BMB
reports

OsHSF7 gene in rice, *Oryza sativa* L., encodes a transcription factor that functions as a high temperature receptive and responsive factor

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Three novel Class A genes that encode heat shock transcription factor (HSF) were cloned from *Oryza Sativa* L using a yeast hybrid method. The *OsHSF7* gene was found to be rapidly expressed in high levels in response to temperature, which indicates that it may be involved in heat stress reception and response. Over-expression of *OsHSF7* in transgenic Arabidopsis could not induced over the expression of most target heat stress-inducible genes of HSFs; however, the transcription of some HSF target genes was more abundant in transgenic plants following two hours of heat stress treatment. In addition, those transgenic plants also had a higher basal thermotolerance, but not acquired thermotolerance. Collectively, the results of this study indicate that *OsHSF7* might play an important role in the response to high temperature. Specifically, these findings indicate that *OsHSF7* may be useful in the production of transgenic monocots that can over-express protective genes such as HSPs in response to heat stress, which will enable such plants to tolerate high temperatures. [BMB reports 2009; 42(1): 16-21]

INTRODUCTION

In eukaryotes, the heat-shock response is a reaction to elevated temperature and various chemical stressors that is conserved among cells and organisms. Functional analysis of the promoter regions of these heat shock-inducible genes has lead to the identification of heat shock elements (HSE) that contain the palindromic consensus sequence, (AGAAn)(nTTCT), which is responsible for the inducible expression of heat shock genes and the accumulation of HSPs (1, 2). Most heat shock factors (HSFs) can specifically bind to HSE in the upstream promoter region of heat shock genes to regulate the expression of these genes. The plant HSF family has more members than the vertebrate HSF family. Indeed, there are 20 to 30 different plant HSFs, most of which have been derived from tomatoes, Arabidopsis and soybeans.

Until now, some plant HSFs have been cloned and characterized from various species using various molecular cloning technologies (2-4,19). In plants, the network of *HSF* genes is highly flexible and specialized; therefore, details regarding the overall HS response network were initially unclear. However, many studies have been conducted to evaluate the HS response network in Arabidopsis and tomato (5-7). The results of these studies demonstrated that HsfA1 was constitutively expressed in tomatoes. Specifically, HsfA1 was found to be the master regulator of the heat response (8), and over-expression of *LpHsfA1* was shown to lead to the accumulation of several HSPs capable of conferring thermotolerance to transgenic plants. Conversely, no master regulator of HsfA1 was identified in Arabidopsis. HsfA1a and HsfA1b have also been shown to be the key activators responsible for regulation of the early phase of heat shock response (9). In subsequent studies, the two AtHsfAs were found to regulate the expression of several heat shock protein genes as well as some functional genes involved in protein biosynthesis, processing, signaling, metabolism and transport (10). Recently, studies evaluating the effect of T-DNA knockout and over-expression of *HsfA2* in Arabidopsis lines have shown that it is the strongest member of the HS family expressed in response to heat stress conditions (11-13).

Although the flexible network of HSF genes has been well studied in tomatoes, Arabidopsis and soybeans, there is little information available regarding the structure and function of HSFs in rice. Therefore, it is essential to isolate and characterize the rice HSFs so the entire network of plant heat stress responses can be understood. In this study, we used the yeast one-hybrid system to screen a rice cDNA library. During screening, three novel cDNA clones that encoded heat shock factor, *OsHSF5, OsHSF7* and *OsHSF9*, were identified. Following exposure to high temperatures, the expression of

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Received 21 May 2008, Accepted 8 July 2008

Keywords: Heat-shock factor, Heat tolerance, OsHSF7, Rice, Yeast one-hybrid

some *HSPs* and *GolS* genes was greatly elevated in transgenic Arabidopsis plants that over-expressed the *OsHSF7* gene, which resulted in transgenic plants having a higher basal thermotolerance than wild-type plants.

