Molecular Cloning and Characterization of a Novel Gene Encoding DREB Protein from *Buchloe dactyloides* (Nutt.) Engelm

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Abstract

A 209bp expressed sequence tag (EST) was obtained with the degenerate primer technology, then based on the sequence of EST, the full-length cDNA of 1050 nucleotides was cloned from Buffalo grass by rapid amplification of cDNA ends (RACE). It was designated as *BdDREB2*, encoding a protein of 255 amino acids. The protein molecular weight was 28.39kDa, and the theoretical isoelectric point was 5.70. The *BdDREB2* probably localized in nucleus and did not have signal peptide. A typical AP2/EREBP conserved domain was found in the coding region. The amino acid sequence compared by blast revealed high homology with DREB proteins of other plants, especially with the Buchloe-dactyloi DREB protein.

The semi-quantitative analysis of expression of transcription showed: the expression of *BdDREB2* gene reached the maximum after being treated by 20%PEG for 6h; *BdDREB2* gene has the highest expression level in the root; the expression was significantly increased with the stress of drought (20% PEG) and high salt (3% NaCl), but not obvious with the stress of low temperature.

Plant expression vector was constructed and transformed into the tobacco by the Agrobacterium-mediated methods. PCR amplification with the transgenic tobacco genome DNA indicated that the *BdDREB2* gene had

integrated in tobacco genome. RT-PCR showed that the *BdDREB2* gene can be transcribed into mRNA in tobacco.

Keywords: *Buchloe dactyloi* (Nutt.) Engelm, Cloning, Semi-quantification analysis, Expression, Transgenic tobacco

1. Introduction

Transcription factor is a kind of DNA-binding protein, which is also called trans-acting factor. It can specially bind with cis-acting elements of eukaryotic gene promoter, and then activates or inhibit the transcription, so the gene production can make appropriate adjustment reaction to environmental signals. Transcription factor was first found in the maize (Sang et al. 2004). A typical transcription factor has four function domains: DNA binding domain, transcription regulation domain, nuclear localization signal (NLS) and oligomerization domain (Liu *et al.*2000， Yang et al. 2004). An AP2/EREBP transcription factor (Riechmann et al. 1998， Wessler et al. 2005) was obtained in this experiment. And these family members are different in the transcriptional activation domain (TAD), but they all have very conserved DNA binding domain (AP2/EREBP domain) and the basic amino acids of nuclear localization in the N-terminal.

DREB (Dehydration Responsive Element Binding protein) transcription factor can combine with the DRE/CRT cis-acting element. Firstly, the plant was induced by drought, high salt or low temperature. With the signal transduction, DERB transcription factor bound with the DRE cis-acting element to regulate the gene expression. The accumulation of gene products caused the physiological and biochemical changes, ultimately, enhanced the plant resistance of abiotic stresses (Wang et al.2004, Xie et al. 2006, Dimosthenis et al. 2002, Dubouzet et al. 1998). Yamaguchi-Shinozaki et al.（1994） detected the protein factor of binding with the DRE cis-acting element for the first time, which was named as DRBF1, and since then, the genes of DREB transcription factor from various plants were cloned successively. DREB can be divided into two categories: DREB1 and DREB2. DREB1 is induced by low temperature and DREB2 is affected by drought and high salt stresses（Zhuang et al. 2010）. DREB genes have been cloned from plants of Arabidopsis thaliana (Yamaguchi-Shinozaki et al. 1994, Nakano et al. 2006, Riechmann et al. 2000), grape, rye, wheat, tomato (Jaglo et al. 2001), barley (Choi et al. 2002), rice (Dubouzet et al. 2003, Fu et al. 2007, Qing et al. 2007), brassica (Gao et al. 2002, Cong et al. 2008), mountain spinach (Shen et al. 2003), corn (Dimosthenis et al. 2002, Qin et al. 2003), grapevine (Jaillon et al. 2007) and poplar (Zhuang et al. 2008). DREB transcription factors in turfgrass of Bermudagrass (Xie et al. 2005) and tall fescue (Yang et al. 2006) had been reported, but there was no report on the buffalo grass.

