DOI: http://dx.doi.org/10.22092/cbj.2013.100446

Expression analysis of cold-induced transcription factor genes in rice (*Oryza sativa* L.)

Kh. Edrisi-Maryan[a*], H. Samizadeh-Lahiji[b], M. M. Sohani[c], and H. Hassani[d]

[a] Graduate School of Biotechnology, Faculty of Agriculture, University of Guilan, Rasht, Iran.
[b, c, d] Faculty of Agriculture, University of Guilan, Rasht, Iran.
[*] Corresponding author's E-mail address: Kh.edrisi@yahoo.com

Received: August 2012
Accepted: December 2102

ABSTRACT

Edrisi-Maryan, Kh., Samizadeh-Lahiji, H., Sohani, M. M., and Hassani, H. 2013. Expression analysis of cold-induced transcription factor genes in rice (*Oryza sativa* L.). **Crop Breeding Journal 3(1): 19-24.**

Rice plants are injured at the seedling stage in early spring in northern Iran. In order to study rice response to cold stress, the expression of *OsDREB1A*, *OsAP37*, *OsMYB3R-2*, and *OsNAC6* genes encoding transcription factors was observed in 14-day-old seedlings of cold-tolerant genotype PR and cold-sensitive cv. Hashemi during cold stress (5°C for 24 h) using the differential display method and three replications for each genotype. PCR products were quantified using Total Lab (ver. 1.10) software and significant differences in gene expression were found using Wilcoxon Test of SPSS (ver. 18) software. Transcription factors play an important regulatory role in the response to abiotic stress and interact with *cis*-elements in the promoter regions of several stress related genes. Different genes displayed different expression patterns in tolerant and susceptible plants during cold stress. While the expression level of *OsNAC6* gene increased in both genotypes after cold stress, the expression level of *OsDREB1A*, *OsAP37*, and *OsMYB3R-2* genes upregulated significantly in PR and decreased in the Hashemi genotype. Results suggest that the expression of genes encoding transcription factors changes in response to cold stress and so could be an informative resource in breeding and biotechnology projects aimed at developing cold tolerance in rice.

Keywords: cold stress, differential display, gene expression, *Oryza sativa*, transcription factor

INTRODUCTION

Rice seedlings are particularly sensitive to chilling in early spring, which causes slow development, yellowing, withering, reduced tillering, and stunted growth (Andaya and Mackill, 2003). Plant response to cold stress is accompanied by morphological, biochemical, and molecular changes (Wang *et al*., 2003). Abiotic stress-related genes can be classified into three groups: genes encoding for signal transduction components; functional proteins; and transcription factors (Gao *et al*., 2008).

The expression of stress-related genes is largely regulated by specific transcription factors (Oh *et al*., 2009). The promoters of stress responsive genes typically have *cis*-regulatory elements such as DRE/CRT, ABRE, and MYCRS/MYBRS and are regulated by various upstream transcriptional factors (Dai *et al*., 2007). Transcription factors such as *OsDREB1A*, *OsAP37*, *OsMYB3R-2*, and *OsNAC6* are proteins that act together with other transcriptional regulators, including chromatin remodeling/modifying proteins, to employ or obstruct RNA polymerases to the DNA template (Udvardi *et al*., 2007). Transcription factors thus upregulate the expression of many downstream genes resulting in abiotic stress tolerance (Agarwal and Jha, 2010).

The dehydration responsive element binding proteins (DREBs) are important AP2/ERF plant transcription factors that induce a set of abiotic stress-related genes through ABA-independent pathways, thus imparting stress tolerance to plants. DREB1 and DREB2 are two main subgroups of the DREB subfamily, that are involved in two different signal transduction pathways under cold and dehydration, respectively (Yamaguchi-Shinozaki and Shinozaki, 1994). Aspartic Protease (AP2) factors appear to be widespread in the genomes of plants such as rice and Arabidopsis (Nakano *et al*., 2006). Members of the AP2 family have been implicated in diverse functions in cellular processes such as flower development, spikelet meristem determinacy, plant growth, and stress tolerance (Gutterson and Reuber, 2004).