RESULTS

Screening for rice cDNA encoding heat shock transcription factors

Two plasmids were employed in the yeast one-hybrid screening system. The plasmid pPC86 rice cDNA expression library was used to express GAL4-cDNA fusion proteins, and the bait plasmid harbored a dimeric HSE derived from the promoter of the soybean (*Glycine max*) Hsp17.3B-Cl gene. Following transformation of the URA-marked bait plasmid containing the HSE sequence and the TRP-marked pPC86 plasmid carrying the rice cDNA library into the yeast cells, approximately 4×10^6 yeast transformants were obtained. After the two plasmids were transformed into strain EGY48 separately, thirty positive yeast colonies, which were identified based on a deep-blue color when grown on the filters containing X-gal, were selected. The vectors of 15 positive clones were then transformed back into yeast strain EGY48 containing a reporter plasmid, after which twelve colonies were still blue when grown on the X-gal plates. We then assembled three complete nucleotide sequences encoding heat shock factor and analyzed these sequences using Blastx. These three genes were then named *OsHSF5, OsHSF7* and *OsHSF9* and deposited into the GeneBank database under accession numbers AY344487, AY344489 and AY344491, respectively.

Nucleotide sequence and predicted amino acid sequence analysis of three rice cDNAs

Analysis of the deduced amino acid sequences revealed that all three proteins contained a highly conserved DNA binding domain (DBD) close to the N-terminus and an oligomerization domain with a hydrophobic heptad repeat (HR-A/B) located adjacent to the nuclear localization signal region. Furthermore, all three OsHSFs contained the plant-specific insertions of 21 amino acid residues between HR-A and HR-B, as well as the short activator peptide motif (AHA motif) in the C-terminal domain (Fig. 1A). These characteristics demonstrated that the three proteins belonged to Class A heat shock factor. Interestingly, an insertion of 20 amino acid residues was observed in the conserved intron position of the OsHSFs, and there were obvious splice sequences in two borders of the introns (Fig. 1A).

We then compared the amino acid sequences of the three OsHSFs with those of all Arabidopsis HSFs present in the TIGR *Arabidopsis thaliana* database using CLUSTALX. The results sug-