Buffalo grass [Buchloe dactyloides (Nutt.) Engelm.] belongs to gramineae family, eragrostis subfamily, buchloe plant. Buffalo grass is warm-season turfgrass, grows slowly and suitable wide temperature range for cultivation. The most prominent feature is its drought tolerant. It can survive in the summer of persistent drought for 2-3 months. It can still grow lushly in the arid, semi-arid arrears of 300-600mm annual rainfall (Sun 1989). Buffalo grass is used primarily for grazing; however, it has many good turf characteristics, such as low plants, better adaptability, stress resistance, less fertilizer requirement and easy maintenance, so it is used as landscape plants gradually. If we could obtain the DREB gene successfully and over-express it in the buffalo grass, it would enhance the resistance of buffalo grass and it would be beneficial for the use of buffalo grass.

A normal way of obtaining new gene is using the degenerate primer. We isolated the BdDREB2 gene EST of buffalo grass by this way. The full-length cDNA was obtained by the RACE technology. The gene was analyzed and transformed into tobacco. This experimentation might provide insight into the physiological processes of stress response in higher plants.

2. Materials and methods

2.1 Plant materials and growth conditions

The seed of Buffalo grass (*Buchloe dactyloides* (Nutt.) Engelm) was obtained from CAAS (Chinese Academy of Agricultural Sciences). We removed the glume, washed the seeds and soaked germination for 1 day, then placed the seeds in the quartz sand tray. The seedings were growing under a regime of 16 h light and 8 h dark at 28℃.

2.2 Amplification of the conserved fragment BdDREB2 cDNA

We searched various plants DREB transcription factors in the GenBank, then made sequence homology analysis by BLAST tools was carried out. According to the principle of degenerate primer, we designed a pair of degenerate primers DREBF and DREBR(Tab. 1), being used to amplify the conserved fragment of *BdDREB2* cDNA. We treated the 14-days-old seedlings by 20% PEG to analyze the gene expression level. Total RNA was isolated from the whole seedling by a guanidine isothiocyanate extract method. Any contaminated genomic DNA was removed by incubating the total RNA with RNase-free DNase (Promega) at 37℃ for 30min. The total RNA

was used to synthesize the cDNA according to the manufacturer's recommendation of Reverse Transcriptase M-MLV（Takara，Japan. The primers DREBF and DREBR were used to amplify the conserved fragment. PCR product was separated by electrophoresis on a 1% agarose gel stained with ethidium bromide, purified using the DNA gel extraction kit (Takara, Japan). The products were cloned into the pMD18-T (Takara, Japan) vector and then transformed into E.coli DH5α. Recombinant plasmids were sequenced by Beijing Genomics Institution (Beijing, China).

2.3 Amplification of full-length BdDREB2 cDNA

We used total RNA as template, synthesized 5'-RACE-Ready-cDNA and 3'-RACE-Ready-cDNA according to the manufacturer's recommendation of SMART^{TM} RACE cDNA amplification kit (Clontech, USA).Based on the EST sequence obtained, gene-specific primers:GSP5 and GSP3 (Tab. 1) were used to amplify the 5'-cDNA end and 3'-cDNA end, respectively, with Advantage TM2 Enzyme kit (Clontech).

To obtain the full-length cDNA sequence, we used the 3'-RACE cDNA as template, PCR reaction was carried on using gene-specific primer D1 and the UPM (Tab. 1).

2.3 Bioinformatics analysis

We used the DNAMAN to splice the cDNA fragment and predict the location of the ORF; analyzed the conserved sequence in the Conserved Domains (http://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and ExPASy Scan prosite (http://www.expasy.org/tools/scanprosite/); predicted the functional sites by ExPASy PROSITE scan (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_prosite.h); In the WebLab,we used Pepstats to predict pI/MV, used Condon Usage to statistical analysis of codon, predicted transmembrane segment in tmap; Potential signal peptide cleavage site was identified by SignalP 3.0; The subcellular localization was analyzed in ExPASy PSORT; After BLAST in NCBI, the high similarity sequences were homology analyzed by the DNAMAN software.

2.4 Semi-quantitative RT-PCR analysis

The seeds were washed by distilled water, then soaked germination for 1 day, placed the seeds in the quartz sand tray. The seedling were grown under a regime of 16 h light and 8 h dark at 28℃, 10 days later treated according the following groups:（a）20% PEG for 0.5h,1h,3h,6h,12h and 24h; (b) 4℃ for 8h; (c) 3％ NaCl for 6h. We weighed the equal weight of seedlings, quickly placed in liquid nitrogen for RNA extraction.

