BMB
Reports

Over-expression of *OsHsfA7* enhanced salt and drought tolerance in transgenic rice

Ai-Ling Liu[1,2,#], Jie Zou[2,#], Cui-Fang Liu[2], Xiao-Yun Zhou[1,2], Xian-Wen Zhang[1,2], Guang-Yu Luo[2] & Xin-Bo Chen[1,2,]*

[1]Key Laboratory for Crop Germplasm Innovation and Utilization of Hunan Province, Hunan Agricultural University, [2]College of Bioscience and Biotechnology, Hunan Agricultural University, Changsha, 410128, China

Heat shock proteins play an important role in plant stress tolerance and are mainly regulated by heat shock transcription factors (Hsfs). In this study, we generated transgenic rice over-expressing *OsHsfA7* and carried out morphological observation and stress tolerance assays. Transgenic plants exhibited less, shorter lateral roots and root hair. Under salt treatment, over-expressing *OsHsfA7* rice showed alleviative appearance of damage symptoms and higher survival rate, leaf electrical conductivity and malondialdehyde content of transgenic plants were lower than those of wild type plants. Meanwhile, transgenic rice seedlings restored normal growth but wild type plants could not be rescued after drought and re-watering treatment. These findings indicate that over-expression of *OsHsfA7* gene can increase tolerance to salt and drought stresses in rice seedlings. [BMB Reports 2013; 46(1): 31-36]

INTRODUCTION

As sessile organism, plants have evolved a variety of mechanisms to rapidly respond to extreme environmental conditions by synthesizing increased amounts or new isoforms of diverse functional proteins. Among these proteins, heat shock proteins (Hsps, also known as heat stress proteins) are a group of proteins that function as molecular chaperones in regulating cellular homeostasis and promoting survival under stressful conditions (1, 2). The induction of Hsps under stresses is primarily regulated by the heat shock transcription factors (Hsfs) that act by binding to the highly conserved heat shock element in the promoters of target genes (3). In many species, Hsfs are the terminal components of signal transduction chain mediating the activation of genes responsive to heat stress and a large number of chemical stressors (4-6).

*Corresponding author. Tel: +86-731-84635290; Fax: +86-731-846 35290; E-mail: xinbochen@live.cn
#A.L. Liu and J. Zou have contributed equally in this work.
http://dx.doi.org/10.5483/BMBRep.2013.46.1.090

Received 25 April 2012, Revised 30 July 2012, Accepted 15 August 2012

Keywords: Drought tolerance, *OsHsfA7*, Rice, Root morphology, Salt tolerance

Hsfs display a modular structure with an N-terminal DNA-binding domain characterized by a helix-turn-helix motif, an adjacent bipartite oligomerization domain composed of hydrophobic heptad repeats, a cluster of basic amino acid residues essential for nuclear import (the nuclear localization signal, or NLS) and a C-terminal activation domain (AHA motifs) (7, 8). In plants, the Hsf system is more complex than in any other organisms investigated so far. Based on sequence homology and domain architecture, plant Hsfs have been divided into three conserved classes, A, B and C (8). The rice genome contains thirteen class A, eight class B and four class C Hsfs. Furthermore, AHA motifs are crucial for the activity of class A Hsfs which have been reported to play a central role in the induction of various genes involved in defense under stressful conditions (9).

Many researches suggested that HsfAs not only responded to high temperature but also to oxidative stress, high salinity, chilling and other stresses. Over-expression of *HsfA1* genes enhanced thermotolerance in *Arabidopsis*, soybean and tomato (10-12). The transgenic *Arabidopsis* over-expressing *OsHsfA2e* exhibited tolerance to high salinity stress (13). Over-expression of *AtHsfA1b* improved resistance to chilling in transgenic tomato (14). It was reported that over-expression of *HsfA2* conferred increased tolerance to high light, salt, oxidative, osmotic and anoxia stresses (15-18). Shim *et al*. (19) demonstrated that transgenic rice and wheat over-expressing *HsfA4a* enhanced Cd tolerance.

