Expression of a rice DREB1 gene, OsDREB1D, enhances cold and high-salt tolerance in transgenic Arabidopsis

Yang Zhang1,2,*, Chen Chen1,2,*, Xiao-Fen Jin2, Ai-Sheng Xiong2, Ri-He Peng2, Yi-Huan Hong1, Quan-Hong Yao2,∗ & Jian-Min Chen1,∗

1College of Bioscience and Biotechnology, Yangzhou University, 88 Daxue Road, Yangzhou 225009, 2Biotechnology Research Institute, Shanghai Academy of Agricultural Sciences, Shanghai, 201106, China

OsDREB1D, a special DRE (dehydration responsive element binding protein) homologous gene, whose transcripts cannot be detected in rice (Oryza sativa L.), either with or without stress treatments, was amplified from the rice genome DNA. The yeast one-hybrid assay revealed that OsDREB1D was able to form a complex with the dehydration responsive element/C-repeat motif. It can also bind with a sequence of LTRE (low temperature responsive element). To analyze the function of OsDREB1D, the gene was transformed and over-expressed in Arabidopsis thaliana cv. Columbia. Results indicated that the over-expression of OsDREB1D conferred cold and high-salt tolerance in transgenic plants, and that transgenic plants were also insensitive to ABA (abscisic acid). From these data, we deduced that this OsDREB1D gene functions similarly as other DREB transcription factors. The expression of OsDREB1D in rice may be controlled by a special mechanism for the redundancy of function. [BMB reports 2009; 42(8): 486-492]

INTRODUCTION

Land plants are greatly affected by environmental stresses such as drought, high-salt, and extreme temperature. These stresses induce various biochemical and physiological responses in plants. Expression of a variety of genes has been demonstrated to be induced by these stresses in a variety of plants, especially the DREB transcription factors. These proteins bind to a dehydration-responsive element (DRE) with the core sequence A/GCCGAC identified as a cis-acting promoter element, and regulate downstream gene expressions in response to drought, high-salt, and cold stresses (1).

Many DRE-binding transcription factors have been identified in kinds of plants, such as eucalyptus (Eucalyptus globules), sweetpotato (Ipomoea batatas) and tall fescue (Festuca arundinacea) (2-4). The function of these transcription factors was further studied, especially in Arabidopsis and rice. Strong tolerance to freezing stress was observed in transgenic Arabidopsis plants that over-express CBF1 (DREB1B) cDNA under control of the cauliflower mosaic virus (CaMV) 35S promoter (5). The over-expression of the CBF3 (DREB1A) also resulted in higher tolerance to drought, high-salt, and freezing stress (6-8). Novillo reported that CBF2/DREB1C negatively regulates the expression of CBF1/DREB1B and CBF3/DREB1A, which guarantees the proper induction of downstream genes and the accurate development of Arabidopsis tolerance to freezing and related stresses (9). In contrast to the three identified CBF/DREB1 homologs, which were induced under cold stress, but not under ABA treatment, CBF4 gene expression was up-regulated by drought stress and ABA treatment, but not by low temperature. Over-expression of CBF4 in transgenic Arabidopsis plants improved tolerance to freezing and drought stress (10). In rice, the OsDREB1-type proteins were defined as those that show high homology to Arabidopsis DREB1A proteins (11). The expression of both OsDREB1A and OsDREB1B was induced by cold. Transgenic rice plants over-expressing the OsDREB1A genes showed growth retardation under normal growth conditions and improved tolerance to drought, high-salt and low-temperature stresses like the transgenic Arabidopsis plants over-expressing OsDREB1A (11, 12). The over-expression of these DREB-type transcription factors in transgenic Arabidopsis plants induced expression of Arabidopsis CBF-targeted genes involved in cold acclimation and drought adaptation (10).

In this study, we report the OsDREB1D, a gene encoding a protein that is the closest homolog of CBF/DREB1 in rice. However, the expression of OsDREB1D is not detected in rice plants, either with or without stress treatment. The over-expression of OsDREB1D under the CaMV 35S promoter resulted in the tolerance to freezing and high-salt stress in transgenic plants. The transgenic plants are insensitive to ABA.

