

## CBF genes expression under drought stress in wheat

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### Abstract

Plants respond to drought by induction of a series of regulatory genes that form the signaling network. Many genes respond to drought stress at the transcriptional level and the products of these genes function in the stress response and tolerance. A major transcription system that controls abscisic acid-independent gene expression in response to dehydration has been identified. The system includes the DRE/CRT *cis*-acting element and its DNA-binding protein. AP2/ERF proteins are a class of protein that is unique to plants and plays a vital role in biotic and abiotic stress response. C-repeat/dehydration-responsive element binding factors (CBF/DREBs) are a family of APETALA2 transcription factors that bind to DRE/CRT *cis*-element and regulate the expression of stress-responsive genes. cDNA-amplified fragment length polymorphism is a powerful and useful tool for analyzing the gene expression profiles of plants that are exposed to abiotic stresses. CDNA-AFLP was used for transcriptome analysis in wheat varieties Tabassi, a drought-stress tolerant genotype and Taifun, a drought-stress sensitive genotype, under drought stress and normal condition. After sequencing and BLAST searches, special TDF was expressed only in Tabassi under drought stress, show homology with CCAAT-binding transcription factor complex WHPA1 in *Triticum aestivum* and also CBF conserved domain was observed. CBF genes, also known as DREBs, and their transcriptional targets that trigger a suite of pathways that collectively protect the plant from the harmful effects of abiotic stresses and enhances resistance to drought. So, we can consider and confirm, CBF genes as a factor for tolerant and response to drought stress.

**Keywords:** CBF transcription factors, cDNA-AFLP, drought stress, wheat.

### Introduction

Common wheat (*Triticum aestivum* L.) is the major source of calories and protein for a large segment of the world population and is the most important grain crop in Iran. Wheat production in many regions of the world is below average because of adverse environmental condition (9). Abiotic stresses such as drought, high or low temperatures, salinity and others reduce wheat productivity. Drought is the main environmental constraint, which occurs in many parts of the world every year, often having devastating effects on crop productivity.

The expression of many genes is induced by drought, and their gene products function directly in stress tolerance and regulation of gene expression and signal transduction in stress responses (20). Among the regulatory proteins, transcription factors (TFs) have a central role in activating defense gene expression (2, 18). Most of the stress related TFs are grouped into several large families, such as AP2/ERF, bZIP, NAC, MYB, MYC, Cys2His2, zinc finger and WRKY (13).

One class of protein that is unique to plants and plays a vital role in biotic and abiotic stress response is the AP2/ERF proteins (1). The AP2/ERF protein coding genes constitute a large super family, which has been further divided into three groups namely the AP2, ERF, and RAV families based on their sequence similarities and numbers of AP2/ERF domains (7, 10). The DREBs (dehydration responsive element binding) also referred as CBF (C-repeat binding factor) proteins, belonging to ERF subfamily and play crucial role in plants in response to abiotic stresses and therefore received considerable attention in past decades.

The dehydration responsive element (DRE) as a *cis*-acting element was found in the promoter regions of many drought- and low-temperature-inducible genes (12). All DREB genes feature three conserved regions, an EREBP/AP2 DNA binding domain, an N-terminal nuclear localization signal, and conserved Ser/Thr rich region adjacent to the EREBP/AP2 domain. DREB TFs play key roles in plant stress signaling transduction pathway, they can specifically bind to DRE/CRT element (G/ACCGAC) and activate the expression of many stress inducible genes. Although the obtained transgenic crops, mainly wheat, by different types of gene transfer technology all exhibit drought resistance to some extent, they have many shortfalls related to agronomical performance and/or development (6, 16).