We extracted the RNA of plant treated above, and reverse transcription was carried on after the quantitative analysis by UV spectrophotometer. Then amplified by the house-keeping gene primers 18S1,18S2 and the *BdDREB2* gene primers BdDREBa, BdDREBb（Tab. 1）. Thermocycling for 20,24,28,32,36,40,44 cycles to confirm the plateau. PCR products were separated by electrophoresis on a 1% agarose gel stained with ethidiium bromide.

2.5 Construction of the plant expression vector

We designed a pair of primers: BdDREB-1 (5'-GCTCTAGAATGGAGCGGGTGGAGGTGC-3'） and BdDREB-2 (5'-CGGGATCCTGAGAAGAGACTGAAC-3'). The underlines were restriction site *Xba*I and *Bam*HI. The reverse transcript cDNA was used as template. PCR product was purified with the DNA gel extraction kit (Takara, Japan). Then we cloned the product into the pMD18-T (Takara, Japan) vector, named pMD-BdDREB, sequenced by Beijing Genomics Institution (Beijing,China). After obtaining the accurate sequence, we extracted the plasmids pBI121（stored at -80℃ in our laboratory）and pMD-BdDREB, then digested the plasmids by *Xba*I and *Bam*HI restriction endonucleases. The aimed fragments were obtained by the DNA gel extraction kit (Takara, Japan). The products were linked by T4-DNA ligase at 16℃ for 6h. We named the constructed vector PBI-BdDREB (Fig. 1).

2.6 Tobacco transformation and analysis

The plasmid was transformed into Agrobacterium LBA4404, and the Agrobacterium LBA4404 harboring PBI-BdDREB was transformed in tobacco with leaf-disc method. (Horsch et al. 1985). The transgenic tobacco genomic DNA was obtained by the CTAB method and the wild type was used as control. Primers used for amplification were GUSF, GUSR; P35S-1, P35S-2; BdDREB-1, BdDREB-2 (Tab. 2).The total RNA was isolated from the transgenic tobacco and wild type tobacco by a guanidine isothiocyanate extract method.

3. Results

3.1 Isolation of the full-length cDNA

According to the degenerate primer cloning method, we obtained a 209bp fragment. Two primers were designed

to obtain 5'-RACE end and 3'-RACE end. A 653bp fragment was isolated by using 3'-RACE and a 426bp fragment was obtained by using 5'-RACE. The 1050bp full-length cDNA of Buffalo grass DREB protein was amplified from cDNA with primers D1 and UPM, tentatively designated *BdDREB2* (GenBank accession no. EF512460).

3.2 Sequence analysis of BdDREB2

Sequence analysis indicated that the full-length cDNA contained an open reading frame of 755bp, encoding a putative protein of 254 amino acids. Ahead of the original code ATG and after the stop code TAG, there were an 83-bp 5'-UTR and a 212-bp 3'-UTR. Its theoretical pI and MV were 5.70 and 28.39 kDa. A typical AP2/EREBP conserve sequence of 57 amino acids was found in the sequence (Fig. 2).

The structural domains and functional sites analysis showed that in addition to the AP2/EREBP conserved domain, there were also many functional sites, such as N-myristoylation sites, Casein kinase II sites, cAMP- and cGMP -dependent protein kinase sites and Protein kinase C sites. These sites enhanced or inhibited the functional protein by combined with corresponding activator protein or inhibitor protein. (Fig. 3)

The codon usage analysis of the sequence indicated in the C-terminal end, there was a nucleus localization signal region which was rich of the alkalescent amino acids (mainly Lys, Arg), in the N-terminal end there was a transcription active region which was rich of the acidic amino acids (mainly Asp, Glu). In addition, the *BdDREB2* gene was rich of hydrophobic amino acids (Ala, Val, Gly).

The amino acid sequence was used to search in the protein databank and showed that it could be aligned with other DREB transcription factors from different species (Fig.4). Homologous comparison indicated the BdDREB2 gene had the highest homology with the Cynodon dactylon DREB gene (Fig.5)

The signal peptide analysis showed that the sequence had no signal peptide. The subcellular localization analysis showed that the possibility in the nucleolus was 88%. It was unlikely localized in other organelles.

3.3 Semi-quantitative RT-PCR analysis

The plateau was ascertained by the different cycles PCR. The result was 18S gene amplified by 28 cycles and *BdDREB2* gene amplified by 36 cycles.