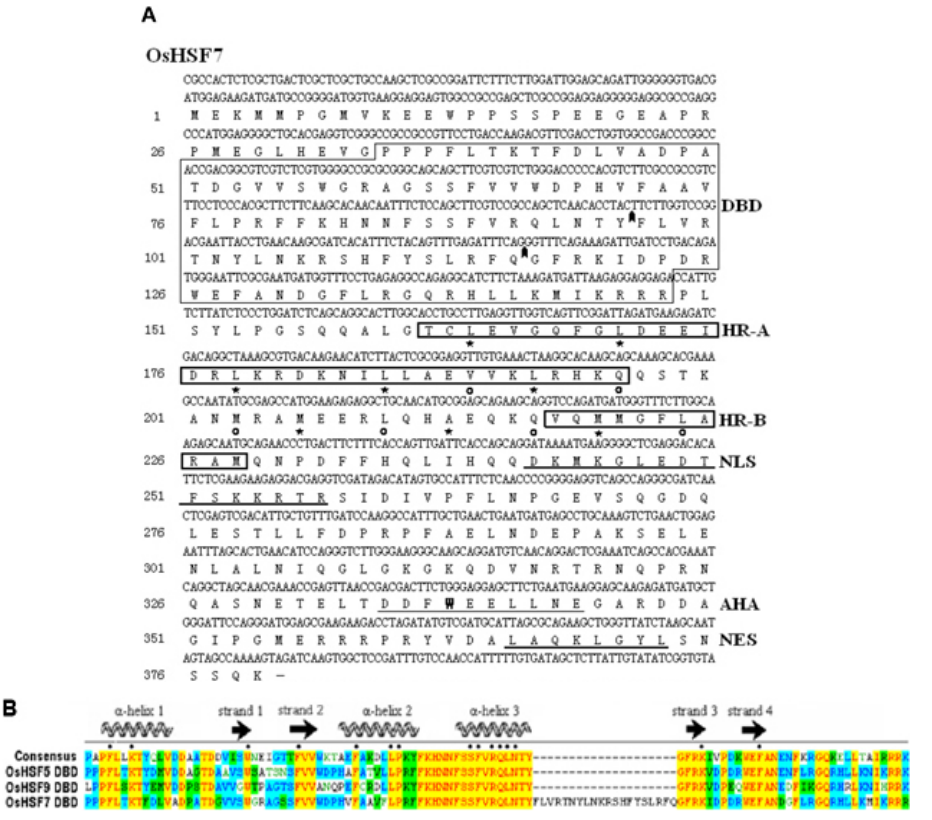

Fig. 1. (A) Full-length sequence of OsHSF7 cDNA and its deduced amino acid sequence. The putative domains are identified and indicated. The overlapping heptad repeats (HR-A/B) are indicated by stars and circles. (B) Comparison of the conserved DNA binding domain sequences and the structures of OsHSF5, OsHSF7 and OsHSF9. Identical amino acid residues and conserved amino acid residues in the aligned sequences are displayed in yellow and light-blue, respectively. The secondary-dimensional structure is marked above the corresponding sequences. The residues with conserved and important functions are indicated by filled circles.

gested that the DBD and HR-A/B region of OsHSF5 and OsHSF7 were more similar to AtHsfA7, AtHsfA6 and AtHsfA2 (At2g26150) than to any other Class A type HSF, while that of OsHSF9 was more homologous to AtHsfA4 (At5g45710). In addition, all three OsHSFs contained a hydrophobic leucine-rich HR-C region (nuclear export signal [NES]) in the C-terminus of the proteins.

Expression of the *OsHSF7*

Quantitative real-time PCR (qRT-PCR) was performed to detect the expression patterns of the *OsHSF7* gene following various stress factor shocks. The results revealed that the accumulation of *OsHSF7* mRNA was quickly (10 min) and strongly (up to 200 times) elevated in response to high temperature, but that the *OsHSF7* mRNA levels peaked after 10 min of exposure to heat, after which they decreased rapidly (Fig. 2A). These results indicate that the transcript of the *OsHSF7* gene was enhanced by heat stress and suggest that OsHSF7 might act as a fast response factor to heat stress.

To further evaluate the accumulation pattern of *OsHSF7* mRNA in different organs and different growth periods, RNA was extracted from the roots, shoots, leaves, and flowers, as well as from mature rice plants, immature seeds in the booting stage and mature seeds in the grain filling stage and then subjected to qRT-PCR analysis. As shown in Fig. 2B, a considerable amount of *OsHSF7* mRNA was present in the leaves and flowers, but the other tissues contained very low levels of *OsHF7*.

Over-expression of *OsHSF7* in Arabidopsis

To analyze the potential function of the *OsHSF7* gene in Arabidopsis, we generated transgenic plants that over-expressed *OsHSF7*. The phenotype and growth of transgenic plants that over-expressed *OsHSF7* were then compared with those of the wild type Arabidopsis plants after sowing. The results revealed no obvious difference between the 35S:OsHSF7 transgenic plants and the wild type.

To determine the level of *OsHSF7* expression, semi-quantitative RT-PCR was conducted using five 35S:OsHSF7 transgenic plants and five wild type plants (Fig. 3A). The results revealed that the *OsHSF7* transcript was expressed at different levels in different lines, which indicates that its expression may depend on positional effects. Therefore, the three lines that expressed the greatest levels of *OsHSF7* transcript, TP4, TP1 and TP3, were used for further analysis.

The effect of over-production of *OsHSF7* on the expression of the downstream of HSFs genes in transgenic Arabidopsis

To determine if over-production of the OsHSF7 gene influences the expression of HSFs in transgenic Arabidopsis, we evaluated the expression of six HSPs, *GolS1-3* and *Apx 1-4*, in wild-type and transgenic plants. In addition, we compared the expression of these genes in D35S:OsHSF7 and wild type plants with and without heat stress treatment. The mRNA levels of all six *HSP* genes was increased markedly in all of the Arabidopsis plants following heat stress (42°C) treatment. Specifically, the transcription of HSP17.7, HSP18.2, HSP21, HSP70, HSP83.1 and HSP101 increased by 180, 80, 260, 20, 40 and 99-fold, respectively, in the transgenic plants, while the transcription increased by 10, 60, 60, 70, 5 and 20-fold, respectively, in the wild type plants. In addition, with the exception of HSP18.2, HSP70 and HSP101 in transgenic plant line 4, the expression of the HSPs did not differ significantly between the D35S:OsHSF7 transgenic plants and wild type plants that were subjected to the 22°C treatment. However, following heat treatment for 2 h, the mRNA levels of HSPs in

