



Isolation and characterization of Soybean DREB 3 transcriptional activator

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ABSTRACT

Cotton (*Gossypium hirsutum*) is an important cash crop and its productivity is significantly hampered by Abiotic stresses, such as drought and high salinity. High salt imposes negative impacts on growth, agronomy traits, seed quality and quantity and thus reduces the yield of cotton. To cope with salt stress, cotton plant has to develop several tolerance mechanisms, including: (i) maintenance of ion homeostasis; (ii) adjustment in response to osmotic stress; (iii) restoration of osmotic balance; and (iv) other metabolic and structural adaptations. The regulatory network for abiotic stress responses in higher plants has been studied extensively in model plants such as *Arabidopsis thaliana*. A novel soybean DREB (dehydration-responsive element-binding protein) functions as an important transcriptional activator and may be useful in improving plant tolerance to abiotic stresses in plants. Based on its similarity with AP2 domains, DREB3 was classified into A-5 subgroup in DREB subfamily in AP2/EREBP family. A DREB orthologue, DREB3, a 528 bp fragment of DREB3 containing the DNA-binding domain was amplified using the primer pair 5# -CCCTCTAGAGAATTTCATGGCGAAACCCAGCAGC-3# (forward) and 5#-CCCCTCGAGCGGCATTTCCGGCACATA-3# (reverse). The amplified product was electrophoresed using 1.5% agarose gel and the fragment was eluted. The eluted fragment was quantified using nanodrop and then subjected to poly -AAA -tailing and cloned into pGEM-T easy vector kit. The soybean DREB 3 gene functions as an important transcriptional activator and may be useful in improving of plant tolerance to abiotic stress in cotton plants.

1.INTRODUCTION

Abiotic stresses, such as drought and high salinity, can limit the geographical distribution of plants and limit the growth and yield of economically important species. Substantial efforts have been devoted to determine the nature of the injury caused by these stresses and the plant-protection mechanisms involved in tolerance responses.

Drought poses a serious threat to the sustainability of cotton yields in rainfed agriculture and it is the most important limiting factor for crop production and it is becoming an increasingly severe problem in many regions of the world. In addition to the complexity of drought itself plant responses to drought are complex and different mechanisms are adopted by plants when they encounter drought. These mechanisms can be (i) drought escape by rapid development which allows plants to finish their cycle before severe water stress, (ii) drought avoidance by, for instance, increasing water uptake and reducing transpiration rate by the reduction of stomatal conductance and leaf area, (iii) drought tolerance by maintaining tissue turgor during water stress via osmotic adjustment which allows plants to maintain growth under water

stress, and (iv) resisting severe stress through survival mechanisms. However, this last mechanism is typically not relevant to agriculture. The maintenance of high plant water status and plant functions at low plant water potential, and the recovery of plant function after water stress are the major physiological processes that contribute to the maintenance of high yield under cyclic drought periods (Blum, 1996).

Some genes are up-regulated and others are down-regulated in plants under drought stress. This regulation occurs at distinct levels, from the moment of stress detection to the production of biologically active proteins. An important step forward was the identification of a *cis*-acting dehydration responsive element (DRE), the function of which is important for the expression of genes responding to dehydration in *Arabidopsis thaliana*. This element has a C-repeat sequence of 5bp (CCGAC) that is present in one or multiple copies in the promoter region of many plant genes related to dehydration response. The transcription factor DREB1 (dehydration-responsive element-binding protein) specifically interacts with the element DRE and induces the expression of genes involved in the stress response of *A. thaliana*. Twelve stress-induced genes were identified as DREB1 target genes by Seki *et al.*, (2001), and Fowler and Tomashow (2002) described 41 genes as DREB target genes.

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The DREB transcription factor has an important role in regulating abiotic stress-related genes. Therefore, it is possible to improve the abiotic stress tolerance of various crop plants by manipulating the expression of DREBs. The function of some of these gene products seems to be related to structure and basic cell function maintenance during water deficit, low temperatures and high salinity (Kasuga *et al.*, 2004) research using model plants, such as *A. thaliana* and tobacco (*Nicotiana tabacum*), can help to identify genes with key functions in defense mechanisms in other plant species.