### Materials and methods

*Triticum aestivum* Tabassi, a drought-stress resistant genotype and Taifun, a drought-stress sensitive genotype were selected and used for the present study. In grain filling stage, Relative Water Content (RWC) was measured after imposing stress conditions until reaching leaf relative water content (RWC) of ~50%. Harvested leaves were immediately frozen in liquid nitrogen and stored at -80°C for RNA extraction.

cDNA-AFLP was performed as described by Bachem et al., used for transcriptome analysis under drought stress and normal condition. Total RNA from leaves was extracted from four different wheat samples (Tabassi and Taifun with or without drought-stress) using a Trisol reagent and concentration of total RNAs were measured by using spectrophotometer. First strand cDNA was synthesized using oligo d[T]<sub>18</sub> and M-MuLV reverse transcriptase starting with about 10 ug total RNA. The second strand cDNA was acquired by a reaction containing DNA polymerase I, RNaseH and DNA ligase. Double-strand cDNA was digestion with *Mse* I and *Pst* I restriction enzymes in a two-step reaction. *Mse* I and *Pst* I anchors were ligated to two ends of cDNA fragments using T4 DNA ligase. The pre-amplifications were performed with *Mse* I and *Pst* I primers with non selective nucleotides. 32 primer combinations were used for the selective amplification, with two selective bases for each primer. Polymerase chain reaction (PCR) products were separated on a 6% sequencing gel and visualized by silver staining.

Bands corresponding to differentially expressed genes were cut from the gels. The resulting sequences were compared to nucleotide and protein sequences in publicly available databases using BLAST sequence alignments (<http://www.ncbi.nlm.nih.gov/>).

### Results and Discussion

In this study, we used a cDNA-amplified fragment length polymorphism (AFLP) to analyse genes that are differentially expressed in both Tabassi and Taifun with or without drought-stress. Selective amplifications with 32 primer combinations allowed the visualization of TDFs that were specifically expressed in tabassi under drought stress.

After sequencing and BLAST searches, special TDF was expressed only in Tabassi under drought stress, show homology with CCAAT-binding transcription factor complex WHPA1 in *Triticum aestivum* and also CBF conserved domain was observed. CBF genes, also known as DREBs, and their transcriptional targets that trigger a suite of pathways that collectively protect the plant from the harmful effects of abiotic stresses and enhances resistance to drought. Majority of CBFs from different plant species are reported to be significantly up regulated in response to abiotic stresses. It is well known that expressions of the A-1 group of genes is induced by low temperature, but not by drought or high-salt stress, while A-2 group genes are regulated by salt and drought, but not by cold (3, 8). However, recent reports have shown some conflicts with respect to these trends. A number of *DREB1/CBF*s genes such as *BrCBF* from Chinese cabbage (5), *MbDREB1* from apple (19), *OsDREB1F* from rice (14), *VviDREB1* from *Vaccinium vitis-idaea* (15) have been reported which are not only responsive to cold but also to high-salt stress, drought, exogenous ABA treatment. Different expression profiles are also reported within the same CBF family. For example, in *Vitis* sp., the *CBF4* gene was mostly induced by cold while the *CBF1*, *CBF2* and *CBF3* genes showed better response to drought compared to cold (17). Also, expression of the *DREB2* genes namely *DREB2A* and *DREB2B*, are induced by dehydration and high-salinity stresses (7, 8, 11). Overexpression of *OsDREB1A* in *Arabidopsis* revealed induction of stress responsive genes and enhanced tolerance to stress (3). Similarly, transgenic rice overexpressing *Arabidopsis DREB1* improved drought and chilling tolerance (4). This studies showed that The CRT/DRE *cis*-acting elements have been identified in promoters of many stress inducible genes from various plants such as wheat and some grasses, suggesting existence of a similar regulatory system in other plants also (11). DREB/CBF proteins are therefore important TFs in plants which regulate expression of various stress-responsive genes, generally in an ABA-independent manner through binding to *DRE/CRT cis*-elements and help plants to sustain single or multiplicative effects of different abiotic stresses.

Thus, according to this TDF was specifically expressed in Tabassi (a drought-stress tolerant genotype) under drought stress, we can also consider and confirm, CBF genes as a factor for tolerant and response to drought stress.