Another transcription factor family, NAC, is reported to participate in abiotic and biotic responses. The NAC family of plant-specific transcription factors is one of the largest in the plant genome, with 106 and 149 members in Arabidopsis

and rice, respectively (Xiong *et al*., 2005). The MYB family of proteins is universally found in both plants and animals; these transcription factors play important roles in many physiological processes under normal or unfavorable growth conditions. The R1R2R3-type MYB protein is induced by cold, drought, and salt stress in rice (Yanhui *et al*., 2006).

Identification of novel genes and screening for differentially expressed genes in response to stress is a straight forward approach for studying the molecular basis of a biological system (Lievense *et al*., 2001). Subtractive hybridization, microarray, and differential display are different methods for detecting, isolating, and studying gene expression in response to abiotic stresses (Kim *et al*., 2007). A method used to isolate genes induced by special stresses is a PCR-based differential display method that is based on the detection of differentially expressed cDNA from two or more samples that are separated in adjacent lanes of sequencing gels (Liang and Pardee, 1992).

Drought stress responsive genes (Tyagi and Chavdra, 2006) and cold related genes (Kim *et al*., 2007) in rice have been identified using the RT-PCR method. Here, we studied the response at the molecular level of 14-day-old seedlings of two rice genotypes, PR (cold tolerant) and Hashemi (cold sensitive) to 24 h of 5°C cold stress and compared the expression changes of four genes encoding transcription factors (*OsDREB1A*, *OsAP37*, *OsMYB3R-2*, and *OsNAC6*) using the differential display method.

MATERIALS AND METHODS

Plant materials

This study was performed at the University of Guilan's biotechnology laboratory. Two rice genotypes were obtained from the Rice Research Institute (http://berenj.areo.ir) in Rasht, Iran. Cultivar Hashemi (HA) and genotype PR27137-CR153 (PR) are cold-sensitive and cold-tolerant, respectively (Alah gholipour *et al*., 2010). Seeds were surface sterilized with ethanol (75% v/v) for 5 min and with diluted commercial bleach (1:3 v/v) NaOCl for 10 min, and then rinsed three times with sterile distilled water. Fourteen-day-old seedlings were exposed to 5°C cold for 24 h (Liu *et al*., 2007). Seedlings were harvested, frozen in liquid nitrogen, and stored at -70 for further analysis.

RNA extraction

Total RNA was isolated from the cold-treated and control plants using RNX-Plus (CinnaGen Co., Iran) according to the manufacturer's instructions. The quality of RNA was analyzed on 1% agarose gel (Fig. 1).

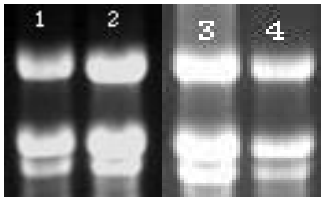

Fig. 1. Total RNA extracted from PR and Hashemi genotypes in 1) PR in 25°C, 2) HA in 25°C, 3) PR in 5°C, and 4) HA in 5°C.

RT-PCR

First-strand cDNA was synthesized using 50 ng RNA, Oligo-dt primer, and M-MLV reverse transcriptase (Fermentase). PCR reaction was set up in a 20-µl volume of 2-µl cDNA, 0.2 µM of gene-specific primers, 10 mM of dNTP and one unit of Taq DNA polymerase. The reaction included an initial 5-min denaturation at 94°C, followed by 40 cycles of (94°C, 40 s; Tm of each primer, 40 s; 72°C, 2 min), and a final 5-min extension at 72°C. PCR products were then separated on a 1/5% agarose gel. The *OsGAPDH* (*Oryza sativa* glyceraldehyde-3-phosphate dehydrogenase) gene was used as the positive control (Jain *et al*., 2006) to quantify the initial amount of RNA (Fig. 2). Four pairs of gene-specific primers were used in this study (Table 1). PCR bands were quantified using Total Lab software ver. 1.10 (Chivers *et al*., 2006). Significant differences in gene expression were determined using Wilcoxon test of SPSS (ver. 18) software.