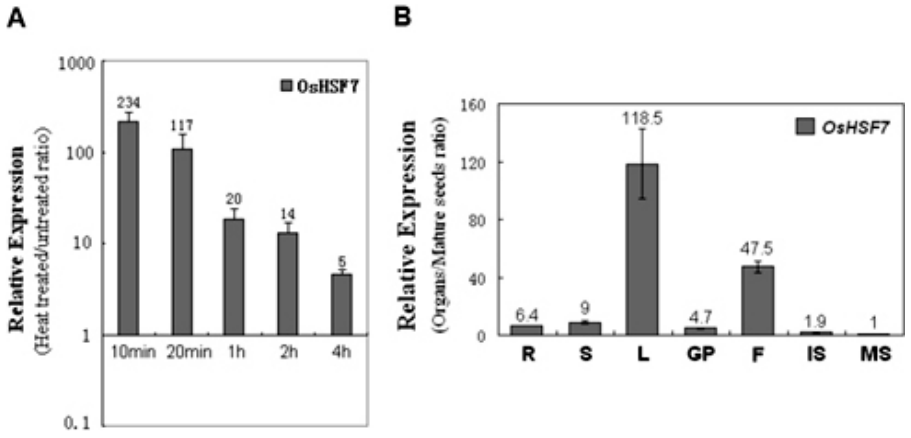

Fig. 2. (A) time-course of *OsHSF7* transcript accumulation during heat shock. Two-week-old seedlings were used for heat treatment. The *OsHSF7* transcripts were normalized to its expression without heat stress treatment. (B) Expression of three *OsHSF7* in different organs and different growth periods. Relative transcript levels of *OsHSF7* in roots (R), stems (S), leaves (L), and the following growing points (GP); flowers (F), immature seeds in the booting stage (IS) mature seeds in the grain filling stage (MS). The *OsHSF7* transcripts were normalized to the expression of the gene in mature seeds in the grain filling stage. Values are the means ± SD of assays conducted during at least two independent experiments that were each repeated three times.

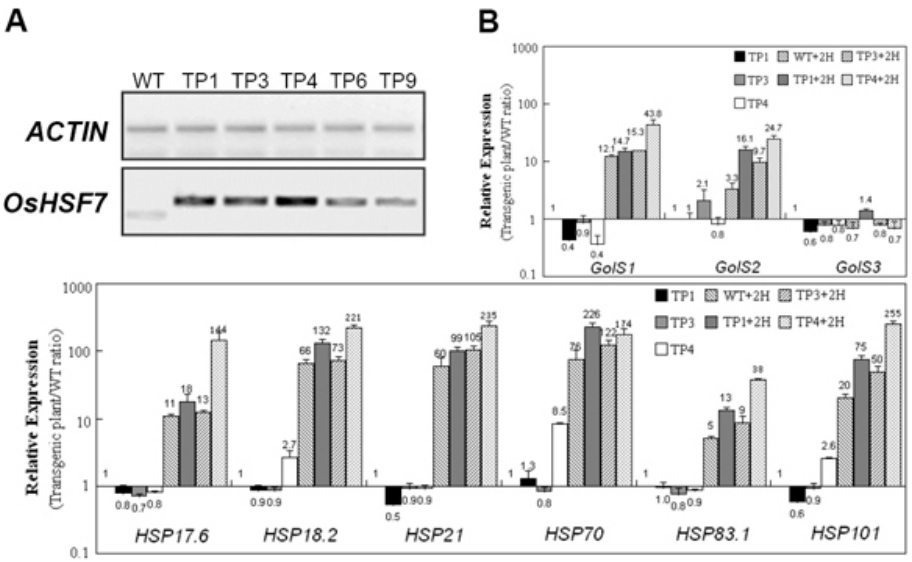

Fig. 3. Levels of the transcription of *OsHSF7*, six *HSP* genes and three *GolS* genes in wild type and D35S:OsHSF7 plants during non-heat and heat treatment. (A) Over-expression of the *OsHSF7* gene was analyzed by semi-quantitative RT-PCR. (B) Analysis of the expression of putative downstream genes in Arabidopsis. Relative levels were calculated and normalized with respect to the expression of each gene in untreated wild type plants. Values are the means ± SD of assays that were repeated three times.

the transgenic plants were higher than those in wild type plants, with the most pronounced increase being that of the *HSP101* mRNA, which was 3.5, 2.5 and 13-fold higher in D35S:OsHSF7 lines 1, 3 and 4, respectively (Fig. 3B).

