions of *OsMSR4*, we generated *Arabidopsis* plants expressing that gene. Two independent transgenic lines, L-3 and L-4, were chosen for further study because of their higher levels of expression (Supplemental Fig. S2). Under normal growing conditions, the transgenic plants were indistinguishable in phenotype from the WT. However, in the presence of 300 mM mannitol, the WT germination rate was 58 % versus 85 % and 87 % for the two transgenic lines (Fig. 3a). When exposed to 275 mM mannitol, the primary roots from WT seedlings were only 2.46 cm long compared with 3.0 and 3.2 cm for the transgenics (Fig. 3b). Whereas the modified plants had a 38 % survival rate after water was withheld for 12 days, only 15 % of the WT plants survived the drought treatment (Fig. 3c).

To investigate the physiological basis for this improvement in drought tolerance by transgenic *Arabidopsis*, we measured proline levels after irrigation was withheld for 8 days. Proline concentrations were 0.112 and 0.124 mmol g^{-1} FW in transgenic lines versus 0.068 mmol g^{-1} FW in the WT control (Fig. 3d).

ABA sensitivity is decreased in transgenic *Arabidopsis* plants

To determine whether *OsMSR4* is involved in regulating the response of transgenic *Arabidopsis* to ABA, we compared sensitivity between WT and transgenic plants during the germination and seedling stages. Seeds from the transgenic lines germinated 2 days earlier than those of the WT when placed on treatment media supplemented with

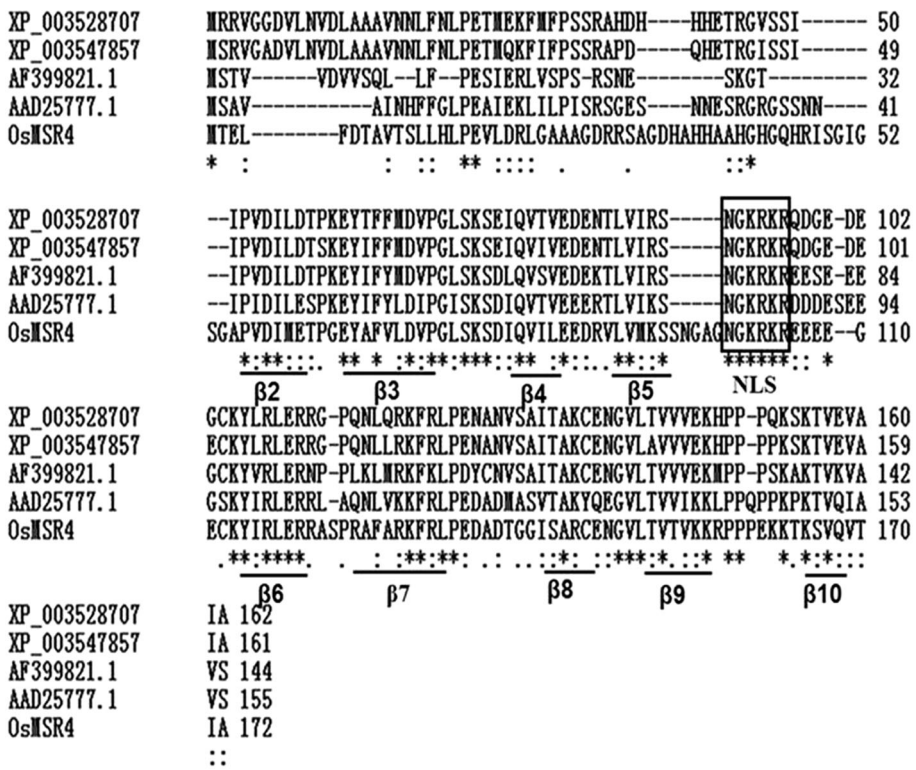

Fig. 1 Alignment of deduced amino acid sequences for *OsMSR4* with other Class III sHSPs from *Solanum peruvianum* (AF399821.1), *Arabidopsis thaliana* (AAD25777.1), and *Glycine max* (XP_003528707 and XP_003547857). Numbers on right side indicate positions of amino acids. Protein sequences were aligned online via Clustalw2 (http://www.ebi.ac.uk/Tools/msa/clustalw2/). Consensus keys: '*', single, fully conserved residue; ':', conservation of strong groups; '.', conservation of weak groups; and '–', no consensus. 'NLS', nuclear localization signal