Techniques such as real-time quantitative polymerase chain reaction (RT-qPCR), allow precise quantification of the mRNA levels of genes of interest when their expressions are compared under various conditions or treatments (Volkov *et al.*, 2003). For coping up with Abiotic stresses like drought, salt, and extreme temperature disturb plant growth and productivity plants respond to these stresses by altering molecular, cellular, and physiological responses. Various functional and regulatory proteins play a pivotal role in controlling abiotic stress tolerance. Investigations on physiological, biochemical, and molecular aspects of plant stress tolerance have unravelled aspects of the intrinsic mechanisms developed during evolution to mitigate against stresses (Vij and Tyagi, 2007) Among these genes, many are transcription factors involved in responses to drought, high salt, and cold stresses, and regulate the expression of downstream target genes through specific binding to cis acting elements in the promoters of down-regulated genes (Bartels and Sunkar, 2005; Vinocur and Altman, 2005). These genes were classified into several large families, such as AP2/EREBP, bZIP, NAC, MYB, MYC, Cys2His2 zinc-finger, and WRKY (Umezawa *et al.*, 2006). In Arabidopsis, 145 AP2/EREBP transcription factors were classified into five subfamilies, including DREB (dehydration-responsive element-binding protein). Genes belonging to the DREB subfamily were thought to be important switches to regulate expression of many stress-inducible genes. The DREB subfamily was further divided into 6 subgroups (A-1 to A-6). Subgroups A-1 and A-2, harbouring the DREB1-type and DREB2-type genes, respectively, are the largest ones that are involved in two ABA-independent pathways [Liu *et al.*, 1998].

DREB3/CBF (C-repeat binding factor) like genes, belonging to the A-5 subgroup, are induced by high temperature and activate the expression of many drought stress-responsive genes, also the DREB3 gene is involved in an ABA-independent cold stress-responsive signal pathway. Furthermore, analysis of the GmDREB3 promoter elucidated its cold-induced modulation. Over expression of DREB3 enhanced tolerance to cold, drought, and high salt stresses in transgenic Arabidopsis. Physiological analyses indicated that the fresh weight and over expression of DREB3 transgenic Arabidopsis under cold stress were higher than those of wild-type controls. DREB3 transgenic tobacco accumulated higher levels of free proline under drought stress and retained higher leaf chlorophyll levels under high salt stress than wild-type tobacco. The consistent trend was that the transgenic tobacco plants accumulated higher levels of free proline

than the wild type under drought stress, and that plants transferred with DREB3 are likely to be more tolerant to drought and high salt stresses homology comparisons of DREB genes in the A-5 subgroup showed that GmDREB3 shared high similarity in the AP2 domain and low similarity in the full-length sequence with six other members, which might be due to the different rates of evolution within the AP2 conserved domain and other parts of the DREB proteins.

The AP2/ERF family TFs, which are found mainly in plants, contain the AP2 DNA-binding domain and act as nodes of a regulatory network in a plant's response to cold and other sources of stress. The AP2/ERF family TFs have been shown to have important roles in transcriptional regulation for increasing tolerance to drought, salt, low temperature and diseases in plant (Ito *et al.*, 2006; Qin *et al.*, 2007; Chen *et al.*, 2008 & 2009; Li *et al.*, 2009; Navarro *et al.*, 2009). There are 147, 167, 132 and 200 AP2/ERF family TFs that have been identified in thale cress (*Arabidopsis thaliana*), rice (*Oryza sativa*), grapevine (*Vitis vinifera*) and poplar (*Populus trichocarpa*) genomes, respectively. There are also 148 AP2/ERF family TFs that have been identified in soybean (*Glycine max*) by expressed sequence tags (ESTs) analysis. Sakuma divided the AP2/ERF family TFs of Arabidopsis into five subfamilies: (1) APETALA2 (AP2), (2) dehydration responsive element-binding (DREB), (3) related to ABI3/VP (RAV), (4) ethylene-responsive element-binding factor (ERF) and (5) other proteins. The DREB and ERF subfamilies were each divided into six subgroups, designated A1–A6 and B1–B6, respectively.