Rice is one of the most important crops in the world. The growth and productivity of rice are often threatened by environmental factors. In the last decade, many efforts have been undertaken to generate stress tolerant rice by manipulating the expression of stress-responsive genes (13, 14, 20-22). We previously demonstrated that the expression of rice *OsHsfA7* gene can be induced by heat shock treatment and other abiotic stresses (23). In this study, we generated 35S::OsHsfA7 transgenic rice plants which exhibited different phenotype and higher survival rate under abiotic stresses compared with WT. Our results suggest that transgenic rice over-expressing *HsfA7* can increase resistance to high salinity and drought stresses.

RESULTS

Bioinformatics analysis of OsHsfA7

The cDNA of *OsHsfA7* with accession number AK064271 is

ISSN: 1976-6696 (print edition) / 1976-670X (electronic edition)

composed of 1,209 bp, coding for a protein of 402 aa with a predicted molecular mass of 43.9 kDa (pI 7.05). Putative HsfA7 proteins were retrieved through a BLASTP search using rice OsHsfA7 (LOC_Os01g39020) as the query. Their putative protein sequences were aligned using the DNAMAN program. OsHsfA7 shares 31.7% and 62.3% identity at the amino acid level with HsfA7 of Medicago and Sorghum. Phylogenetic analysis revealed that genetic relationship between *Oryza sativa* and Sorghum was closer (Fig. S1).

Root morphology in OsHsfA7-OE transgenic rice

Eight independent T0 rice transgenic lines were obtained after selection on hygromycin media and analyzed by RT-PCR. All the subsequent T1 and T2 lines showed conformed over-expression and similar root morphology and stress responses. The two highly over-expressed T2 lines (OE-1 and OE-8) were used as representative for later characterization. Root morphology of the WT and OsHsfA7-OE transgenic plant seedlings was shown in Fig. 1. OsHsfA7-OE plants exhibited longer young roots (including primary root and adventitious root) (Fig. 1A) but shorter and less lateral roots (Fig. 1B) and root hair (Fig. 1C) compared with the WT. Average radicle length of 5 d OsHsfA7-OE seedling was 3.7 cm, and that of WT was only 1.5 cm (Fig. S2A), the results showed that the young roots of transgenic seedlings grew faster than the control. Moreover, the roots of OsHsfA7-OE at tillering stage were thicker, sparser, more wide distributed and almost no lateral roots compared with that of wild type.

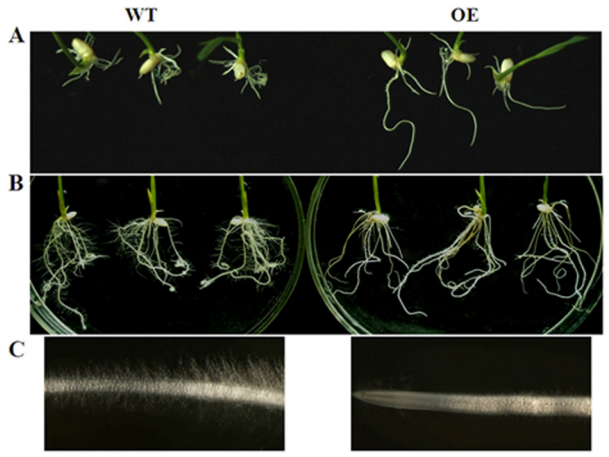

Fig. 1. Root morphology of wild-type and OsHsfA7-OE transgenic plants. (A, B) Primary root, adventitious root and lateral root in wild type and T2 transformants. Plants were grown in MS medium and photographed at 5 d (A) and 15 d (B). (C) Root hair of 7 d rice seedlings was observed by stereoscope.