In the early period of simulated drought condition (20%PEG treatment), the expression of *BdDREB2* gene increased with the prolongation of drought stress time, and reached its maximum in 6 hours then stabilized (Fig. 6).

In the normal condition, *BdDREB2* gene has a certain level of expression. Relatively, the underground part has higher expression than the aerial part; the expression of PEG treated seeding was significantly increased compared with the control group, particularly in root (Fig. 7).

The expression of *BdDREB2* gene has obviously increased after high salt and drought treatment. However, it was not significantly before and after low temperature treatment (Fig. 8).

3.4 Transgenic tobacco analysis of PCR and RT-PCR

The *BdDREB2* gene was amplified with genomic DNA of transgenic tobacco genome DNA. The result indicated that *BdDREB2* gene had integrated into the tobacco genome. We amplified the *BdDREB2* gene with cDNA of transgenic tobacco. And the result showed the gene was not silenced at the transcription level, it may be translated into protein in transgenic plants.

4. Discussion

In this study, we isolated the full-length cDNA of *BdDREB2* from *Buchloe dactyloides* (Nutt.) Engelm. Sequence analysis of *BdDREB2* indicated that there was a typical AP2/EREBP domain with 57 amino acids in the coding region. This domain has very high similarity with DREB transcription factor from other plants. There were two functional amino acids (Valine and Glutamate) in the 14-bit and 19-bit amino acid site, which have been confirmed of specific bind in the DRE cis-acting element.

We defined it was a member of EREBP subfamily according the number of conserve domain. The report showed, the class of DREB1（A～C）gene has a conservative C/SEV/LR amino acid sequence between the 14-bit Valine and 19-bit Glutamate, but it is AEIR in the class of DREB2（A～B）gene (Zhuang et al. 2010). *BdDREB2* gene is AEIR between 14-bit and 19-bit, therefore, it is a member of DREB2.

Yamaguchi-Shinozaki et al. (1994) isolated the DRE binding protein from Arabidopsis thaliana for the first time and named DREB1, thereafter, the cloning of DREB transcription factor developed greatly. Many DREB genes have been isolated from various plants. DREB gene family was enriched and improved continuously. However,

there was not report about DREB gene in Buffalo grass.

BdDREB2 has the typical characteristic of DREB transcription factor (Riechmann et al. 1998, Wang et al. 2005), there is a nuclear localization signal which is rich in basic amino acids (Lys and Arg) in the N-terminal. There is a transcription activation domain that rich of acidic amino acids (Asp and Glu) in the C-terminal. So we predicted that this gene had the similar function with other DREB gene. It played important roles in the regulation of abiotic stress response and the activation of transcription of stress resistant genes.

The results of semi-quantitative PCR showed that the gene transcription expression level in plants was obvious different in different tissues and stressed conditions. *BdDREB2* gene expressed in the whole plant and with highest expression level in roots. And these results were consistent with some reports before (Jiang et al. 2001). The expression was not obvious increased in the early stage of stress conditions, and the expression was gradually increased till 6h treated.

DREB family was divided into two sub-families, DREB1 and DREB2. DREB1 was induced by low temperature, and DREB2 was induced by drought and high salt (Kizis et al. 2001, Sakuma et al. 2002, Zhao et al. 2006, Yamaguchi et al. 2006). In our study, the expression of *BdDREB2* gene was obvious increased after being treated by high salt and PEG; but was not obvious increased after low temperature treatment. So we confirmed the *BdDREB2* gene belonged to the DREB2 family.

We obtained the transgenic tobacco by the Agrobacterium-mediated method. PCR and RT-PCR analysis showed that *BdDREB2* gene had integrated into the tobacco genome and may be transcribed into mRNA. To verify the authenticity of transgenic tobacco, we should verify the expression of protein by Western Blotting. More research should carry out to understand functions of *BdDREB2*.