Similar to the HSPs, after two hours of heat treatment the transcript levels of *GolS2* were higher in transgenic plants than that in wild type plants (Fig. 3B), while the transcript levels of the *GolS3* gene did not differ significantly among plants.

Effect of OsHSF7 expression on heat tolerance

The ability of transgenic plants over-expressing *OsHSF7* to tolerate heat was compared to that of wild-type plants. Briefly, plants were grown in pots at 22°C for approximately 4 weeks, after which they were subjected to 42°C for approximately 16 hours. We found that only 22% of the wild-type plants survived, but 52% of the D35S:OsHSF7 plants survived, indicating that they were more tolerant of heat stress (Fig. 4).

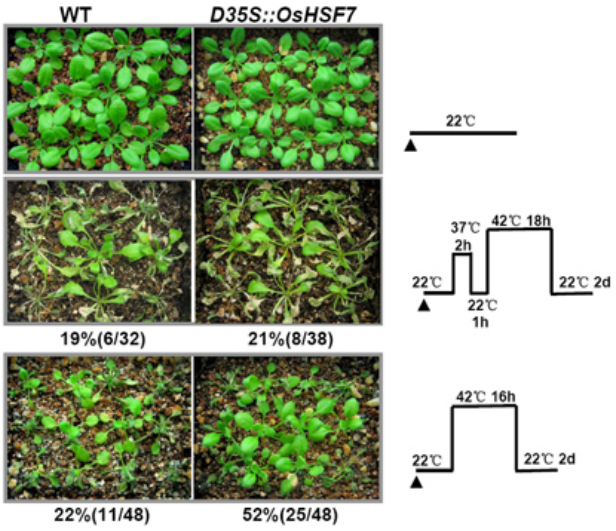

Fig. 4. Basal thermotolerance of the D35S:OsHSF7 transgenic Arabidopsis. Three-week-old wild type and transgenic plants were grown under normal conditions, after which the wild type and D35S:OsHSF7 plants were treated by the different HS regimes shown to the right of each section. Percentages of the surviving plants, the numbers of surviving plants and the total number of tested plants are shown under the photographs.

DISCUSSION

When compared to the three Arabidopsis HSFs (AtHsfA7, AtHsfA6 and AtHsfA2) and the HSFs of rice, OsHSF7 had longer alternative splicing DNA binding domain that included a 20 amino acids insertion in the conserved intron position of heat shock factor (Fig. 1B). However, even though OsHSF7 had a longer DNA binding domain than most other HSFs in plants, it was still capable of interacting with HSE as well as other HSFs in yeast cells. This indicates that the splicing pattern of OsHSF7 may function as a transcription activator in rice under specific conditions and during specific growth periods.

The results of quantitative PCR demonstrated that expression of the *OsHSF7* gene was strongly induced by heat stress within 10 minutes of HS treatment. The expression patterns of *LpHsfA2* and *AtHsfA2* differed from that of *OsHSF7* in tomato and Arabidopsis. Specifically, a high level of LpHsfA2 accumulated in response to prolonged heat stress (HS), and it played a crucial role from the point of view of different forms in the cells as well by acting as a potential co-regulator and activator during the heat stress response (12) In contrast, the expression of AtHsfA2 was significantly induced by heat stress as

well as by high levels of light and H_2O_2 (17). We also investigated the transcript levels of *OsHSF7* in response to H_2O_2 stress; however, the results revealed that the expression *OsHSF7* was not induced by H_2O_2 (data not show). Taken together, these findings suggest that the HSFs in the branch are involved in the response to heat stress and that there is a different regulation mode for HsfA2 subgroups among rice, tomatoes and Arabidopsis. Moreover, the HsfA2 subgroup in rice is more complex, which might indicate that it has a more sophisticated heat shock regulation system.