The cis-acting elements and transcription factors that function in regulation of stress-related gene expression have been analyzed to elucidate the molecular mechanisms of environmental stresses. AP2/EREBP transcription factors are crucial in regulating plant responses to different stresses and in imparting stress endurance to plants (Xu *et al.* 2008). The AP2/ERF family of transcription regulators is characterized by the presence of the AP2 DNA-binding domain. Sakuma characterized the large AP2/ERF gene family in Arabidopsis thaliana on the basis of number of repetitions and sequence of the AP2 domain. They divided the 144 members of this gene family into five subfamilies: DREB, ERF, AP2, RAV, and others. The DREB and ERF subfamilies (120 proteins) have a single AP2 domain and a conserved WLW motif. The DREB protein from different plant species shows differential binding to ACCGAC and GCCGAC cis-elements of the Arabidopsis rd29A promoter. AtDREB2A and OsDREB2A proteins showed similar binding to both the DREs, whereas, PgDREB2A protein showed preferential binding to the ACCGAC element as compared to GCCGAC .The DREs function as cis-acting elements, involved in the induction of rd29A expression by low-temperature or high-salt stress. DRE-related motifs have been reported in the promoter regions of cold- and drought-inducible genes such as kin1, cor 6.6 and rd17 (Iwasaki *et al.* 1997; Wang *et al.* 1995). A similar motif (CRT; TGGCCGAC) was also reported in the promoter region of cold inducible cor15a gene Plants-protect themselves by different mechanisms involved in tolerance

responses some genes are up-regulated and others are down-regulated in plants under drought stress. This regulation occurs at distinct levels, from the moment of stress detection to the production of biologically active proteins.

MATERIALS AND METHODS

Plant material

Soybean plant leaves were used for isolation of DREB3 gene. For this seeds were grown in soil at 25° C and 70% humidity under 14 h light and 10 h darkness. The seeds were surface sterilized and planted on MS medium. After emergence, seedlings of Soybean plants were transferred to pots.

RESULTS & DISCUSSION

Isolation and characterization of DREB3 genes from soybean

The genes encoding the DRE-binding protein from soybean, leaf and seed samples were used for isolation of DNA by Phenol-Chloroform method and CTAB method with liquid Nitrogen. The Genomic DNA was quantified by Nanodrop, and then amplified at 55 °C. The 528 bp fragment of GmDREB3 containing the DNA-binding domain was amplified using the primer pair:

DREB3-1, 5#CCCTCTAGAGAATTCATGGCGAAACCCA
GCAGC-3# (forward); and

DREB3-2, 5#CCCCTCGAGCGGCATTTCCGGCACATA-3#
(reverse).

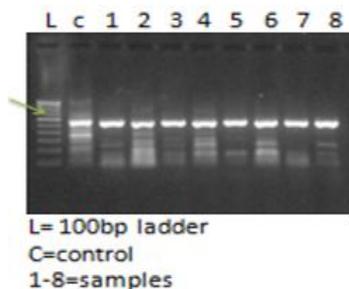


Fig. 1: Electrophoresis of the DREB 3 fragment from the Soybean DNA.

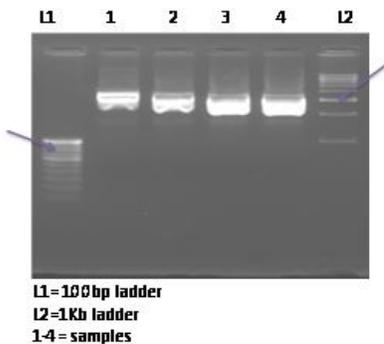


Fig. 2: Electrophoresis of the DREB 3 fragment from the pGEM-T vector clone.

The amplified product was electrophoresed using 1.5% agarose (Invitrogen) gel and was eluted using QIAGEN gel elution kit (Fig1). The eluted fragment was quantified using nanodrop and