Fig. 2. The expression of *OsGAPDH* gene as a positive control: 1) PR in 25°C, 2) HA in 25°C, 3) PR in 5°C, and 4) HA in 5°C.

RESULTS AND DISCUSSION

The expression of four cold-induced genes encoding transcription factors, namely, *OsDREB1A*, *OsNAC6*, *OsAP37*, and *OsMYB3R-2* were screened in this study using the RT-PCR method; results displayed different expression patterns in PR and HA rice genotypes under cold stress.

DREB: Dehydration responsive element binding; *AP*: Aspartic proteases; *MYB3R-2*: R1R2R3 MYB gene; *NAC* gene: NAM, ATAF1, -2, and CUC2 gene.

OsDREB1A gene

OsDREB1A expression was induced by cold treatment in the PR genotype but suppressed significantly in the HA cultivar (Fig. 3, Table 2). These expression patterns in two genotypes indicates that timing is an important factor in stress-related gene response in cold-tolerant genotypes

Table 1. Name and accession number of genes, specific primer sequences and Tm (melting temperature), and references for each primer used in this study.

Gene name/ accession no.	Primer sequence	Temp.	Reference
OsDREB1A	5'-GCT CCG ATT ACG AGT CTT C-3'	60˚C	Dubouzet *et al*., 2003
(Os09g35030)	5'-TTC TCC GAC GAA CTC CTC-3'		
OsAP37	5'-ATG GCG CCC AGA GCA GCT AC-3'	66˚C	Oh *et al*., 2009
(AK061380)	5'-CTA GTT CTC TAC CGG CGG CG-3'		
OsNAC6	5'-CAT GGC CGG TGA ACT TTG AC-3'	65˚C	Liu *et al*., 2007
(AV028185)	5'-CTC GTC GTC GTT CAG TCC AG-3'		
OsMYB3R-2	5'-CAT GGC CGG TGA ACT TTG AC-3'	64˚C	Dai *et al*., 2007
	5'-CTC GTC GTC GTT CAG TCC AG-3'		
OsGAPDH	5'-AAG CCA GCA TCC TAT GAT CAG ATT-3'	63˚C	Jain *et al*., 2006
(AK064960)	5'-CGT AAC CCA GAA TAC CCT TGA GTT T-3'		

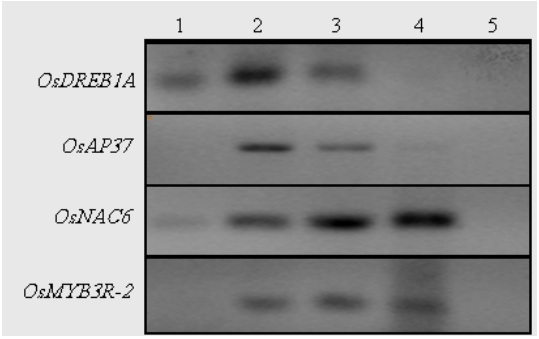

Fig. 3. PCR product of *OsDREB1*, *OsAP37, OsNAC6*, *OsMYB2-3R* genes: 1) PR in 25˚C, 2) PR in 5˚C cold stress, 3) Hashemi in 25˚C, 4) Hashemi in 5˚C, 5) negative control.

Table 2. Quantification results of gene expression using Total Lab sotfware.

Gene name	bp	PR in 25˚C (ng)	PR in 5˚C (ng)	HA in 25˚C (ng)	HA in 5˚C (ng)
OsDREB1A	198	35.295 ± 0.4	82.217±1.5	38.948 ± 0.5	0.0
OsAP37	700	0	88.302 ± 5.4	69.966 ± 1.4	13.27 ± 0.6
OsNAC6	162	129.037± 0.5	239.964 ±1.2	274.155 ±0.9	285.067± 0.3
OsMYB3R-2	162	0	22.168 ± 0.7	30.878 ± 0.5	17.612 ± 0.4

Note: The average value of the gene expression measured in three replicates is given in the table. Expression levels of genes that are undetectable by the software are equal to zero.