References

Cathala, G. Savouret, J.F. Mendez, B. West, B.L. Karin, M. Martial, J.A.,& Baxter, J.D. (1983). A method for isolation of intact, transcriptionally active ribonucleic acid. *DNA*, 2, 329-335. http://dx.doi.org/10.1089/dna.1983.2.329

Choi, D.W. Rodriguez, E.M., & Close, T.J. (2002). Barley Cbf3 gene identification, expression pattern, and map location. *Plant Physiol*, 129, 1781-1787. http://dx.doi.org/10.1104/pp.003046

Cong, L. Chai, T.Y., & Zhang, Y.X. (2008). Characterization of the novel gene BjDREB1B encoding a DRE-binding transcription factor from Brassica juncea L. Biochem Biophys Res Commun,371,702-706. http://dx.doi.org/10.1016/j.bbrc.2008.04.126

Dimosthenis Kizis & Montserrat PageÁ s. (2002). Maize DRE-binding proteins DBF1 and DBF2 are involved in rab17 regulation through the drought-responsive element in an ABA- dependent pathway. *The Plant Journal*, 3(6), 679-689. http://dx.doi.org/10.1046/j.1365-313X.2002.01325.x

Dubouzet, J.G. Sakuma, Y. Ito Y., Kasuga, M. Dubouzet, E.G. Miura, S. Seki, M. Shinozaki, K., & Yamaguchi-Shinozaki, K. (1998). Os-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.

Dubouzet, J.G. Sakuma, Y. Ito, Y. Kasuga, M. Dubouzet, E.G. Miura, S. Seki, M. Shinozaki, K. & Yamaguchi, S.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. http://dx.doi.org/10.1046/j.1365-313X.2003.01661.x

Fu, X.Y. Zhang, Z. Peng, R.H. Xiong, A.S. Liu, J.G. Wu, L.J. Gao, F. Zhu, H. Guo, Z.K. & Yao, Q.H. (2007). Isolation and characterization of a novel cDNA encoding ERF/AP2-type transcription factor OsAP25 from Oryza sativa L. *Biotechnol Lett*, 29, 1293-1299. http://dx.doi.org/10.1007/s10529-007-9370-1.

Gao, M.J. Allard, G. Byass, L. Flanagan, A.M. & Singh, J. (2002). Regulation and characterization of four CBF transcription factors from Brassica napus. *Plant Mol Biol*, 49, 459-471. http://dx.doi.org/10.1023/A:1015570308704

Horsch, R. Fry, J.E. Hoffmann, N. Eichholtz, D. Rogers, S. & Fraley, R.T. (1985). A simple and general method for transferring genes into plants. *Science*, 227, 1229-1231. http://dx.doi.org/10.1126/science.227.4691.1229

Jaglo, K.R. Kleff, S. Amundsen, K.L. *et al*. (2001). Components of the Arabidopsis C-repeat/ Dehydration Responsive element binding factor cold-response pathway are conserved in Brassica napus and other plantspecies. *Plant Physiol*, 127, 910-917. http://dx.doi.org/10.1104/pp.010548

Jaillon, O. Aury, J.M. Noel, B. Policriti, A. Clepet, C. Casagrande, A. Choisne, N. & Aubourg, S. (2007). The grapevine genome sequence suggests ancestral hexaploidization in major angiosperm phyla. *Natur*e, 449, 463-467. http://dx.doi.org/10.1038/nature06148

Jiang, X.M. Huang, M.R. & Wang, M.X. (2001). A Review On Salt and Drought Resistant Gene Engineering in Plants. *Journal of Nanjing Forestry University (Natural Sciences Edition)*, 25(5), 57-62.

Kizis, D. Lumbreras, V. & Page`s, M. (2001). Role of AP2/EREBP transcription factors in gene regulation during abiotic stress. *FEBS Lett*, 498, 187-189. http://dx.doi.org/10.1016/S0014-5793(01)02460-7

Liu, Q. Zhang, G.Y. & Chen, S.Y. (2000). The Structure and Regulation of Plant Transcription Factors.*Chienese Science Bulletin*, 45(14), 1465-1471.

Nakano, T. Suzuki, K., Fujimura, T. & Shinshi, H. (2006). Genomewide analysis of the ERF gene family in Arabidopsis and rice. *Plant Physiol*, 140, 411-432. http://dx.doi.org/10.1104/pp.105.073783

Qin, F., Li, J., Zhang, G.Y., *et al*. (2003). Isolation and structural analysis of DRE-binding transcription factor from maize (*Zea mays* L.). *Acta Botanica Sinica*, 45(3), 331-339.

Qin, Q.L. Liu, J.G. Zhang, Z. Peng, R.H. Xiong, A.S. Yao, Q.H. & Chen, J.M. (2007). Isolation, optimization, and functional analysis of the cDNA encoding transcription factor RdreB1 in *Oryza Sativa* L. *Mol Breed*, 19, 329-340. http://dx.doi.org/10.1007/s11032-006-9065-7

Riechmann, J. L. & Meyerowitz, E.M. (1998). The AP2/EREBP family of plant transcription factors. *Biol Chem*, 379,633-646.