then subjected to poly -AAA -tailing using Invitrogen Taq Polymerase, and the 528 bps fragment was eluted and cloned into pGEM-T easy vector. The 528 bps fragment was reamplified from the pGEM-T easy vector and the product was confirmed by electrophoresis (Fig 2). Most research on the DREB subfamily has focused on the A-1 and A-2 subgroups, and little is known about the characteristics and functions of members of the other subgroups. Studies on A-5 subgroup members, such as PpDBF1, GmDREB2, and GhDBF1 (Huang *et al.*, 2006; Liu *et al.*, 2007; Chen *et al.*, 2007), suggested that the A-5 subgroup, like the A-1 subgroup, are important genetic resources, potentially useful for the improvement of crop stress tolerance. In this study, a novel DREB subfamily A-5 subgroup member, GmDREB3, was isolated from soybean. It was hypothesized that a gene transfer event might have introduced an AP2 gene from lower organisms to the common ancestor of the moss and plant, and then the AP2 genes began to spread in the genome by transposition and homing recombination (Magnani *et al.*, 2004). During the course of evolution, AP2 genes diverged and acquired new functions by transposition and duplication events, and the DREB gene subfamily might have evolved from such events (Liu *et al.*, 2007)

REFERENCES

- Bartels D, Sunkar R. Drought and salt tolerance in plants. *Critical Reviews in Plant Sciences*. 2005; 24: 23–58.
- Benedict C, Skinner JS, Meng R, Chang Y, Bhalerao R, Huner NA, Finn CE, Chen TH, Hurry V. The CBF1-dependent low temperature signaling pathway, regulon and increase in freeze tolerance are conserved in populus spp. *Plant, Cell and Environment*. 2006; 29:1259–1272.
- Blum A. Crop responses to drought and the interpretation of adaptation. *Plant Growth Regulation*. 1996; 20: 135–148.
- Chen JQ, Dong Y, Wang YJ, Liu Q, Zhang JS. 2003. An AP2/EREBP-type transcript-factor gene from rice is cold-inducible and encodes a nuclear-localized protein. *Theoretical and Applied Genetics*; 107: 972–979.
- Chen L, Zhang Z, Liang H, Liu H, Du L, Xu H, Xin Z. Overexpression of TiERF1 enhances resistance to sharp eyespot in transgenic wheat. *J Exp Bot*. 2008; 59:4195–4204.
- Chen M, Xu Z, Xia L, Li L, Cheng X, Dong J, Wang Q, Ma Y. Cold-induced modulation and functional analyses of the DREbinding transcription factor gene, GmDREB3, in soybean (*Glycine max* L). *J Exp Bot*. 2009; 60:121–135.
- Fowler S and Thomashow. MF. Arabidopsis transcriptome profiling indicates that multiple regulatory pathways are activated during cold acclimation in addition to the CBF cold response pathway. *Plant Cell*. 2002; 14: 1675-1690.
- Gutterson N, Reuber TL. Regulation of disease resistance pathways by AP2/ERF transcription factors. *Curr Opin Plant Biol*. 2004; 7:465–471.
- Guo A, He K, Liu D, Bai S, Gu X. DATF: a database of Arabidopsis transcription factors. *Bioinformatics*. 2005; 21:2568–2569.
- Hong JP, Kim WT. Isolation and functional characterization of the Ca-DREBLP1 gene encoding a dehydration-responsive element binding-factor-like protein 1 in hot pepper (*Capsicum annuum* L. cv. Pukang). *Planta*. 2005; 220: 875–888.
- Ito Y, Katsura K, Maruyama K, Taji T, Kobayashi M, Seki M, Shinozaki K, Yamaguchi-Shinozaki K. Functional analysis of