Keywords: Abiotic stress, Arabidopsis, DREB-type transcription factors, Rice, Transgenic plants
RESULTS

Identification and phylogenetic analysis of the OsDREB1D

Dubouzet searched the rice genome database with amino acid sequence of the DREB1A protein, and found a genomic clone showed high homology in the conserved region (accession No. AP023482, nucleotide 1489-2250). The gene was named as OsDREB1D. Of interest, that OsDREB1D mRNA was not detected in rice plants either with, or without stress treatments (11). We amplified it by PCR, and cloned it from rice genome DNA. OsDREB1D contained an open reading frame of 253 amino acids.

We were unable to detect the OsDREB1D mRNA in any organs including roots, leaves, shoots, growing points, spike at late bolting stage and seeds of rice (Oryza sativa L.) with, or without stress treatments. To predict the function of OsDREB1D, phylogenetic analysis was carried out based on the similarities of AP2 domains in AP2/EREBP proteins from rice and Arabidopsis (Supplementary Fig. 1). The OsDREB1D was closest to OsDREB1A on the phylogenetic tree. These two genes show 70% identity at the amino acid level over the entire ORFs (data not shown), and they are the highest homologs in the genes included in the phylogenetic tree. It seems likely that OsDREB1D and OsDREB1A may be orthologous and play similar roles under stress conditions.

Over-expression of OsDREB1D gene improved stress tolerance in transgenic Arabidopsis

The high degree of sequence similarity between OsDREB1D and OsDREB1A suggested that the proteins were probably functional homologs. To test this hypothesis, the OsDREB1D gene was constitutively over-expressed under the control of the CaMV 35S promoter in transgenic Arabidopsis plants. Three independent homozygous T3 transgenic lines, named as 8217-1, 8217-2, 8217-4, were chosen to undergo physiological experiments. Although the expression levels of OsDREB1D were different in each independent line, it was readily detectable (Fig. 1A). We treated them with several stresses including cold, heat, high-salt, and drought. The transgenic plants and wild-type plants showed no difference when treated by heat stress or drought stress (data not shown). For high-salt tolerance analysis, seeds of the transgenic lines and wild-type plants were germinated on MS media containing 0, 75 and 100 mM NaCl, respectively, at 22°C. The germination rates of the transgenic and the wild-type lines were compared. Seeds were considered to have germinated when the seed coat was broken. After 5 d in 75 mM NaCl, 65% to 80% seeds of three selected transgenic lines germinated, compared to 40% of wild-type seeds. In medium containing 100 mM NaCl, 40% to 50% seeds of transgenic lines germinated in contrast to only 20% of wild-type seeds. High-salt stress inhibited germination in the wild-type.
OsDREB1D in transgenic Arabidopsis thaliana
Yang Zhang, et al.

seeds more severely than in all transgenic plants. The germination rates of transgenic plants on the plates without salt were almost the same as those of the wild-type plants (Fig. 1B-D). These results indicated that the transgenic lines displayed strong tolerance against high-salt stress.

Three-week-old plants were used to compare the degree of cold tolerance between transgenic and wild-type lines. After cold acclimation, the plants were placed under normal growth conditions for one week. The constitutive over-expression of the OsDREB1D gene resulted in an increase in cold tolerance (Fig. 2). The degree of cold tolerance was correlated with the level of OsDREB1D expression, in which the 8217-2 and 8217-4 transgenic lines exhibited higher level of expression and greater cold tolerance than the 8217-1 plants. These results indicate that the OsDREB1D protein also plays an important role in cold tolerance.

DNA-binding activity analysis of OsDREB1D using yeast one-hybrid assays
As OsDREB1D belonged to the DREB subgroup, we tested the DRE-binding activity using yeast one-hybrid assays. A similar motif was identified as C-repeat (CRT) and low temperature-responsive element (LTRE) in cold-inducible genes (13, 14). For the 35S:OsDREB1D enhances the cold tolerance, the binding activity between OsDREB1D and the cis-element LTRE was also tested. The yeast containing only bait plasmid pG221 did not display blue while the yeast containing bait plasmid pG221 and the expression plasmid display blue (Fig. 3A). This result demonstrated that OsDREB1D can bind with both DRE and LTRE.