Riechmann, J.L. Heard, J. Martin, G. Reuber, L. Jiang, C. Keddie, J. Adam, L. Pineda, O. Ratcliffe, O.J. Samaha, R.R. Creelman, Pilgrim M. Broun, P. Zhang, J.Z. Ghandehari, D. Sherman, B.K. & Yu, G. (2000). Arabidopsis transcription factors: genome-wide comparative analysis among eukaryotes. *Science*, 290, 2105-2110. http://dx.doi.org/10.1126/science.290.5499.2105

Sakuma, Y. Liu, Q. Dubouzet, J.G. Abe, H. Shinozaki, K. & Yamaguchi, S.K. (2002). DNA- binding specificity of the ERF/AP2 domain of Arabidopsis DREBs, transcription factors involved in dehydration and cold-inducible gene expression. Biochem Biophys Res Commun, 290,998-1009. http://dx.doi.org/10.1006/bbrc.2001.6299.

Sang, X.H. Wu, Z.Y. Huang, C.L. Zhang, L.S. (2004). Progresses on Transcription Factors Related to Plant Stress-Tolerance. *Chinese Bulletinof Botany*, 21 (6), 700-708.

Shen, Y.G. Yan, D.Q. Zhang, W.K. et al. (2003). Novel halophyte EREBP/AP2 type DNA binding protein improves salt tolerance intransgenic tobacce. *Acta Botanica Sinica*, 45 (1), 82-87.

Sun, J.X. (1989). *Lawn Science*, GanSu Science and Technology Press.

Wang, S.X. Wang, Z.Y. & Peng, Y.K. (2004). Dehydration Responsive Element Binding (DREB)Transcription Activator and Its Function in Plant Tolerance to Environmental Stresses. *Plant Physiology Communication*, 40(1), 7-13.

Wessler, S.R. (2005). Homing into the origin of the AP2 DNA binding domain. *Trends Plant Sci,* 10, 54–56. http://dx.doi.org/10.1016/j.tplants.2004.12.007.

Xie, Y.L. Wang, Z.A. Zhang, S.P. (2006). DREB-a special transcription factors response to abiotic-stress in plan. *Journal of Qinghai University (Nature science)*, 24 (2), 54-58.

Xie, Y.L. Wang, Z.Z. Liu, Q. & Zhang, S.P. (2005). Cloning and Functional Identification of Stress-resistant *BeDREB* Genes from Bermuda grass. *Chinese Journal of Biochemistry and Molecular Biology*, 21(4), 521-527.

Yamaguchi, S.K. & Shinozaki, K. (2006). Transcriptional regulatory networks in cellular responses and tolerance to dehydration and cold stresses. *Annu Rev Plant Biol*, 57,781-803. http://dx.doi.org/10.1146/annurev.arplant.57.032905.105444

Yamaguchi-Shinozaki, K. 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 (2), 251-264.

Yang, W.L. Liu, J.M. Liu, Q. Gong, Y.D. & Zhao, N.M. (2006). Isolation and Characterization of a DREB-like transcription factor gene from Tall Fescue. *Journal of Nuclear Agricultural Sciences,* 20(3), 187-192. http://dx.doi.org/10.1146/annurev.arplant.57.032905.105444.

Yang, Z.R. Wang, X.C. & L, X.m. (2004). Advance on the Study of Transcription Factors in Higher Plants. *Hereditas*, 26(3), 403-408.

Zhao, T.J. Sun, S. Liu, Y. Liu, J.M. Liu, Q. Yan, Y.B. & Zhou, H.M. (2006). Regulating the drought-responsive element (DRE)-mediated signaling pathway by synergic functions of trans-active and transinactive DRE binding factors in Brassica napus. *J Biol Chem*, 281, 10752-10759. http://dx.doi.org/10.1074/jbc.M510535200

Zhuang, J. Cai, B. Peng, R.H. Zhu, B. Jin, X.F. Xue, Y. Gao, F. Fu, X.Y. Tian,Y.S. Zhao, W. Qiao, Y.S. Zhang, Z. Xiong, A.S. & Yao, Q.H. (2008). Genome-wide analysis of the AP2/ERF gene family in Populus trichocarpa. Biochem Biophys Res Commun, 371, 468-474. http://dx.doi.org/10.1016/j.scienta.2009.08.002

Zhuang, J. Xiong, A.S. Peng, R.H. Gao, F. Zhu, B. Zhang, J. Fu, X.Y. Jin, X.F. Chen, J.M. Zhang, Z. Qiao, Y.S. & Yao, Q.H. (2010). Analysis of Brassica rapa ESTs: gene discovery and expression patterns of AP2/ERF family genes. *Mol Biol* Rep., 37, 2485-2492. http://dx.doi.org/10.1007/s11033-009-9763-4.