The results of several studies have demonstrated that the functions of HSFs depend on the trimerization of homologous and heterogenous genes and the interaction of HSFs with HSP or other transcription factors. For example, if HSFB1 acts as a novel type coactivatior it may be able to cooperate with HSFA1a or other activators to control expression of the housekeeping gene in tomatoes (16). In this study, over-expression of the OsHSF7 cDNA in transgenic Arabidopsis plants resulted in increased expression of *GolS2* and many *HSPs* after 2 hours of heat stress treatment, which led to enhanced tolerance to heat stress. There are two possible explanation for these findings: (i) heat stress induced the folding, processing and trimerization of OsHSF7 protein, which eventually results in the over-production of some HSPs; (ii) heat stress accelerates the production of some Arabidopsis HSFs that can interact with OsHSF7 and regulate the expression of *HSPs* or other genes.

Sequencing of the Arabidopsis genome and analyzing the 1000 bp putative promoter sequence of 22,810 genes revealed that approximately 33% of those genes contained a consensus HSE motif. This indicates that heat shock proteins (HSPs) are the major target of HSFs, but not the only target (17) The results of several recent studies demonstrated that the HSE motif was present in the promoter of ascorbate peroxide 1 (*Apx1*), galactinol synthase 1 (*GolS1*) and many other genes responsible for H_2O_2 signalling and defense (14, 15). To determine if OsHSF7 could induce the expression of these genes, we evaluated the expression profiles of *Apx1-4, tApx, sApx* and *GolS1-3*. No changes in the expression of *Apx* genes were observed in transgenic plants (data not show). However, the results of previous studies demonstrated that *Hsp101* is required for survival in response to severe heat stress and for the acquisition of thermotolerance (18). The results of the present study indicate that the accumulation of *HSP101* mRNA was higher in D35S:OsHSF7 plants than in wild type plants, which may result in the transgenic plants having a higher tolerance to heat stress.

MATERIALS AND METHODS

Plants, materials, growth conditions and treatments

To conduct the stress treatments, rice seeds (*Oryza sativa IR36*) were surface sterilized with 1% (v/v) NaOCl and then geminated in water at 37°C overnight, after which they were grown hydroponically at 25°C under a 12 h light:dark photoperiod for approximately 10 days, with light being provided by cool-white fluorescent lamps (150-200 $mol \cdot m^{-2} \cdot s^{-1}$). High shock stress was then induced by placing the seedlings in a growing room at 45°C ± 1 under constant illumination for 10 min to 4 h. The organs of mature rice plants at such as roots, stems and leaves, as well as samples collected from plants during different growing points such as the tillering stage, immature seeds in the booting stage, the spikes during the late booting stage, and mature seeds during the grain filling stage were harvested and frozen in liquid nitrogen for subsequent RNA extraction and further analyses.

Construction of a cDNA library and plasmid

The poly (A+) mRNA was derived from total RNA of 15-day-old rice seedlings that had been stressed by incubation at 40 ± 1°C for 2 h using the polyATract mRNA Isolation System (Promega, Madison, WI, USA). The rice cDNA was then synthesized using a SuperScript™ RT Kit (Invitrogen, Carlsbad, CA, USA). Next, the cDNA was ligated into a pPC86 vector, which is a Trp-marked yeast expression plasmid that contains a GAL4 activating domain under the control of the yeast alcohol dehydrogenase (*ADC1*) promoter.

Oligonucleotides containing two tandem copies of the wild-type HSE were synthesized by PCR using the following primers: hseZ5′-CAAGGACTTTCTCGAAAGTACTATACAAGG ACTTTCTCGAAAGTACTATACACTGACTGTCGTGATGGATC CTG-3′ and hseF5′-CAGGATCCATCACGACAGTCAGTG- 3′. A 74-bp fragment was then isolated and inserted into a bait plasmid, pLGΔ-265UP1, that was subsequently referred to as pLGΔ-265UP1 HSE. This plasmid was a URA-marked *E.coli*-yeast shuttle plasmid that carried the *lacZ* reporter gene under the control of the CYC1 minimal promoter.