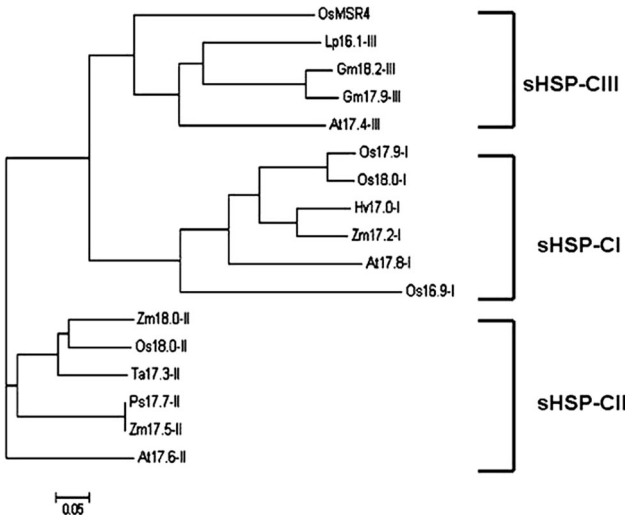

Fig. 2 Phylogenetic tree of deduced amino acid sequences from *OsMSR4* and other plant sHSPs. Tree was constructed using MEGA software (Version 5.1) and Neighbor-Joining method, with pairwise deletion and Poisson correction model. Accession numbers: Lp16.1-III (*Solanum peruvianum*, AF399821.1), Gm18.2-III (*Glycine max*, XP_003528707), Gm17.9-III (*G. max*, XP_003547857), At17.4-III (*Arabidopsis thaliana*, AAD25777.1), Os18.0-1 (*Oryza sativa*, AAC78393), Os17.9-1 (*O. sativa*, EU846987), Os16.9-1 (*O. sativa*, GU120337), Hv17.0-1 (*Hordeum vulgare* subsp. *vulgare*, CAA69172), Zm17.2-I (*Zea mays*, NP_001105442), At17.8-1 (*A. thaliana*, NP_172220), Zm18.0-II (*Z. mays*, CAA38012), Os18.0-II (*O. sativa*, ABA29610), Ta17.3-II (*Triticum aestivum*, CAA41218), Ps17.7-II (*Pisum sativum*, AAA33670), Zm-17.5-II (*Z. mays*, ACG43108), and At17.6-II (*A. thaliana*, CAA45039.1)

ABA. Moreover, the average percentage of seedlings with leaves was 6.3 % versus 29.2 % and 31.3 % for the transgenics when plants were exposed to media containing 0.6 μM ABA (Fig. 4a).

Expression of stress-related genes is altered in transgenic *Arabidopsis*

We used qRT-PCR to investigate whether changes in sensitivity to ABA and drought tolerance in transgenic plants are accompanied by altered expression of three stress-related genes. Under both normal and stress conditions, transcript levels for *RD29A*, *P5CS1*, and *NCED3* were significantly higher in the transgenic *Arabidopsis* lines than in the WT (Fig. S3).