Enhanced salt and drought tolerance by over-expression of *OsHsfA7*

After irrigating with 200 mM NaCl for 10 d, leaf apex of WT plants became brown and dried, whereas over-expressing *OsHsfA7* leaves remained green (Fig. 2B). Meanwhile, leaf REC and MDA content were lower in the transgenic lines than in the WT (Fig. 2E, F). In addition, hydroponic seedlings were treated with 200 mM NaCl for 24 h then transferred to 1/2 MS solution for recovery. After 4 d, leaves of WT were completely curled and wilted, while those of OsHsfA7-OE were rolled only in the tip part (Fig. 2C). After 10 d recovery, leaves of WT were withered and almost all plants were completely dead, while most of the transgenic plants remained alive and only the leaf tips scorched (Fig. 2D). The survival rates of the transgenic seedlings were 67.5% for OE-1 line and 70.8% for OE-8 line (Fig. S2B). These results indicate that over-expression of *OsHsfA7* can improve salt resistance of transgenic rice.

To examine the tolerance to drought stress, three-week-old plants were withheld water for 10 d and then re-watered for additional 10 d. Both WT and transgenic plants suffered severe blast after un-watering (Fig. 3B), whereas there was remarkable

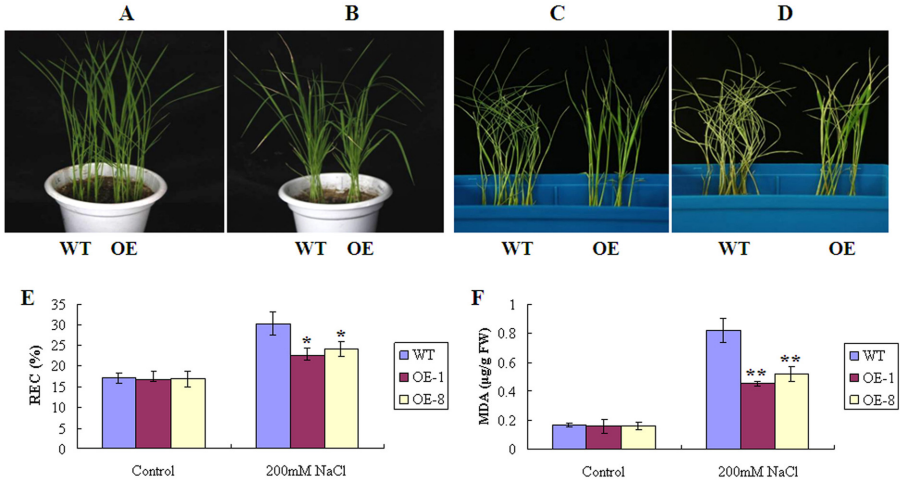

Fig. 2. Influence of high salt stress on rice seedlings. (A) Seedlings before treatment. Three-week-old *OsHsfA7* transgenic plants and WT control plants were grown in soil in a tray. (B) Seedlings were subjected to 200 mM NaCl for 10 d. (C, D) Hydroponic seedling treatment. WT and OsHsfA7-OE grown in 1/2 MS liquid medium for 2 weeks were treated by 200 mM NaCl for 24 h, they were photographed after recovery for 4 d (C) and 10 d (D). (E, F) Assay of REC and MDA content. Three-week-old seedlings were treated with 200 mM NaCl for 10 d. The data are the means ± SD of three independent experiments. The values with significant differences according to t-tests are indicated by asterisks (*$P < 0.05$; **$P < 0.01$).