Yeast one-hybrid screening of the rice cDNA library

The lithium acetate protocol was used to transfer pLGΔ-265UP1 HSE into yeast EGY48, after which the pPC86 vector was transferred into yeast that contained the bait plasmid. Next, the transformed yeast were selected by cultivation on media without Ura and Tyr, after which the colonies were overlaid onto nitrocellulose filters and incubated overnight at 30°C. The yeast permeated filters were then treated with X-Gal (5-bromo-4-chloro-3-indolyl β-D-galactoside) to identify yeast that possessed β-galactosidase activity. Plasmid DNA from the positive clones was then isolated and transformed into *E.coli* strain MC8, after which the transformants were selected by culture on M9 minimal medium containing all amino acids except tryptophan. The plasmid DNA was then transformed back into the yeast reporter strain to confirm the β-galactosidase activity.

Real-time reverse transcriptase (RT)-PCR analysis

The total RNA was extracted from rice samples using the method described above, after which it was treated with DNase I (Promega, Madison, WI, USA) to remove genomic DNA contamination. The first strand cDNA was then synthesized us-

ing a Reverse Transcription System (Promega, Madison, WI, USA) for real-time PCR. Next, amplification of specific regions in the targeted genes was conducted using an iQ SYBR Green Supermix Kit (BioRad, Hercules, CA, USA), after which real-time detection of the production was performed using a Mini Opticon Real Time PCR System (BioRad, Hercules, CA, USA). The real-time PCR primer sequences were designed using the Primer 3 Software (http://www.genome.wi.mit.edu/cgi-bin/primer/primer3.cgi). All cDNA samples were analyzed in duplicate or triplicate using cDNA derived from two sets of independently grown plants. The relative changes in gene expression were then quantified using the $2^{-\Delta\Delta Ct}$ method.

Transgenic plants over-expressing the *OsHSF7* cDNA

Agrobacterium tumefaciens strain GV3101 was used to transform the *Arabidopsis thaliana* cv. Columbia using the floral dip method. The plasmid used for the transformation of Arabidopsis was derived from pCAMBIA1304, after which the full-length *OsHSF7* cDNA was digested with *Bam*HI and *Sac*I and cloned into the binary vector under the control of an enhanced double CaMV 35S promoter.

Acknowledgements

The research was supported by 863 Program (2006AA10Z117); Shanghai Rising-Star Program (08QH14021); Shanghai International Scientific and Technological Cooperation of (08540706500); National Natural Science Foundation (30670179); Shanghai Natural Science Foundation (07ZR14094, 08ZR1417200) and Jiangsu Natural Science Foundation (BK2007080).