(Kiedrowski *et al.*, 1992) such as PR, compared with cold-sensitive ones. The *OsDREB1A* gene encoding one of the important transcription factors causes the expression of cold responsive genes by identifying C-repeat regions in the promoter of these genes. DRE contains the TACCGACAT core sequence shown to be linked to dehydration, low temperature, and high salt responses, as well as abscisic acid treatment (Yamaguchi-Shinozaki and Shinozaki, 1994). CBF/DREB1 transcription factors have also been identified in wheat, rye, tomato, and rice, and all of them showed a rapid response to cold stress (Dubouzet *et al*., 2003). The overexpression of *OsDREB1A* in rice induces the accumulation of soluble sugars, including raffinose, sucrose, glucose, and fructase, which may act as osmoprotectants (Ito and Kurata, 2006). *AtDREB1* in Arabidopsis was induced within 10 min at 4˚C (Liu *et al*., 1998). *OsDREB1A* and *OsDREB1B* were induced early (within 40 min) after cold exposure, but not on ABA treatment (Dubouzet *et al*., 2003). Transgenic Arabidopsis and rice plants overexpressing *OsDREB1A* also displayed tolerance to low temperatures, high salinity, and drought (Dubouzet *et al*., 2003; Ito *et al*., 2006).

***OsAP37* gene**

The expression of *OsAP37* gene was up-regulated in PR but significantly suppressed in HA after cold stress (Fig. 3, Table 2). AP2 factors are widespread in plants, with the genomes of rice and Arabidopsis predicted to contain 139 and 12 AP2 genes, respectively (Nakano *et al*., 2006). The AP2 family has been found to be involved in diverse functions such as plant growth and stress tolerance (Gutterson and Reuber, 2004). Based on these diverse functions, the involvement of the AP2 family in stress response has been relatively well characterized. Oh *et al*. (2009) showed that *AP37* gene encoding a transcription factor caused increased tolerance to low temperatures, drought, and salinity during the vegetative phase. The expression of *OsAP37* as a transcription factor may prevent cold injury indirectly through induction and expression of stress responsive genes, which often

encode osmoprotectants.

***OsNAC6* gene**

Upregulation of *OsNAC6* was a result of cold treatment in the PR genotype, but expression was not significantly enhanced in the HA cultivar under the same treatment (Fig. 3, Table 2). NAC family transcription factors are plant-specific and contain a highly conserved N-terminal DNA-binding domain and a diversified C-terminal domain (Hu *et al*., 2008); they are reported to participate in plant response to abiotic and biotic stress. One hundred and forty NAC or NAC-like genes were identified in rice and divided into five subgroups based on phylogenetic analysis (Fang *et al*., 2008). All of the known stress-related NAC genes were grouped into family III, including two well-characterized stress-responsive NAC genes, *SNAC1*, and *OsNAC6/ SNAC2*. Unlike other transcription factors, NAC expression pathway is ABA-dependant (Zheng *et al*., 2009). Some members of the NAC family have been shown to be involved in plant stress responses (Lu *et al*., 2007). Using Northern blot analysis, Xiong *et al*. (2002) found that *OsNAC6* is strongly induced by cold. Ohnishi *et al*. (2005) showed that *OsNAC6* is induced by salt, cold, drought, and abscisic acid in rice. Hence NAC transcription factors play a crucial role in physiological adaptation that allows successful plant propagation under abiotic stress conditions.