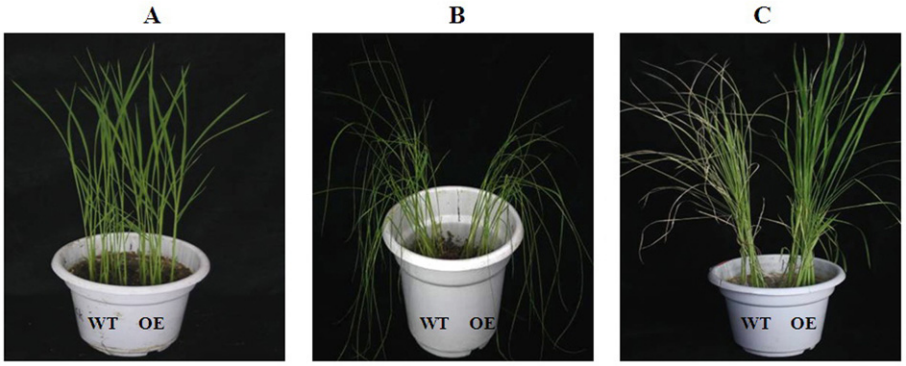

Fig. 3. Phenotype of the *OsHsfA7* over-expression transgenic plants in response to drought treatment. Three-week-old *OsHsfA7* transgenic plants and WT control plants were grown in soil in a tray. (A) Seedlings before treatment. (B) Seedlings were un-watered 10 d for drought treatment. (C) Seedlings were re-watered for 10 d after the treatment.

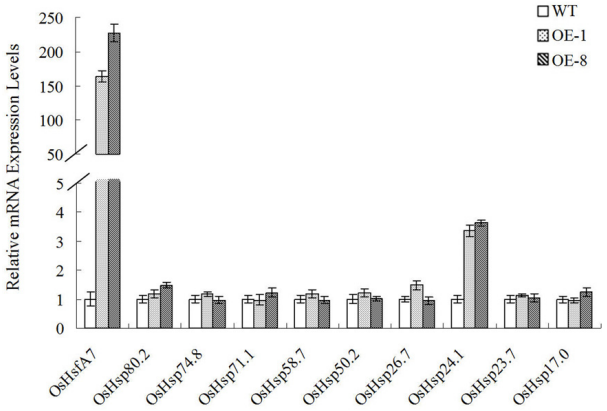

Fig. 4. Relative expression levels of *OsHsfA7* and Hsp genes in WT and transgenic rice under normal conditions analyzed by real-time qPCR. *OsUBQ5* was used as an internal control. The result is the average of three independent experiments and expression levels of these genes in WT were taken as 1, the error bars indicate ± SD. *OsHsp80.2, OsHsp74.8* and *OsHsp50.2* belong to *Hsp90* family. *OsHsp71.1, OsHsp58.7* and *OsHsp23.7* belong to *Hsp70* family. *OsHsp26.7, OsHsp24.1* and *OsHsp17.0* belong to *sHsp* family.

difference after re-watering. While most of the WT plant leaves showed further withered and could not be rescued, the majority of OsHsfA7-OE plants restored normal growth (Fig. 3C). The survival rates of the transgenic seedlings were 77.4% for OE-1 line and 82.8% for OE-8 line (Fig. S2C).

In addition, three-week-old seedlings of WT and transgenic plants with similar vigor were used for 47°C high temperature treatments for 90 min. After treatment, leaf tips of both WT and transgenic plants were rolled or withered, no significant difference was observed. After recovery for 7 d, both transgenic plants and WT plants remained alive and new leaves grew out. Meanwhile, we tested the thermo-tolerance of transgenic and WT plants at booting stage, no significant difference was found in seed setting rate after treatment.

Expression of putative downstream genes regulated by OsHsfA7

To investigate the putative downstream genes regulated by OsHsfA7, nine *Hsp* genes from three major Hsp families (Hsp90s, Hsp70s and sHsps) were selected to detect their relative expressions in WT and OsHsfA7-OE plants under normal growth conditions by RT-PCR. It was found that only *OsHsp24.1* was up-regulated in the OsHsfA7-OE plants, while expressions of the other eight *Hsp* genes showed no obvious difference between WT and transgenic rice (Fig. 4).

DISCUSSION

Individual Hsfs have unique functions during development. For example, HsfA9 was characterized as a specialized Hsf for embryogenesis and seed maturation in sunflower and *Arabidopsis* (24, 25). HsfA5 transcripts are mainly found in pollen together with HsfA4 in *Arabidopsis* (26). A rice HsfA4d mutant (*Spl7*) showed spontaneous necrotic lesions in mature leaves (27). In the present study, transgenic rice plants over-expressing *OsHsfA7* exhibited different root than WT. Noticeably, lateral roots and root hair of the OsHsfA7-OE rice were shorter and less than those of the WT at seedling stage. In the other hand, tap roots of OsHsfA7-OE rice were longer compared with WT. Our results indicate that OsHsfA7 has an important role in root growth and development.