### References

- Agarwal, P. K., Agarwal, P., Reddy, M. K. and Sopory, S. K., 2006. Role of DREB transcription factors in abiotic and biotic stress tolerance in plants. *Plant Cell Reports*, **25**: 1263-1274.
- Chen, W. and Zhu, T., 2004. Networks of transcription factors with roles in environmental stress response. *Trends Plant Science*, **9**: 591-596.
- Dubouzet, J. G., Sakuma, Y., Ito, Y., Kasuga, M., Dubouzet, E. G., Miura, S., 2003. *OsDREB* genes in rice, *Oryza sativa* L., encode transcription activators that function in drought-, highsalt- and cold-responsive gene expression. *Plant journal*, **33**: 751-763.
- Ito, Y., Katsura, K., Maruyama, K., Taji, T., Kobayashi, M., Seki, M., 2006. Functional analysis of rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant and Cell Physiology*, **47**: 141-153.
- Jiang, F., Wang, F., Wu, Z., Li, Y., Shi, G., Hu, J. and Hou, X., 2011. Components of the *Arabidopsis* CBF cold-response pathway are conserved in non-heading Chinese cabbage. *Plant Molecular Biology Reporter*, **29**: 525-532.
- Kern, M. J., 2002. Food, feed, fiber, fuel and industrial products of the future: challenges and opportunities. Understanding the strategic potential of plant genetic engineering. *Agriculture and Crop Science*, **188**: 291-302.
- Lata, C. and Prasad, M., 2011. Role of DREBs in regulation of abiotic stress responses in plants. *Journal of Experimental Botany*, **62**: 4731-4748.
- Liu, Q., Kasuga, M., Sakuma, Y., Abe, H., Miura, S., Goda, H., 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**: 391-406.
- Maghraby, M. A., Moussa, M. E., Hana, N. S., and H. A. Agrama, 2005. Combining ability under drought stress relative to SSR diversity in common wheat. *Euphytica*, **141**: 301-308
- Nakano, T., Suzuki, K., Fujimura, T. and Shinshi, H., 2006. Genome wide analysis of the ERF gene family in *Arabidopsis* and rice. *Plant Physiology*, **140**: 411-432.
- Nakashima, K. and Yamaguchi-Shinozaki, K. 2010. Promoters and transcription factors in abiotic-stress responsive gene expression. *Physiological, Molecular and Genomic Foundation*, Springer, The Netherlands, pp: 199-216.
- Shinozaki, K. and K. Yamaguchi-Shinozaki, 2007. Gene networks involved in drought stress response and tolerance. *Journal of Experimental Botany*, **58**: 221-227.
- Umezawa, T., Fujita, M., Fujita, Y., Yamaguchi-Shinozaki, K. and Shinozaki, K., 2006. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Current Opinion in Biotechnology*, **17**: 113-122.
- Wang, Q., Guan, Y., Wu, Y., Chen, H., Chen, F. and Chu, C., 2008. Overexpression of a rice *OsDREB1F* gene increases salt, drought, and low temperature tolerance in both *Arabidopsis* and rice. *Plant Molecular Biology*, **67**: 589-602.
- Wang, Q. J., Xu, K. Y., Tong, Z. G., Wang, S. H., Gao, Z. H., Zhang, J. Y., 2010. Characterization of a new dehydration responsive element binding factor in central arctic cowberry. *plant cell tissue and organ culture*, **101**: 211-219.
- Wang, W. X., Vinocur, B. and A. Altman, 2003. Plant Responses to Drought, Salinity and Extreme Temperature: Towards Genetic Engineering for Stress Tolerance. *Planta*, **218**: 1-14.
- Xiao, H., Tattersall, E. A. R., Siddiqua, M. K., Cramer, G. and Nassuth, A., 2008. *CBF4* is a unique member of the CBF transcription factor family of *Vitis vinifera* and *Vitis riparia*. *Plant Cell Environment*, **31**: 1-10.
- Xu, Z. S., Chen, M., Li, L. C. and Ma, Y. Z., 2008. Functions of the ERF transcription factor family in plants. *Botany*, **865**: 969-977.
- Yang, W., Liu, X. D., Chi, X. J., Wu, C. A., Li, Y. Z., Song, L. L., 2011. Dwarf apple *MbDREB1* enhances plant tolerance to low temperature, drought, and salt stress via both ABA-dependent and ABA-independent pathways. *Planta*, **233**: 219-229.
- Zheng, J., Fu, J., Gou, M., Huai, J., Liu, Y., Jian, M., Huang, Q., Guo, X., Dong, Z., Wang, H. and G. Wang, 2010. Genome-wide transcriptome analysis of two maize inbred lines under drought stress. *Plant Molecular biology*, **72**: 407-423.