***OsMYB3R-2* gene**

The expression of *OsMYB3R-2* was up-regulated in the PR genotype after cold treatment, but was not significantly enhanced in the HA cultivar. The suppression of *OsMYB3R-2* gene in the HA cultivar may be an indication of weak signal perception or a signal transduction pathway that may cause HA's sensitivity to cold-stress (Fig. 3, Table 2). The MYB transcription factors participate in the ABA-dependent pathway. A MYB domain is usually composed of one to three imperfect repeats, each with about 52 amino acid residues that adopt a helix-turn-helix conformation intercalating in the major groove of the DNA (Yanhui *et al*., 2006). Plant MYB proteins are categorized into three major groups: (1) R2R3-MYB with two adjacent repeats; (2) R1R2R3-MYB with three adjacent repeats; and (3) MYB-related proteins, usually containing a single MYB repeat (Stracke *et al*., 2001). The R1R2R3-type MYB protein is located in the nucleus in rice and is induced by cold, drought, and salt stress. The enhanced stress tolerance of *35s;OsMYB3R-2* Arabidopsis plants reveals that *OsMYB3R-2* could mediate signal transduction, regulating some stress-responsive genes involved in CBF-dependent or CBF-independent pathways (Dai *et al*., 2007). *OsMYB3R-2* transgenic plants showed enhanced tolerance to freezing, drought, and salt stress and decreased sensitivity to ABA (Dai *et al*., 2007). Ma *et al*. (2009) showed that *OsMYB3R-2* functions in both stress and developmental processes in rice. Transgenic plants overexpressing *OsMYB3R-2* exhibited enhanced cold tolerance. Cold treatment greatly induced the expression of *OsMYB3R-2*, which encodes an active transcription factor.

CONCLUSIONS

The differential expression of various stress-induced genes was studied in one cold-tolerant and one cold-sensitive genotype. This investigation showed that cold induced genes encoding transcription factors expressed in higher levels and that genes encoding transcription factors may confer stress tolerance in tolerant genotypes through the activation of genes whose products are involved either in the regulation of gene expression for cold adaptation or for protection of cells from chilling injury. Our observations suggest that the differences between the studied genotypes regarding cold tolerance may be due to the timing of expression. Kiedrowski (1992) suggested the hypothesis of quantitative differences in the resistant and susceptible responses of genes to abiotic stress. Thus quantitative differences in the expression of genes involved in tolerance induction during abiotic stress could also be the result of the timing and intensity of transcriptional activation of involved genes rather than their presence/absence or other mechanisms. Thus it is important to enhance the genetic control capability of one or several key regulatory factor(s) to make the plant achieve multiple stress resistance (Qiang *et al*., 2000).

This investigation showed that cold induced genes encoding transcription factors, namely *OsDREB1A*, *OsAp37*, *OsNAC6*, and *OsMYB3R-2* that regulate various stress responsive gene expressions, showed higher levels of expression in tolerant genotype. Functional analysis of these transcription factors should thus provide more information on the intricate regulatory networks involved in abiotic stress responses, and the cross-talk between different signaling pathways during stress adaptation.

Furthermore, considering transcription factors as candidate genes in breeding and other crop improvement programs will give us a clear understanding of abiotic stress related signal transduction events and will eventually lead us to develop crop varieties with superior stress tolerance through genetic manipulation (Lata *et al*., 2011). The results of this study suggest that the expression

of genes encoding transcription factors changes in response to cold stress and so could be an informative resource in breeding and biotechnology projects aimed at developing cold tolerance in rice.

References

Agarwal, P. K., and B. Jha. 2010. Transcription factors in plants and ABA dependent and independent abiotic stress signaling. Biol. Plantarum 54: 201-212.

Alah gholipour, M., S. Moradi, A. Nahvi, and N. Niyazi. 2010. Study and selection in segregating generation populations in order to identify cold-resistant rice lines. Final Report of a Research Project. Iran Rice Research Institute. (In Persian.)

Andaya, V.C., and D. J. Mackill. 2003. Mapping of QTLs associated with cold tolerance during the vegetative stage in rice. J Exp Bot. 54: 2579-2585.

Chivers, J. E., W. Gong, E. King, J. Seybold, J. Mak, L. Donnelly, N. Holden, and R. Newton. 2006. Analysis of the dissociated steroid RU24858 does not exclude a role for inducible genes in the anti-inflammatory actions of glucocorticoids. Mol. Pharmacol. 70: 2084-2095.