REFERENCES

1. Morimoto, R.I. (1998) Regulation of the heat shock transcriptional response: cross talk between family of heat shock factors, molecular chaperones, and negative regulators. *Genes Dev*. **12**, 3788-3796.
2. Nover, L., Bharti, K., Döring, P., Mishra, S.K., Ganguli, A. and Scharf, K.D. (2001) Arabidopsis and the heat stress transcription factor world: how many heat stress transcription factors do we need. *Cell Stress Chaperones* **6**, 177-189.
3. Baniwal, S.K., Bharti, K., Chan, K.Y., Fauth, M., Ganguli, A., Kotak, S., Mishra, S.K., Nover, L., Port, M., Scharf, K.D., Tripp, J., Zielinski, D. and von Koskull-Döring, P. (2004) Heat stress response in plants: a complex game with chaperones and more than 20 heat stress transcription factors. *J. Biosci*. **29**, 471-487.
4. Yamanouchi, U., Yano, M., Lin, H., Ashikari, M. and Yamada, K. (2002) A rice spotted leaf gene, *Spl7*, encodes a heat stress transcription factor protein. *Proc. Natl. Acad. Sci. U.S.A*. **9**, 7530 -7535.
5. Schöffl, F., Prändl, R. and Reindl, A. (1998) Regulation of the heat-shock response. *Plant Physiol*. **117**, 1135-1141.
6. Bharti, K., Schmidt, E., Lyck, R., Bublak, D. and Scharf, K.D. (2000) Isolation and characterization of HsfA3, a new heat stress transcription factor of Lycopersicon peruvianum. *Plant J*. **22**, 355-365.
7. Döring, P., Treuter, E., Kistner, C., Lyck, R., Chen, A. and Nover, L. (2000) The role of AHA motifs in the activator function of tomato heat stress transcription factors HsfA1 and HsfA2. *Plant Cell* **12**, 265-278.
8. Mishra, S.K., Tripp, J., Winkelhaus, S., Tschiersch, B., Theres, K., Nover, L. and Scharf, K.D. (2002) In the complex family of heat stress transcription factors, HsfA1 has a unique role as master regulator of thermotolerance in tomato. *Genes Dev*. **16**, 1555-1567.
9. Lohmann, C., Eggers-Schumacher, G., Wunderlich, M. and Schoffl, F. (2004) Two different heat shock transcription factors regulate immediate early expression of stress genes in Arabidopsis. *Mol. Genet. Genomics* **271**, 11-21.
10. Busch, W., Wunderlich, M. and Schöffl, F, (2005) Identification of novel heat shock factor-dependent genes and biochemical pathways in Arabidopsis thaliana. *Plant J*. **41**, 1-14.
11. Li, C., Chen, Q., Gao, X., Qi, B., Chen, N., Xu, S., Chen, J. and Wang, X. (2005) AtHsfA2 modulates expression of stress responsive genes and enhances tolerance to heat and oxidative stress in Arabidopsis. *Sci. China C Life Sci*. **48**, 540-550.
12. Nishizawa, A., Yabuta, Y., Yoshida, E., Maruta, T., Yoshimura, K. and Shigeoka, S. (2006) Arabidopsis heat shock transcription factor A2 as a key regulator in response to several types of environmental stress. *Plant J*. **48**, 535-547.
13. Charng, Y.Y., Liu, H.C., Liu, N.Y., Chi, W.T., Wang, C.N., Chang, S.H. and Wang, T.T. (2007) A heat-inducible transcription factor, HsfA2, is required for extension of acquired thermotolerance in Arabidopsis. *Plant Physiol*. **143**, 251-262.
14. Panikulangara, R.J., Eggers-Schumacher, G., Wunderlich, M., Stransky, H. and Schöffl, F. (2004) Galactinol synthase1: a novel heat shock factor target gene responsible for heat-induced synthesis of raffinose family oligosaccharides in Arabidopsis. *Plant Physio*l. **136**, 3148-3158.
15. Storozhenko, S., Pauw, P.D., Montagu, M.V., Inzé, D. and Kushnir, S. (1998) The heat-shock element is a functional component of the Arabidopsis APX1 gene promoter. *Plant Physiol*. **118**, 1005-1014.
16. Bharti, K., von Koskull-Döring, P., Bharti, S., Kumar, P., Tintschl-Körbitzer, A., Treuter, E. and Nover, L. (2004) Tomato heat stress transcription factor HsfB1 represents a novel type of general transcription coactivator with a histone-like motif interacting with HAC1/CBP. *Plant Cell* **16**, 1521-1535.
17. Miller, G. and Mittler, R. (2006) Could heat shock transcription factors function as hydrogen peroxide sensors in plants. *Ann. Bot*. **98**, 279-288.
18. Hong, S.W., Lee, U. and Vierling, E. (2003) Arabidopsis *hot* mutants de-fine multiple functions required for acclimation to high temperatures. *Plant Physiol*. **132**, 757-767.
19. Zhang, X., Henriques, R. and Lin, S.S. (2006) Agrobacterium-mediated transformation of Arabidopsis thaliana using the floral dip method. *Nat. Protoc.* **1**, 641-646.
20. Zhu, B., Xiong, A.S., Peng, R.H., Xu, J., Zhou, J., Xu, J.T., Jin, X.F., Zhang, Y., Hou, X.L. and Yao, Q.H. (2008) Heat stress protection in Aspen sp1 transgenic Arabidopsis thaliana. *BMB Rep*. **41**, 382-387.