There is increasing evidence that individual member of a Hsf family may play distinct role in response to various environmental stresses (9, 13, 15, 19, 28). The growth of OsHsfA7-OE seedlings in soil was unaffected under high salt stress, while the leaf apex of the control became scorched. Meanwhile, leaf REC and MDA content were lower in the transgenic lines than in the WT. Electrolyte leakage is an indirect measure of damage done to plant cell membranes, and lower REC indicates that less membrane damage occurred (29). MDA is one of the end products of lipid peroxidation damage from free radicals (30). Salt-tolerant plants have a more perfect defense mechanism to maintain low levels of MDA (31). These findings suggested that the changes of physiological index in plants are highly adapted

to stress resistance. In addition, the transgenic rice plants over-expressing *OsHsfA7* had increased tolerance to drought stress in our study. These results indicate that *OsHsfA7* may play a role as a member of natural defense system against high salinity and drought stresses.

Over-expression of *HsfA1* and *HsfA2* genes in *Arabidopsis,* tomato and soybean have been reported to enhance plant heat stress resistance (14-16, 32, 33). Nevertheless, no obvious heat tolerance of OsHsfA7-OE transgenic plants was observed in our experiment. A recent investigation showed that over-expression of *OsHsfC1b* improved tolerance to salt in rice (34). In addition, *HsfA3* was confirmed as part of drought stress signaling (35). These results indicated that a certain Hsf could be responsible for some specific kinds of abiotic stresses and different Hsfs could have diverse role in response to abiotic stresses.

Under abiotic stresses, different Hsfs can specifically bind to heat shock elements of some *Hsps* and subsequently activate their transcription (3-6). Almoguera *et al*. (24) found that the expression *HaHsp17.6G1* and *HaHsp17.7G4* were regulated by sunflower HsfA9. Busch *et al*. (36) demonstrated HsfA1a and (or) HsfA1b regulated the expression of *Hsp70* and *Hsp101* in double mutants. Over-expression of LlHsfA2 activated the downstream genes including *Hsp101, Hsp70* and *Hsp25.3* (37). We have checked the expression of nine *OsHsp* genes in OsHsfA7-OE transgenic plants and only *OsHsp24.1* was highly expressed in comparison with WT. It is likely that *OsHsp24.1* was potential target gene of OsHsfA7 and involved in the adaptation to high salinity or drought stress in transgenic rice.

MATERIALS AND METHODS

Phylogenetic analysis

OsHsfA7 homolog protein sequences from various plant species were retrieved from GenBank through a BLASTP search. OsHsfA7 and these homolog Hsfs protein sequences were aligned by DNAMAN software with default gap penalties. The phylogenetic tree was constructed by MEGA4 with the neighbor-joining algorithm using default settings. A bootstrap analysis of 1,000 replicates was performed.

Plasmid construction and rice transformation

The complete ORF of *OsHsfA7* was obtained from full-length cDNA clone AK064271 (National Institute of Agrobiological Sciences, Tsukuba, Japan) by Kpn I and Pst I digestion. Then the ORF fragment was cloned into Kpn I and Pst I digested pCAMBIA1301-Multi (modified from pCAMBIA1301) under the control of the CaMV 35S promoter. The construct was transformed into rice (*Oryza sativa* ssp. *japanica* var. *Nipponbare*) according to the rice genetic transformation method (38).

RNA isolation and real-time qPCR analysis

OsHsfA7-OE and WT were planted according to Liu *et al*. (23). After 3 weeks, rice leaf samples were collected for expression analysis of *OsHsfA7* and 9 *OsHsp* genes (primers listed in Table S1). Total RNA extraction, reverse transcription into cDNA and real-time quantitative PCR was performed according to Zou *et al*. (39). All cDNA samples were analyzed in triplicate from three sets of independent plants. The relative changes in gene expression were quantified using the $2^{-\triangle\triangle Ct}$ method. The data were expressed as mean ± standard error.