Dai, X., Y. Xu, Q. Ma, W. Xu, T. Wang, Y. Xue, and K. Chong. 2007. Over expression of a R1R2R3 MYB gene, *OsMYB3R-2*, increased tolerance to freezing, drought, and salt stress in transgenic Arabidopsis. Plant Physiol. 143: 1-13.

Dubouzet, J. G., Y. Sakuma, Y. Ito, M. Kasuga, E. G. Dubouzet, S. Miura, M. Seki, K. Shinozaki, and K. Yamaguchi-Shinozaki. 2003. *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activation in drought, high-salt and cold-responsive gene expression. Plant J. 33: 751-763.

Fang, Y., J. You, K. Xie, W. Xie, and L. Xiong. 2008. Systematic sequence analysis and identification of tissue-specific or stress-responsive genes of NAC transcription factor family in rice. Mol. Genet. Genomics 280: 547-563.

Gao, J. P., D. Y. Chao, and H. X. Lin. 2008. Toward understanding molecular mechanisms of abiotic stress responses in rice. Rice 1: 36-51.

Gutterson, N., and T. L. Reuber. 2004. Regulation of disease resistance pathways by AP2/ERF transcription factors. Curr. Opin. Plant Biol. 7: 465-471.

Hu, H., J. You, Y. Fang, X. Zhu, Z. Qi, and L. Xiong. 2008. Characterization of transcription factor gene SNAC2 conferring cold and salt tolerance in rice. Plant Mol. Biol. 67: 169-181.

Ito, Y., and N. Kurata. 2006. Identification and characterization of cytokine in signalling gene families in rice. Genet. 382: 57-65.

Ito, Y., K. Katsura, K. Maruyama, T. Taji, M. Kobayashi, K. Shinozaki, and K. Yamaguchi-Shinozaki. 2006. Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. Plant Cell Physiol. 47: 41-153.

Jain, M., A. Nijhawan, K. Tyagi, and J. P. Khurana. 2006. Validation of housekeeping genes as internal control for studying gene expression in rice by quantitative real-time PCR. Bioch. Bioph. Res. Commun. 345: 646-651.

Kiedrowski, S., P. Kawalleck, K. Hahlbrock, I. E. Somssich, and J. L. Dangle. 1992. Rapid activation of a novel plant defense gene is strictly dependent on the Arabidopsis RPM1 disease resistance locus. EMBO J. 11: 4677-4684.

Kim, S. H., J. Y. Kim, S. J. Kim, K. S. An, G. An, and S. R. Kim. 2007. Isolation of cold stress-responsive genes in the reproductive organs, and characterization of the *OsLti6b* gene from rice (*Oryza sativa* L.). Plant Cell Rep. 26: 1097-1110.

Lata, C., A. Yadav, and M. Prasad. 2011. Role of plant transcription factors in abiotic stress tolerance. Pp. 261-296. In: A. Kumar-Shanker, and B. Venkateswarlu (eds.). Abiotic Stress Response in Plants—Physiological, Biochemical and Genetic Perspectives.

Liang, P., and A. B. Pardee. 1992. Differential display of eukaryotic messenger RNA by means of the polymerase chain reaction. Sci. 257: 967-971.

Lievense, S., S. Goormachtig, and M. Holsters. 2001. A critical evaluation of differential display as a tool to identify genes involved in legume nodulation: looking back and looking forward. Nucl. Acids Res. 29(17): 3459- 3468.

Liu, K., L. Wang, Y. Xu, N. Chen, Q. Ma, F. Li, and K. Chong. 2007. Overexpression of *OsCOIN*, a putative cold inducible zinc finger protein, increased tolerance to chilling, salt and drought, and enhanced proline level in rice. Planta. DOI 10.1007/s00425-007-0548-5.

Liu, Q., M. Kasuga, Y. Sakuma, H. Abe, S. Miura, H. Goda, Y. Shimada, S. Yoshida, K. Shinozaki, and K. Yamaguchi-Shinozaki. 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. The Plant Cell 10: 391-406.