OsDREB1D activates the expression of cold-responsive genes
To elucidate the molecular mechanism of OsDREB1D in the cold response, we monitored the expression of cold-responsive genes identified in the regulated pathways by real-time PCR analysis. Under 4°C cold treatment for 24 h, the expression of the tested marker genes, including RD29A, COR15A, KIN1, showed significant induction in both wild-type plants and transgenic plants under cold-stress conditions, consistent with
previous studies (1, 15-18). The expression of these three genes in OsDREB1D-overexpressed transgenic plants was substantially higher than that in wild-type plants (Fig. 3B-D). The variations in the gene expressions among the lines are consistent with the different expression of the target genes. As the three cold-inducible genes contain the DRE element in their promoter regions and have been identified as downstream genes of AtCBF3 in Arabidopsis (19), over-expression of OsDREB1D increases expression of RD29A, COR15A, KIN1, all of which are involved in plant tolerance.

ABA insensitive of transgenic plants

ABA plays an important role in the tolerance response of plants to drought and high salinity. Exogenous application of ABA also induces a number of genes that respond to dehydration and cold stress (20, 21). However, the role of ABA in cold stress-responsive gene expression is not clear. To explore whether OsDREB1D responds to exogenous ABA, we analyzed the sensitivity of the transgenic plants to ABA. First we analyzed the germination rates of the transgenic and wild-type plants on the MS plates containing 0, 1.2, 2.0 μM ABA, respectively. ABA inhibited the germination in the wild-type plants more severely than in the transgenic plants (Fig. 4A). After three weeks, the effect of ABA on the growth of these transgenic plants compared with that of the wild-type plants was also observed. The roots of the transgenic plants were much longer than those of the wild-type plants (Fig. 4B). These results indicate that the OsDREB1D protein does respond to exogenous ABA and may be involved in ABA-dependent pathway.

DISCUSSION

The over-expression of OsDREB1D conferred cold and high-salt tolerance and ABA insensitiveness in transgenic Arabidopsis

In this study, we reported a new DREB-type transcription factor in rice, OsDREB1D. To study its function, the OsDREB1D gene was over-expressed in Arabidopsis. The transgenic plants increased the cold and high-salt tolerance. The similar phenotypes have been observed for the constitutive over-expression of CBF1 (5), CBF3 (6-8) and OsDREB1A (11, 12), indicating that OsDREB1D is, in fact, a new member of the CBF/DREB1 family. Interestingly, the level of cold tolerance that can be achieved by OsDREB1D over-expression is very similar to that of the OsDREB1A gene. And the over-expression of OsDREB1D resulted in constitutive expression of COR15A, RD29A and KIN1, which also happened in over-expression of OsDREB1A (11), suggesting that these two genes may have redundancy in function.

Most CBF/DREB1-type transcription factors have been reported not to be induced by ABA treatment and they are involved in the ABA-independent pathway (15, 22-24). However, recent studies revealed that ABA-dependent pathway can also involve the CRT/DRE elements and AP2-type transcription factors (25, 10). In the ABA treatment assay, we found that the transgenic plants over-expressing the OsDREB1D were less sensitive to ABA than the wild-type plants. The result demon-

![Fig. 4. Comparison of growth inhibition by ABA in transgenic and wild-type plants. (A) The seed germination rates of transgenic and wild-type plants were measured on MS plates containing 1.2, 2.0 μM ABA from 3 to 9 d after sowing. (B) Three-week-old seedlings of wild-type and transgenic plants grown on MS plates with 1.2 μM ABA.](http://bmbreports.org)
strates again that the CRT/DRE elements are also involved in ABA signal transduction.