Drought, salt and heat stress treatments

T2 generation seeds of OsHsfA7-OE homozygous plants were used for stress treatments. After one week germination on 1/2 MS solid medium, the seedlings were transferred to nutritious soil in plastic pots and placed in a growth chamber (14-h-light/10-h-dark cycles) at 30°C and 75% relative humidity. After two weeks, the seedlings were used for the following abiotic stress treatments. For drought tolerance treatment, seedling plants were withheld water for 10 d, and then re-watered for 10 d. For high-salt stress treatment, seedlings were irrigated with 200 mM NaCl solution for 10 d. For heat stress treatment, plants were exposed to 47°C for 90 min. In addition, salt tolerance at seedling stage was evaluated under another stress conditions. After germination on 1/2 MS solid medium for one week, hydroponic seedlings were cultured with 1/2 MS liquid medium for 1 week, and followed by 24 h treatment in 1/2 MS solution containing 200 mM NaCl, and then transferred back to 1/2 MS solution for 4 d and 10 d recovery, respectively. The seedlings were evaluated for their survival percentage based on observations that actively growing seedlings as survivors and the non-growing and wilted seedlings were as non-survivors. All above experiments were repeated three times. The phenotype of *OsHsfA7* transgenic plants and the WT under different treatments was observed and photographed.

Assay for relative electrical conductivity (REC) and malondialdehyde (MDA)

Three-week-old seedlings were treated with 200 mM NaCl for 10 d and the leaf REC and MDA were assayed. The leaf REC was measured at the beginning and at the end of salt treatment as the method described by Yu *et al*. (40). Five seedling shoots were harvested before and after the treatment and finely ground in liquid nitrogen using a mortar and pestle previously chilled with liquid nitrogen and the frozen powder was immediately used for MDA assay. MDA content was measured for salt treatment according to Kuk *et al*. (41). The mean values of REC and MDA were taken from the measurements of three replicates and 'Standard Error' of the means was calculated. Data were analyzed by Excel using *t* test to assess the significance of differences among the means.

Acknowledgements

This study was supported by Natural Science Foundation of China (30870206), National Special Key Science and Technology Project of China (2009ZX08001-026B), Special Key Science and Technology Project of Hunan Province (2009FJ1004-1) and Provincial Youth Foundation of Hunan (09B045).