Discussion

Heat shock proteins are the most ubiquitous and evolutionarily conserved molecular chaperones across all species in response to excessive temperatures and other abiotic stresses (Lindquist and Craig 1988). In plants, the sHSPs are the most prevalent type of HSPs; their abundance and diversity enable plants to survive in adverse environments (Waters et al. 1996). The CIII–related genes in rice, tomato, and *Arabidopsis* contain short introns in the region that encodes the β_4 strand of the ACD. An important characteristic of CIII sHSPs is the highly conserved motif NGKRKR between β_5 and β_6 of the ACD (Siddique et al. 2003). We isolated and cloned a full-length cDNA of *OsMSR4* from rice. Sequence analysis indicated that the encoded protein (Accession AK119621.1) contains an NIL sequence and an ACD in the C-terminal region. It shares significant homology with CIII sHSPs from other plants. Based on alignment and phylogenetic comparisons with sHSP sequences from other species, we confirmed that *OsMSR4* is a member of the plant CIII sHSP gene family, a finding consistent with those from earlier experiments (Sarkar et al. 2009).

The HSPs are produced in cells upon exposure to elevated temperatures and other stresses. Thus, they may play an important role in the plant response to a wide range of stresses (Mahmood et al. 2010). Guan et al. (2004) have reported that plant sHSPs display different expression levels under heat shock treatment and other abiotic stresses. Furthermore, expression of *OsMSR4* is up-regulated in response to salt, drought, or heat (Sarkar et al. 2009). We found that this gene was strongly induced in the leaves and panicles of 'Pei'ai 64S' rice when plants were exposed to cold, heat, or drought stresses at different developmental stages. The greatest accumulation of transcripts was detected in leaves at the booting and heading stages in response to high temperature. This was evidence of a correlation between *OsMSR4* expression and heat shock treatment, similar to activity by other sHSPs. Expression was also significantly increased in leaves and panicles at the booting and heading stages under drought and chilling conditions. This suggested that *OsMSR4* functions in those tissues where it plays an important role in regulating plant responses to such stresses. However, differences in expression patterns observed here versus the findings reported by Sarkar et al. (2009) most likely arose because the plant materials and methods were not the same between these analyses.

We also noted several matches to stress-related *cis*-acting elements, including ABRE and MBS, in the 1-kb putative promoter region of *OsMSR4*. These regulatory elements are pivotal in regulating the transcription of stress-inducible genes involved in ABA signaling and abiotic stress responses (Yamaguchi-Shinozaki and Shinozaki 2005). Therefore, our results suggested that *OsMSR4* participates in modulating plant responses to abiotic stress and exogenous ABA.

Extensive studies have demonstrated that constitutive overexpression of sHSPs in plants is associated with enhanced tolerance to abiotic stress (Jiang et al. 2009; Perez et al. 2009; Xue et al. 2010). For example, transgenic *Arabidopsis* plants