**Why the transcripts of OsDREB1D cannot be detected in rice?**

As mentioned above, the mRNA of OsDREB1D cannot be detected in any organs of rice. However, an independent group submitted the cDNA sequence for OsDREB1D to GeneBank (AF243384.1). Therefore, it is possible that OsDREB1D is expressed in a different specific growth stage or organ other than those found in our experiment. We attempted to over-express OsDREB1D in rice by Agrobacterium-mediated transformation. The transgenic rice plants were confirmed by expression of the GUS gene via histochemical staining, which is an adjacent reporter gene with the cloned OsDREB1D within the T-DNA region. However, we were unable to detect any transcripts of OsDREB1D from transgenic plants (data not shown). On the one hand, the transgenic plants are likely false positive. On the other hand, there may be a special mechanism to control the expression of OsDREB1D. It is likely that there was a certain miRNA which degraded the mRNA once the OsDREB1D was transcribed. However, we did not find any possible miRNA from the DNA sequence of OsDREB1D. In plants, the DNA methylation of promoter regions usually inhibits transcription. In some cases, DNA methylation controls the overall level of expression from a family of repeated genes (26). For example, the expression of tryptophan biosynthesis genes in the Phosphoribosylanthranilate isomerases (PAI) family can be modulated by DNA methylation. In some A. thaliana accessions, a PAI1-PAI4 inverted repeat triggers the silencing of the homologous PAI2 gene (27). If the expression of OsDREB1D is really silenced, it may be related with DNA methylation. What’s more, phylogenetic analysis revealed that OsDREB1D and OsDREB1A are the closest genes on the phylogenetic trees. And the over-expression of OsDREB1D and OsDREB1A in Arabidopsis showed that the function of these two genes was very similar. We predicted that the mRNA of OsDREB1D was likely to be degraded in particular developmental stage for its redundant function.

**MATERIALS AND METHODS**

**Phylogenetic analysis**

The nucleotide and amino-acid sequences were compared with those released in GeneBank databases by using the BLAST program, and associated molecular information were analyzing by BioEdit and Clustal W.

**Generation of transgenic Arabidopsis**

The incubation and growth conditions of A. thaliana were the same as described by Zhang et al (28). The full length OsDREB1D DNA was amplified from rice genome DNA. Reactions were performed using the PCR primers 5’-CCACAGAGAGGTACGAGTACGAGTTGTCCTTCAC-3’; 5’-CATGAGTCTCCGGGAGGAAGTG-3’) according to the sequence of OsDREB1D gene. Semiquantitative RT-PCR was assessed as described previously (33). For real-time PCR, triplicate quantitative assays were performed on 1 μl of each cDNA dilution with the SYBG GreenMaster mix and an ABI 7,900 sequence detection system according to the manufacturer’s protocol (Biorad). The relative quantification method (Delta-Delta CT) was used to evaluate quantitative variation between the replicates examined. The amplification of Actin was used as an internal control to normalize all data. Gene-specific primers for RD29A, 5’-AAGGAAACGACGACAAGGAAG-3’; 5’-CCACCCACCAAGCCAGCCAGAC-3’; for COR15A, 5’-TAAAGGAGGGAGGCTAAGGA-3’; 5’-AGATGATCCGCTGACTCTG-3’; for KIN1, 5’-TACCCTTCAAGGCGGTCAGC-3’; 5’-AGGCCGTTACGTCCCTCAC-3’.

**Yeast one-hybrid assay**

The bait plasmid pG221 and the expression plasmid pPC86 were reconstructed as described previously (34). The plasmid pG221 was first transformed into yeast EGY48 using lithium
acetate protocol as described by Gietz and Woods (35), and then the plasmid pPC86 was transformed into the yeast containing bait plasmid pG221. Then transformed yeast was overlaid onto media containing X-gal (5-bromo-4-chloro-3-indolyl b-D-galactoside) using nitrocellulose filters and tested the β-galactosidase activity.

Acknowledgements

The research was supported by Jiangsu Natural foundation (BK2007080); 863 Program (2006AA10Z117; 2006AA06Z358; 2008AA10Z401); Shanghai Rising-Star Program (08QH14021); National and Shanghai Natural Science Foundation (30670179; 08ZR1417200); The Key Project Fund of the Shanghai Municipal Committee of Agriculture and Shanghai University (BK2007080); 863 Program (2006AA10Z117; 2006AA06Z358; 2008AA10Z401); Shanghai Rising-Star Program (08QH14021); National and Shanghai Natural Science Foundation (30670179; 08ZR1417200); The Key Project Fund of the Shanghai Municipal Committee of Agriculture (No. 2008-7-5). We thank Dr. Genlou Sun, Saint Mary’s University, Canada, for his critical review of the manuscript.