REFERENCES

1. Haslbeck, M. (2002) sHsps and their role in the chaperone network. *Cell Mol. Life Sci.* **59**, 1649-1657.
2. Hartl, F. U. and Hayer-Hartl, M. (2002) Molecular chaperones in the cytosol: from nascent chain to folded protein. *Science* **295**, 1852-1858.
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., Weber, C., Zielinski, D. and von Koskull-Döring, P. (2004) Heat stress response in plants: A complex game with chaperones and more than twenty heat stress transcription factors. *J. Biosci.* **29**, 471-487.
4. Morimoto, R. I. (1998) Regulation of the heat stress transcriptional response: cross talk between family of heat stress factors, molecular chaperones, and negative regulators. *Genes Dev.* **12**, 3788-3796.
5. Schöffl, F., Prändl, R. and Reindl, A. (1998) Regulation of the heat shock response. *Plant Physiol.* **117**, 1135-1141.
6. Wu, C. (1995) Heat shock transcription factors: structure and regulation. *Annu. Rev. Cell Dev. Biol.* **11**, 441-469.
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. Nover, L., Bharti, K., Döring, P., Mishra, S. K., Ganguli, A. and Scharf, K. D. (2001) *Arabidopsis* and the Hsf world: How many heat stress transcription factors do we need? *Cell Stress Chaperones.* **6**, 177-189.
9. Chauhan, H., Khurana, N., Agarwal, P. and Khurana, P. (2011) Heat shock factors in rice (*Oryza sativa L.*): genome-wide expression analysis during reproductive development and abiotic stress. *Mol. Genet. Genomics.* **286**, 171-187.
10. Lohmann. C., Eggers-Schumacher, G., Wunderlich, M. and Schöffl, F. (2004) Two different heat shock factors regulate immediate early expression of stress genes in *Arabidopsis*. *Mol. Genet. Genomics.* **271**, 11-21.
11. 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.
12. Zhu, B., Ye, C., Lü, H., Chen, X., Chai, G., Chen, J. and Wang, C. (2006) Identification and characterization of a novel heat shock transcription factor gene, GmHsfA1, in soybeans (*Glycine max*). *J. Plant Res.* **119**, 247-256.
13. Yokotani, N., Ichikawa, T., Kondou, Y., Matsui, M., Hirochika, H., Iwabuchi, M. and Oda, K. (2008) Expression of rice heat stress transcription factor OsHsfA2e enhances tolerance to environmental stresses in transgenic *Arabidopsis*. *Planta.* **227**, 957-967.
14. Li, H. Y., Chang, C. S., Lu, L. S., Liu, C. A., Chan, M. T. and Charng, Y. Y. (2003) Over-expression of *Arabidopsis thaliana* heat shock factor gene (AtHsfA1b) enhances chilling tolerance in transgenic tomato. *Bot. Bull. Acad. Sin.* **44**, 129-140.
15. Banti, V., Mafessoni, F., Loreti, E., Alpi, A. and Perata, P. (2010) The heat-inducible transcription factor HsfA2 enhances anoxia tolerance in *Arabidopsis*. *Plant Physiol.* **152**, 1471-1483.
16. Li, C. G., Chen, Q. J., Gao, X. Q., Qi, B. S., Chen, N. Z., Xu, S. M., Chen, J. and Wang, X. C. (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.
17. 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.
18. Ogawa, D., Yamaguchi, K. and Nishiuchi, T. (2007) High-level overexpression of the *Arabidopsis* HsfA2 gene confers not only increased themotolerance but also salt/osmotic stress tolerance and enhanced callus growth. *J. Exp. Bot.* **58**, 3373-3383.
19. Shim, D., Hwang, J. U., Lee, J., Lee, S., Choi, Y., An, G., Martinoia, E. and Lee, Y. (2009) Orthologs of the class A4 heat shock transcription factor HsfA4a confer cadmium tolerance in wheat and rice. *Plant Cell.* **21**, 4031-4043.
20. Huang, C., Ding, S., Zhang, H., Du, H. and An, L. (2011) CIPK7 is involved in cold response by interacting with CBL1 in *Arabidopsis thaliana*. *Plant Sci.* **181**, 57-64.
21. Tripathi, V., Parasuraman, B., Laxmi, A. and Chattopadhyay, D. (2009) CIPK6, a CBL-interacting protein kinase is required for development and salt tolerance in plants. *Plant J.* **58**, 778-790.
22. Wang, Y., Wan, L., Zhang, L., Zhang, Z., Zhang, H., Quan, R., Zhou, S. and Huang, R. F. (2012) An ethylene response factor OsWR1 responsive to drought stress transcriptionally activates wax synthesis related genes and increases wax production in rice. *Plant Mol. Biol.* **78**, 275-288.
23. Liu, A. L., Zou, J., Zhang, X. W., Zhou, X. Y., Wang, W. F., Xiong, X. Y., Chen, L. Y. and Chen, X. B. (2010) Expression profiles of class a rice heat shock transcription factor genes under abiotic stresses *J. Plant Biol.* **53**, 142-149.
24. Almoguera, C., Rojas, A., Díaz-Martín, J., Prieto-Dapena, P., Carranco, R. and Jordano, J. (2002) A seed-specific heat-shock transcription factor involved in developmental regulation during embryogenesis in sunflower. *J. Biol. Chem.* **277**, 43866-43872.
25. Kotak, S., Vierling, E., Bäumlein, H. and von Koskull-Döring, P. (2007) A novel transcriptional cascade regulating expression of heat stress proteins during seed development of *Arabidopsis*. *Plant Cell* **19**, 182-195.
26. Baniwal, S. K., Chan, K. Y., Scharf, K. D. and Nover, L. (2007) Role of heat stress transcription factor HsfA5 as specific repressor of HsfA4. *J. Biol. Chem.* **282**, 3605-3613.
27. Yamanouchi, U., Yano, M., Lin, H., Ashikari, M. and Yamada, K. (2002) A rice spotted leaf gene, Sp l7, encodes a heat stress transcription factor protein. *Proc. Natl. Acad. Sci. U. S. A.* **99**, 7530-7535.
28. Liu, H. C., Liao, H. T. and Charng, Y. Y. (2011) The role of class A1 heat shock factors (HSFA1s) in response to heat and other stresses in *Arabidopsis*. *Plant. Cell Environ.* **34**, 738-751.
29. Bajji, M., Kinet, J. and Lutts, S. (2002) The use of the electrolyte leakage method for assessing cell membrane stabil-