Lu, P. L., N. Z. Chen, R. An, Z. Su, B. S. Qi, F. Ren, J. Chen, and X. C. Wang. 2007. A novel drought-inducible gene, ATAF1, encodes a NAC family protein that negatively regulates the expression of stress-responsive genes in Arabidopsis. Plant Mol. Biol. 63: 289-305.

Ma, Q., X. Dai, Y. Xu, J. Guo, Y. Liu, N. Chen, J. Xiao, D. Zhang, Z. Xu, X. Zhang, and K. Chong. 2009. Enhanced tolerance to chilling stress in OsMYB3R-2 transgenic rice is mediated by alteration in cell cycle and ectopic expression of stress genes. Plant Physiol. 150(1): 244-56.

Nakano, T., K. Suzuki, T. Fujimura, and H. Shinshi. 2006. Genome-wide analysis of the ERF gene family in Arabidopsis and rice. Plant Physiol. 140: 411-432.

Oh, S. J., Y. S. Kim, C. W. Kwon, H. K. Park, J. S. Jeong, and J. K. Kim. 2009. Overexpression of the transcription factor AP37 in rice improves grain yield under drought conditions. Plant Physio. 150: 1368-1379.

Ohnishi, T., S. Sugahara, T. Yamada, K. Kikuchi, Y.

Yoshiba, and H. Y. Hirano. 2005. OsNAC6, a member of the NAC gene family, is induced by various stresses in rice. Genes Genet. Syst. 80: 135-139.

Qiang, L., Z. Nanming, K. Yamaguchi-Shinozaki, and K. Shinozaki. 2000. Regulatory role of DREB transcription factors in plant drought, salt and cold tolerance. Chinese Sci. Bull. 45(11): 970-975.

Stracke, R., M. Werber, and B. Weisshaar. 2001. The R2R3-MYB gene family in *Arabidopsis thaliana*. Current Opinion in Plant Biol. 4: 447-456.

Tyagi, A., and A. Chavdra. 2006. Isolation of stress responsive *Psb A* gene from rice (*Oryza sativa* L.) using differential display. Indian J. Bioch. Biophy. 43: 244-246.

Udvardi, M. K., K. Kakar, M. Wandrey, O. Montanri, J. Murray, A. Andraiankaja, J. Y. Zhang, V. Benedito, J. M. I. Hofer, F. Cheng, and C. D. Town. 2007. Legume transcription factors: global regulators of plant development and response to the environment. Plant Physiol. 144: 538-549.

Wang, W., B. Vinocur, and A. Altman. 2003. Plant response to drought, salinity and extreme temperatures: towards genetic engineering for stress tolerance. Planta 218: 1-14.

Xiong, L., K. S. Schumaker, and J. K. Zhu. 2002. Cell signaling during cold, drought, and salt stress. Plant Cell 14: 165-183.

Xiong, Y., T. Liu, C. Tian, S. Sun, J. Li, and M. Chen. 2005. Transcription factors in rice: a genomewide comparative analysis between monocots and eudicots. Plant Mol. Bio and. 59: 191-203.

Yamaguchi-Shinozaki, K., and K. Shinozaki. 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.

Yanhui, C., Y. Xiaoyuan, H. Kun, L. Meihua, L. Jigang, G. Zhaofeng, L. Zhiqiang, Z. Yunfei, W. Xiaoxiao, Q. Xiaoming, S. Yunping, D. Xiaohui, L. Jingchu, D. Xing-Wang, C. Zhangliang, G. Hongya, and Q. Li-Jia. 2006. The MYB transcription factor superfamily of Arabidopsis: expression analysis and phylogenetic comparison with the rice MYB family. Plant Mol. Biol. 60: 107-124.

Zheng, X., B. Chen, G. Lu, and B. Han. 2009. Overexpression of a NAC transcription factor enhances rice drought and salt tolerance. Bioch. Biophy. Rese. Commun. 379: 985-989.