REFERENCES

1. Yamaguchi-Shinozaki, K. and 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.
2. Gamboa, M. C., Rasmussen-Poblete, S., Valenzuela, P. D. and Krauskopf, E. (2007) Isolation and characterization of a cDNA encoding a CBF transcription factor from E. globulus. Plant Physiol. Biochem. 45, 1-5.
3. Kim, Y. H., Yang, K. S., Ryu, S. H., Kim, K. Y., Song, W. K., Kwon, S. Y., Lee, H. S., Bang, J. W. and Kwak, S. S. (2008) Molecular characterization of a CDNA encoding DRE-binding transcription factor from dehydration-treated fibrous roots of sweetpotato. Plant Physiol. Biochem. 46, 196-204.
4. Tang, M., Lu, S., Jing, Y., Zhou, X., Sun, J. and Shen, S. (2005) Isolation and identification of a cold-inducible gene encoding a putative DRE-binding transcription factor from Festuca arundinacea. Plant Physiol. Biochem. 43, 233-239.
5. Jaglo-Ottesen, K. R., Gilmour, S. J., Zarka, D. G., Schabenberger, O. and Thomashow, M. F. (1998) Arabidopsis CBF1 overexpression induces COR genes and enhances freezing tolerance. Science 280, 104-106.
6. Gilmour, S. J., Sebott, A. M., Salazar, M. P., Everard, J. D. and Thomashow, M. F. (2000) Overexpression of the Arabidopsis CBF3 transcriptional activator mimics multiple biochemical changes associated with cold acclimation. Plant Physiol. 124, 1854-1865.
7. Kasuga, M., Liu, Q., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1999) Improving plant drought, salt, and freezing tolerance by gene transfer of a single stress-inducible transcription factor. Nat Biotechnol. 17, 287-291.
8. Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1998) Two transcription factors, DREB1 and DREB2, with an EREBP/AP2 DNA binding domain separate two cellular signal transduction pathways in drought- and low-temperature-responsive gene expression, respectively, in Arabidopsis. Plant Cell 10, 1391-1406.
9. Novillo, F., Alonso, J. M., Ecker, J. R. and Salinas, J. (2004) CBF2/DREB1C is a negative regulator of CBF1/DREB1B and CBF3/DREB1A expression and plays a central role in stress tolerance in Arabidopsis. Proc. Natl. Acad. Sci. U.S.A. 101, 3985-3990.
10. Haake, V., Cook, D., Reichmann, J., Pineda, O., Thomashow, M. F. and Zhang, J. Z. (2002) Transcription factor CBF4 is a regulator of drought adaptation in Arabidopsis. Plant Physiol. 130, 639-648.
11. Dubouzet, J. G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E. G., Miura, S., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2003) OsDREB genes in rice, Oryza sativa L., encode transcription activators that function in drought-, high-salt- and cold-responsive gene expression. Plant J. 33, 751-763.
12. Ito, Y., Katsura, K., Maruyama, K., Taji, T., Kobayashi, M., Seki, M., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2006) Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. Plant Cell Physiol. 47, 141-153.
13. Baker, S. S., Wilhelm, K. S. and Thomashow, M. F. (1994) The 5'-region of Arabidopsis thaliana cor15a has cis-acting elements that confer cold-, drought- and ABA-regulated gene expression. Plant Mol. Biol. 24, 701-713.
14. Jiang, C., Lu, B. and Singh, J. (1996) Requirement of a CCGAC cis-acting element for cold induction of the BN115 gene from winter Brassica napus. Plant Mol. Biol. 30, 679-684.
15. Gilmour, S. J., Zarka, D. G., Stockinger, E. J., Salazar, M. P., Houghton, J. M. and Thomashow, M. F. (1998) Low temperature regulation of the Arabidopsis CBF family of AP2 transcriptional activators as an early step in cold-induced COR gene expression. Plant J. 16, 433-442.
16. Kobayashi, F., Takumi, S. and Nakamura, C. (2008) Increased freezing tolerance in an ABA-hypersensitive mutant of common wheat. J. Plant Physiol. 165, 224-232.
17. Kurkela, S. and Borg-Franck, M. (1992) Structure and expression of kin2, one of two cold- and ABA-induced genes of Arabidopsis thaliana. Plant Mol. Biol. 19, 689-692.
18. Stockinger, E. J., Gilmour, S. J. and Thomashow, M. F. (1997) Arabidopsis thaliana CBF1 encodes an AP2 domain-containing transcriptional activator that binds to the C-repeat/DRE, a cis-acting DNA regulatory element that stimulates transcription in response to low temperature and water deficit. Proc. Natl. Acad. Sci. U.S.A. 94, 1035-1040.
19. Maruyama, K., Sakuma, Y., Kasuga, M., Ito, Y., Seki, M., Goda, H., Shimada, Y., Yoshida, S., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2004) Identification of cold-inducible downstream genes of the Arabidopsis DREB1A/CBF3 transcriptional factor using two microarray systems. Plant J. 38, 982-993.
20. Shinozaki, K., Yamaguchi-Shinozaki, K. and Seki, M. (2003) Regulatory network of gene expression in the drought and cold stress responses. Curr. Opin. Plant Biol. 6, 410-417.
21. Zhu, J. K. (2002) Salt and drought stress signal transduction in plants. Annu. Rev. Plant Biol. 53, 247-273.
22. Medina, J., Bargues, M., Terol, J., Perez-Alonso, M. and Salinas, J. (1999) The Arabidopsis CBF gene family is composed of three genes encoding AP2 domain-containing proteins whose expression is regulated by low temperature but
not by abscisic acid or dehydration. *Plant. Physiol.* **119**, 463-470.