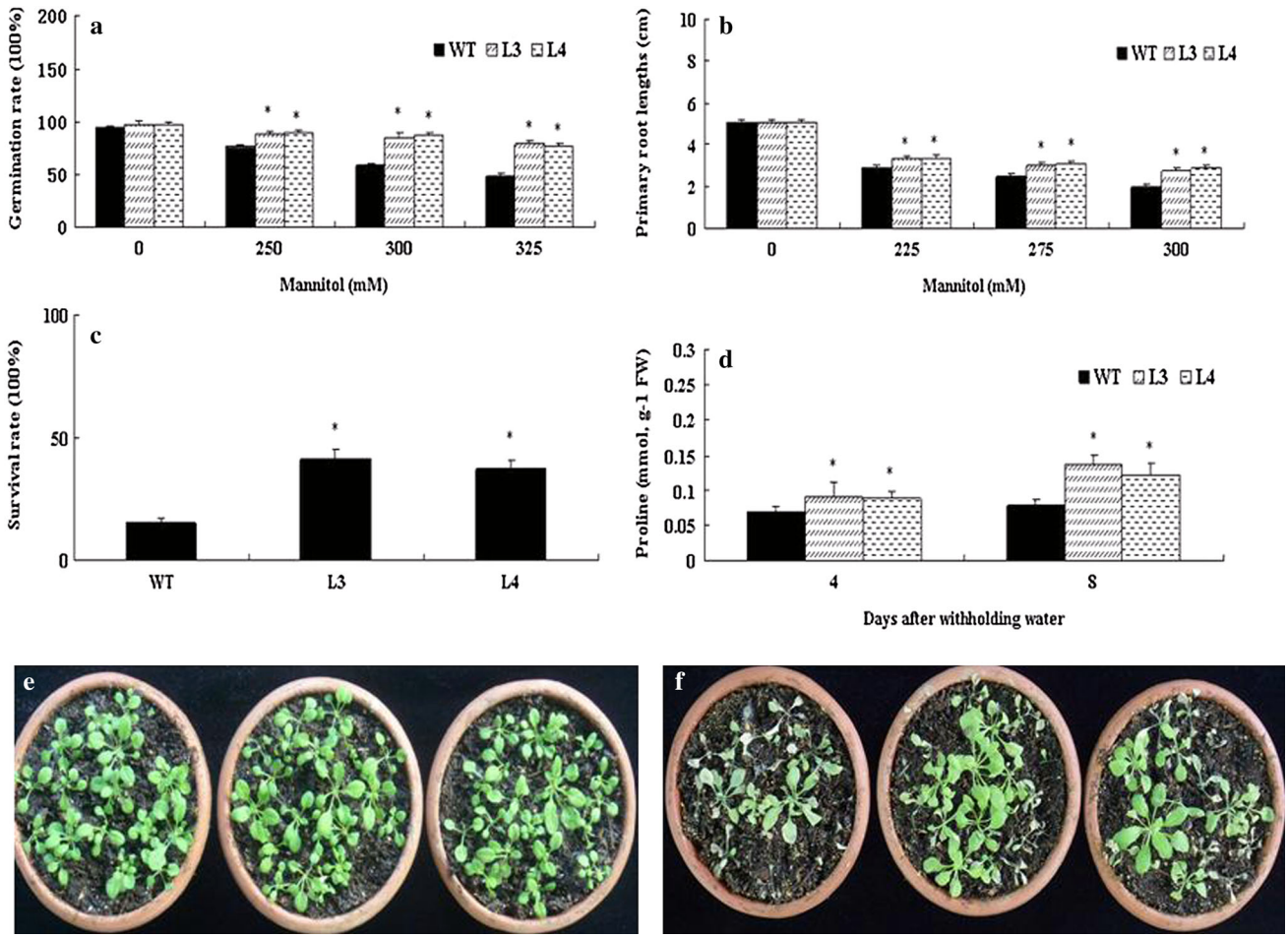

Fig. 3 Effect of expression by *OsMSR4* on drought tolerance in *Arabidopsis* plants. **a** Germination rates for WT and transgenic lines on media with and without mannitol. **b** Lengths of primary roots from individual plants exposed to mannitol. **c** Survival rates after drought treatment. **d** Proline concentrations in transgenic lines and WT. **e** Phenotypes of transgenic lines and WT grown in soil for 3 weeks after germination under normal conditions. **f** Seedlings produced from transgenic lines and WT after watering resumed for 7 days. Asterisks indicate differences statistically significant from WT ($P = 0.05$) under same conditions

expressing RCHSP17.8 are more tolerant of heat, salt, drought, and osmotic stress (Jiang et al. 2009). Although we saw no apparent phenotypic differences between our transgenic and WT *Arabidopsis* plants under normal growing conditions, the transformed lines showed significantly higher rates for seed germination and plant survival, longer primary roots, and greater drought tolerance when irrigation was withheld. We considered these results to be evidence that expression of *OsMSR4* in that species enhances tolerance to water stress at the seedling stage. Furthermore, the transgenic plants accumulated more free proline under drought when compared with the WT.