ity as a water stress tolerance test in durum wheat. *Plant Growth Regul.* **36**, 61-70.
30. Marnett, L. J. (1999) Lipid peroxidation-DNA damage by malondialdehyde. *Mutat. Res-Fund Mol. M.* **424**, 83-95.
31. Xie, Z., Duan, L., Tian, X., Wang, B., Eneji, A. E. and Li, Z. (2008) Coronatine alleviates salinity stress in cotton by improving the antioxidative defense system and radical-scavenging activity. *Plant Physiol.* **165**, 375-384.
32. 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.
33. Liu, J. G., Qin, Q. L., Zhang, Z., Peng, R. H., Xiong, A. S., Chen, J. M. and Yao, Q. H. (2009) OsHSF7 gene in rice, Oryza sativa L., encodes a transcription factor that functions as a high temperature receptive and responsive factor. *BMB Rep.* **42**, 16-21.
34. Schmidt, R., Schippers, J. H., Welker, A., Mieulet, D., Guiderdoni, E. and Mueller-Roeber, B. (2012) Transcription factor OsHsfC1b regulates salt tolerance and development *in Oryza sativa ssp. japonica*. *AoB Plants.* doi:10.1093/aobpla/pls011.
35. Scharf, K. D., Berberich, T., Ebersberger, I. and Nover, L. (2012) The plant heat stress transcription factor (Hsf) family: structure, function and evolution. *Biochim. Biophys. Acta.* **1819**, 104-119.
36. 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.
37. Xin, H., Zhang, H., Chen, L., Li, X., Lian, Q., Yuan, X., Hu, X., Cao, L., He, X. and Yi, M. (2010) Cloning and characterization of HsfA2 from Lily (Lilium longiflorum). *Plant Cell Rep.* **29**, 875-885.
38. Toki, S., Hara, N., Ono, K., Onodera, H., Tagiri, A., Oka, S. and Tanaka, H. (2006) Early infection of scutellum tissue with Agrobacterium allows high speed transformation of rice. *Plant J.* **47**, 969-976.
39. Zou, J., Liu, C. F., Liu, A. L., Zou, D. and Chen, X. B. (2012) Overexpression of *OsHsp17.0* and *OsHsp23.7* enhances drought and salt tolerance in rice. *J. Plant. Physiol.* **169**, 628-635.
40. Yu, X., Peng, Y. H., Zhang, M. H., Shao, Y. J., Su, W. A. and Tang, Z. C. (2006) Water relations and an expression analysis of plasma membrane intrinsic proteins in sensitive and tolerant rice during chilling and recovery. *Cell Res.* **16**, 599-608.
41. Kuk, Y. I., Shin, J. S., Burgos, N. R., Hwang, T. E., Han, O., Cho, B. H., Jung, S. and Guh, J. O. (2003) Antioxidative enzymes offer protection from chilling damage in rice plants. *Crop. Sci.* **43**, 2109-2117.