- rice DREB1/CBF-type transcription factors involved in cold-responsive gene expression in transgenic rice. *Plant Cell Physiol.* 2006; 47:141–153.
12. Iwasaki T, Kiyosue T, Yamaguchi-Shinozaki K, Shinozaki K. The dehydration-inducible Rd17 (Cor47) gene and its promoter region in *Arabidopsis thaliana*. *Plant Physiol.* 1997; 115:1287–1289.
 13. Kasuga M, Miura S, Shinozaki K and Yamaguchi-Shinozaki K. A combination of the *Arabidopsis* DREB1A gene and stress-inducible rd29A promoter improved drought- and low-temperature stress tolerance in tobacco by gene transfer. *Plant Cell Physiol.* 2004; 45: 346-350.
 14. Li Y, Su X, Zhang B, Huang Q, Zhang X, Huang R. Expression of jasmonic ethylene responsive factor gene in transgenic poplar tree leads to increased salt tolerance. *Tree Physiol.* 2009; 29:273–279.
 15. Liu N, Zhong NQ, Wang GL, Li LJ, Liu XL, He YK, Xia GX. Cloning and functional characterization of PpDBF1 gene encoding a DRE-binding transcription factor from *Physcomitrella patens*. *Planta.* 2007; 226:827–838.
 16. Nakano T, Suzuki K, Fujimura T, Shinshi H. Genome-wide analysis of the ERF gene family in *Arabidopsis* and rice. *Plant Physiol.* 2006; 140:411–432
 17. Navarro M, Marque G, Ayax C, Keller G, Borges JP, Marque C, Teulière C. Complementary regulation of four eucalyptus CBF genes under various cold conditions. *J Exp Bot.* 2009; 60:2713–2724
 18. Qin F, Sakuma Y, Li J, Liu Q, Liu YQ, Shinozaki K, Yamaguchi Shinozaki K. 2004.
 19. Cloning and functional analysis of a novel DREB1/CBF transcription factor involved in cold-responsive gene expression in *Zea mays* L. *Plant and Cell Physiology*; 45: 1042–1052.
 20. Qin QL, Liu JG, Zhang Z, Peng RH, Xiong AS. Isolation, optimization, and functional analysis of the cDNA encoding transcription factor RdreB1 in *Oryza sativa* L. *Mol Breed.* 2007; 19:329–340
 21. Seki M, Narusaka M, Abe H, Kasuga M. Monitoring the expression pattern of 1300 *Arabidopsis* genes under drought and cold stresses by using a full-length cDNA microarray. *Plant Cell* 2001; 13: 61-72.
 22. Shen YG, Zhang WK, He SJ, Zhang JS, Liu Q, Chen SY. An EREBP/AP2 type protein in *Triticum aestivum* was a DRE-binding transcription factor induced by cold, dehydration and ABA stress. *Theoretical and Applied Genetics.* 2003; 106; 923–930.
 23. Tardieu F. Plant tolerance to water deficit: physical limits and possibilities for progress. *Comptes Rendus Geoscience.* 2005; 337; 57–67.
 24. Umezawa T, Fujita M, Fujita Y, Yamaguchi-Shinozaki K, Shinozaki K. Engineering drought tolerance in plants: discovering and tailoring genes to unlock the future. *Current Opinion in Biotechnology.* 2006; 17: 113–122
 25. Vij S, Tyagi AK. Emerging trends in the functional genomics of the abiotic stress response in crop plants. *Plant Biotechnology Journal.* 2007; 5: 361–380.
 26. Vinocur B, Altman A. Recent advances in engineering plant tolerance to abiotic stress: achievements and limitations. *Current Opinion in Biotechnology.* 2005; 16: 123–132.
 27. Volkov RA, Panchuk II and Schoffl F. Heat-stress-dependency and developmental modulation of gene expression: the potential of house-keeping genes as internal standards in mRNA expression profiling using real-time RT-PCR. *J. Exp. Bot.* 2003 ; 54: 2343-2349.
 28. Wang H, Datla R, Georges F, Loewen M, Cutler AJ. Promoters from *kin1* and *cor6.6*, two homologous *Arabidopsis thaliana* genes: transcriptional regulation and gene expression induced by low temperature, ABA, osmoticum and dehydration. *Plant Mol Biol.* 1995; 28:605–617
 29. Xu ZS, Chen M, Li LC, Ma YZ. Functions of the ERF transcription factor family in plants. *Botany.* 2008;86:969–977
 30. Zhuang J, Cai B, Peng RH, Zhu B, Jin XF, Xue Y, Gao F, Fu XY, Tian YS, Zhao W, Qiao YS, Zhang Z, Xiong AS, Yao QH. Genome-wide analysis of the AP2/ERF gene family in *Populus trichocarpa*. *Biochem Biophys Res Commun.* 2008; 371:468–474.
 31. Zhuang J, Peng RH, Cheng ZM, Zhang J, Cai B, Zhang Z, Gao F, Zhu B, Fu XY, Jin XF, Chen JM, Qiao YS, Xiong AS, Yao QH. Genome-wide analysis of the putative AP2/ERF family genes in *Vitis vinifera*. *Sci Hort.* 2009; 123:73–81

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