To gain a better understanding of the molecular basis for this enhanced tolerance by plants transformed with *OsMSR4*, we also examined the expression patterns of the well-characterized, stress-related *RD29A* and *P5CS1* and found that transcripts levels of both genes were significantly higher in the *OsMSR4* transgenics than in the WT under drought conditions (Fig. S3). Expression of *RD29A* is known to be induced when plants are dehydrated or exposed to low temperature, high salt, or exogenous ABA (Yamaguchi-Shinozaki and Shinozaki 1994). The *Arabidopsis* gene *P5CS1* appears to catalyze the rate-limiting step in proline biosynthesis and is required for proline accumulations under osmotic stress, activity that is critical to the development of stress tolerance (Kishor et al. 1995). In response to abiotic stress, osmotic adjustments are a crucial process by which plants adapt to such challenges (Chaves et al. 2003). Under drought stress, reactive oxygen species (ROS) are generated and act as messengers, triggering the expression of genes in the signal transduction pathways during the stress-response process (Swindell et al. 2007). Therefore, we propose that excessive *OsMSR4* may bind and hold proteins of transcription factors that are responsible for the induction of acolytes needed for osmotic balance under drought stress. Alternatively, *OsMSR4* expression might reduce the extent of damage of stress-related transcription factors that

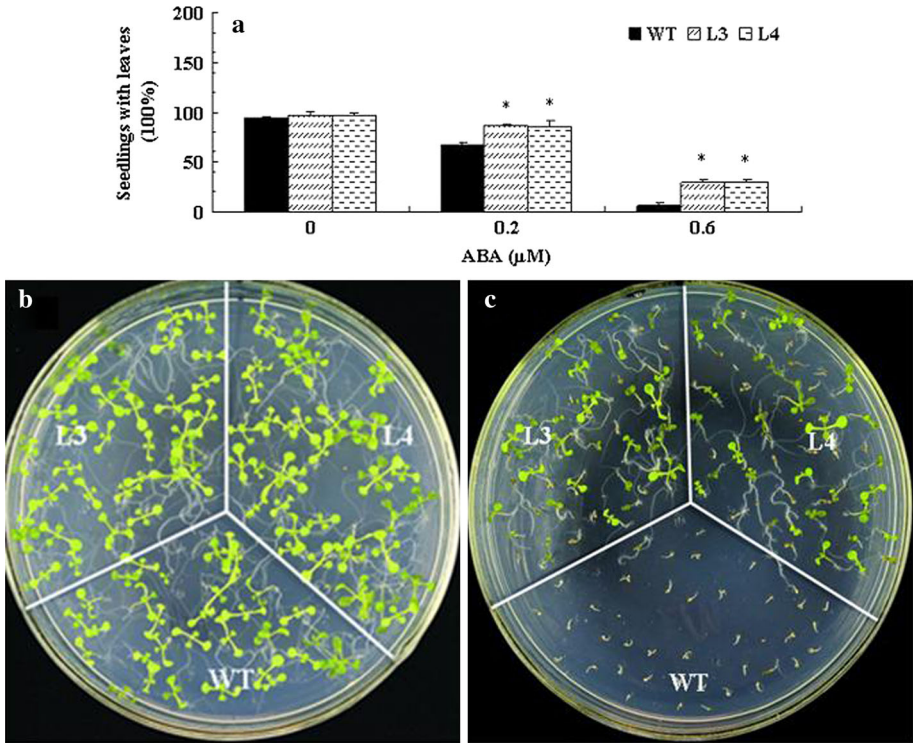

Fig. 4 Sensitivity of WT and transgenic *Arabidopsis* to ABA. **a** Amount of seedlings with leaves as percentage of total number of seeds sown on 1/2 MS media supplemented with different concentrations of ABA. For each concentration, asterisks indicate differences statistically significant from WT ($P = 0.05$). Comparisons between WT and transgenic *Arabidopsis* seedlings on control MS medium (**b**) and treatment medium with 0.6 μM ABA (**c**) at 10 days after stratification

results from ROS accumulation. Afterward, *RD29A*, *P5CS1*, and possibly other stress-related genes are induced in the transgenic plants.