23. Nakashima, K., Shinwari, Z. K., Sakurna, Y., Seki, M., Miura, S., Shinozaki, K. and Yamaguchi-Shinozaki, K. (2000) Organization and expression of two Arabidopsis DREB2 genes encoding DRE-binding proteins involved in dehydration- and high-salinity-responsive gene expression. *Plant. Mol. Biol.* **42**, 657-665.

24. Shinwari, Z. K., Nakashima, K., Miura, S., Kasuga, M., Seki, M., Yamaguchi-Shinozaki, K. and Shinozaki, K. (1998) An Arabidopsis gene family encoding DRE/CRT binding proteins involved in low-temperature-responsive gene expression. *Biochem. Biophys. Res. Commun.* **250**, 161-170.

25. Chen, M., Wang, Q. Y., Cheng, X. G., Xu, Z. S., Li, L. C., Ye, X. G., Xia, L. Q. and Ma, Y. Z. (2007) GmDREB2, a soybean DRE-binding transcription factor, conferred drought and high-salt tolerance in transgenic plants. *Biochem. Biophys. Res. Commun.* **353**, 299-305.

26. Chan, S. W., Henderson, I. R. and Jacobsen, S. E. (2005) Gardening the genome: DNA methylation in Arabidopsis thaliana. *Nat. Rev. Genet.* **6**, 351-360.

27. Bender, J. and Fink, G. R. (1995) Epigenetic control of an endogenous gene family is revealed by a novel blue fluorescent mutant of Arabidopsis. *Cell* **83**, 725-734.

28. 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.

29. Peng, R. H., Huang, X. M., Li, X., Sun, A. J., Yao, Q. H. and Peng, Y. L. (2001) Construction of a plant binary expression vector containing intro-canaraiacin gene and transformation in nicotiana tabacam. *Acta. Phytophysiologica. Sinica.* **27**, 55-60.

30. Matsuura, I., Ugaki, M., Hirochika, H., Ohshima, M., Murakami, T., Gotoh, Y., Katayose, Y., Nakamura, S., Honkura, R., Nishimiya, S., Ueno, K., Mochizuki, A., Tanimoto, H., Tsugawa, H., Otuk, Y. and Ohashi, Y. (1996) Efficient promoter cassettes for enhanced expression of foreign genes in dicotyledonedous and monocotyledonedous plants. *Plant. Cell. Physiol.* **37**, 49-59.

31. Gallie, D. R., Sleet, D. E., Watts, J., Turner, P. C. and Wilson, T. M. A. (1987) A comparison of eukaryotic viral 50-leader sequences as enhancers of mRNA expression in vivo. *Nucl. Acids. Res.* **15**, 8693-8711.

32. Xiong, L., Lee, B., Ishitani, M., Lee, H., Zhang, C. and Zhu, J. K. (2001) FIERY1 encoding an inositol polyphosphate 1-phosphatase is a negative regulator of abscisic acid and stress signaling in Arabidopsis. *Genes Dev.* **15**, 1971-1984.

33. 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.

34. Bnder, J. and Fink, G. R. (1995) Epigenetic control of an endogenous gene family is revealed by a novel blue fluorescent mutant of Arabidopsis. *Cell* **83**, 725-734.

35. Gietz, R. D. and Woods, R. A. (2006) Yeast transformation by the LiAc/SS Carrier DNA/PEG method. *Methods Mol. Biol.* **313**, 107-120.