ABA plays diverse roles in plant development and the adaption to environmental stresses such as drought, high salinity, and low temperature (Lee and Hwang 2009). Under abiotic stress conditions, this hormone is rapidly accumulated and then functions as a secondary messenger in abiotic stress signaling. Exogenous ABA treatment can induce numerous genes that respond to dehydration stress (Li et al. 2013). For example, *NCED3* is thought to be involved in ABA biosynthesis, acting as an important regulator of ABA levels in *Arabidopsis* during periods of water stress, such that transcription is induced (Huo et al. 2013). The involvement of *P5CS1* and *RD29A* in drought tolerance is regulated by ABA-mediated pathways or other pathways (Yoshiba et al. 1999; Shinozaki et al. 2003). We found that *Arabidopsis* plants expressing *OsMSR4* had significantly decreased sensitivity to exogenous ABA. Moreover, expression by *RD29A*, *P5CS1*, and *NCED3* was stronger in the transgenics than in the WT plants under either drought stress or ABA treatment. Therefore, the information obtained from our study suggests that *OsMSR4* functions in response to drought conditions and exposure to ABA by altering the expression of stress-related genes through either ABA-dependent or -independent signal transduction pathways. Future research will focus on transgenic rice plants with *OsMSR4* overexpression and/or RNAi-mediated inhibition while we continue to examine the functioning of that gene.

Acknowledgments This research was supported by the Science and Technology Key Project of Hu Nan Province, P. R. China (S2011F2022).

References

Basha E, O'Neill H, Vierling E (2012) Small heat shock proteins and a-crystallins: dynamic proteins with flexible functions. Trends Biochem Sci 37:106–117

Bates LS, Waldren RP, Teeare ID (1973) Rapid determination of free Pro for water-stress studies. Plant Soil 39:205–207

Chaves MM, Maroco JP, Pereira JS (2003) Understanding plant response to drought-from the genes to the whole plant. Funct Plant Biol 30:239–264

Chen RJ, Dong JL, Liu SB, Xu ZJ, Gao XL (2012) isolation of a novel abscisic acid stress ripening (*OsASR*) gene from rice and analysis of the response of this gene to abiotic stresses. Afr J Biotechnol 11:13873–13881

Clough SJ, Bent AF (1998) Floraldip: a simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. Plant J 16:735–743

Cope LM, Irizarry RA, Jaffee HA, Wu Z, Speed TP (2004) A benchmark for Affymetrix GeneChip expression measures. Bioinformatics 12:323–331

Guan JC, Jinn TL, Yeh CH, Feng SP, Chen YM, Lin CY (2004) Characterization of the genomic structures and selective expression profiles of nine class I small heat shock protein genes clustered on two chromosomes in rice (*Oryza sativa* L.). Plant Mol Biol 56:795–809

Horwitz J (1992) Alpha-crystallin can function as a molecular chaperone. Proc Natl Acad Sci USA 89:10449–10453

Huo HQ, Dahal P, Kunusoth K, McCallum CM, Bradford KJ (2013) Expression of 9-cis-epoxycarotenoid dioxygenase 4 is essential for thermoinhibition of lettuce seed germination but not for seed development or stress tolerance. Plant Cell 25:884–900

Irrizary RA, Bolstad BM, Collin F, Cope LM, Hobbs B, Speed TP (2003) Summaries of Affymetrix Genechip probe level data. Nucleic Acids Res 15:1–8

Jiang CH, Xu JY, Zhang H, Zhang X, Shi JL, Li M, Ming F (2009) A cytosolic class I small heat shock protein, RcHSP17.8, of *Rosa chinensis* confers resistance to a variety of stresses to *Escherichia coli*, yeast and *Arabidopsis thaliana*. Plant Cell Environ 32:1046–1059

Kishor P, Hong Z, Miao GH, Hu C, Verma D (1995) Overexpression of [delta]-pyrroline-5-carboxylate synthetase increases proline production and confers osmotolerance in transgenic plants. Plant Physiol 108:1387–1394

Lee SC, Hwang BK (2009) Functional roles of the pepper antimicrobial protein gene, *CaAMP1*, in abscisic acid signaling, and salt and drought tolerance in *Arabidopsis*. Planta 229:383–391

Li H, Gao Y, Xu H, Dai Y, Deng DQ, Chen JM (2013) ZmWRKY33, a WRKY maize transcription factor conferring enhanced salt stress tolerances in Arabidopsis. Plant Growth Regul 70:207–216

Lindquist S, Craig EA (1988) The heat-shock proteins. Annu Rev Gene 22:631–677

Mahmood T, Safdar W, Abbasi BH, Saqlan Naqvi SM (2010) An overview on the small heat shock proteins. Afr J Biotechnol 9:927–949

Perez DE, Hoyer JS, Johnson AI, Moody ZR, Lopez J, Kaplinsky NJ (2009) BOBBER1 is a noncanonical Arabidopsis small heat shock protein required for both development and thermotolerance. Plant Physiol 151:241–252

Sarkar NK, Kim YK, Grover A (2009) Rice sHsp genes: genomic organization and expression profiling under stress and development. BMC Genom 10:393–398

Shinozaki K, Yamaguchi-Shinozaki K, Seki M (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr Opin Plant Biol 6:410–417

Siddique M, Port M, Tripp J, Weber C, Zielinski D, Calligaris R, Winkelhaus S, Scharf KD (2003) Tomato heat stress protein Hsp16.1-CIII represents a member of a new class of nucleocytoplasmic small heat stress proteins in plants. Cell Stress Chaperones 8:381–394

Swindell WR, Huebner M, Weber AP (2007) Transcriptional profiling of *Arabidopsis* heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. BMC Genom 8:125

Trent JD (1996) A review of acquired thermo tolerance, heat-shock proteins, and molecular chaperones in archaea. FEMS Microbiol Rev 18:249–258

Verslues PE, Zhu JK (2007) New developments in abscisic acid perception and metabolism. Curr Opin Plant Biol 10:447–452

Wang WX, Vinocur B, Altman A (2003) Plant responses to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218:1–14

Wang WX, Vinocur B, Shoseyov O, Altman A (2004) Role of plant heat-shock proteins and molecular chaperones in the abiotic stress response. Trends Plant Sci 9:244–252

Waters ER, Lee GJ, Vierling E (1996) Evolution, structure and function of the small heat shock proteins in plants. J Exp Bot 47:325–338

Xue Y, Peng R, Xiong A, Li X, Zha D, Yao Q (2010) Overexpression of heat shock protein gene hsp26 in *Arabidopsis thaliana* enhances heat tolerance. Biol Plant 54:105–111

Yamaguchi-Shinozaki K, Shinozaki K (1994) A novel cis-acting element in an *Arabidopsis* gene is involved in responsiveness to drought, low-temperature, or high-salt stress. Plant Cell 6:251–264

Yamaguchi-Shinozaki K, Shinozaki K (2005) Organization of *cis*-acting regulatory elements in osmotic- and cold-stress-responsive promoters. Trends Plant Sci 10:88–94

Ye SF, Yu SW, Shu LB, Wu JH, AiZ Wu, Luo LJ (2012) Expression profile analysis of 9 heat shock protein genes throughout the life cycle and under abiotic stress in rice. Chin Sci Bull 57:336–343

Yoshiba Y, Nanjo T, Miura S, Yamaguchi-Shinozaki K, Shinozaki K (1999) Stress-responsive and developmental regulation of Δ^1-pyrroline-5-carboxylate synthetase 1 (*P5CS1*) gene expression in *Arabidopsis thaliana*. Biochem Biophys Res Comm 261:766–772

Zou J, Liu AL, Chen XB, Zhou XY, Gao GF, Wang WF, Zhang XW (2009) Expression analysis of nine rice heat shock protein genes under abiotic stresses and ABA treatment. J Plant Physiol 166:851–861

Zou J, Liu CF, Liu AL, Zou D, Chen XB (2012) Overexpression of OsHsp17.0 and OsHsp23.7 enhances drought and salt tolerance in rice. J Plant Physiol 169:628–635