Table 1. Sets of specific oligonucleotide primers for *BdDREB2*

Primer	Nucleotide sequences
DREBF	5’-CCB GCC AAR GGK TCS AAG AAR GG-3’
DREBR	5’-TAC ATT GCY CTD GCM GCY TCR TC-3’
GSP3	5’-CTC GTT CCC TAC TAC TGC TCT GG-3’
GSP5	5’-GAG CAG CCT CCA GAG CAG TAG GGA ACG AG-3’
D1	5’-GAT GGA GCG GGT GGA GGT GC-3’
UPM (universal primer)	A mix: 5’-CTAATACGACTCACTATAGGGCAAGCAGTGGTATCAACGCAGAGT-3’ and 5’-AAGCAGTGGTATCAACGCAGAGT-3’
18S1	5’-AGT ATG GTC GCA AGG CTG AA-3’
18S2	5’-CAT TCA ATC GGT AGG AGC GA-3’
BdDREBa	5’-CCT GAC TCC ATC GCT GAG ACA AT-3’
BdDREBb	5’-GTG CTG ACG TGC AAC CTG AG-3’

Table 2. Sets of specific oligonucleotide primers

Primer	Nucleotide sequences
GUSF	5'-GCAACTGGACAAGGCACT-3'
GUSR	5'-GAGCGTCGCAGAACATTACA-3'
P35S-1	5'-CTTACGCAGCAGGTCTCATCA-3'
P35S-2	5'-CCACCTTCCTTTTCCACTATCTT-3'
BdDREB-1	5’－GCTCTAGAATGGAGCGGGTGGAGGTGC-3’
BdDREB-2	5’－CGGGATCCTGAGAAGAGACTGAAC-3’

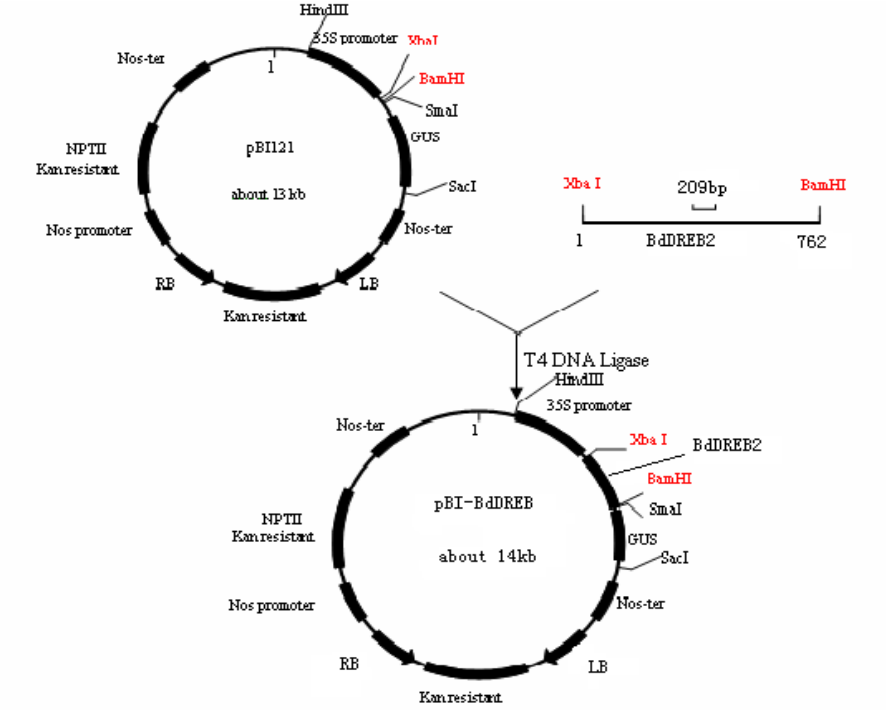

Figure 1. Schemes of constructing of expression vector for BdDREB2 gene

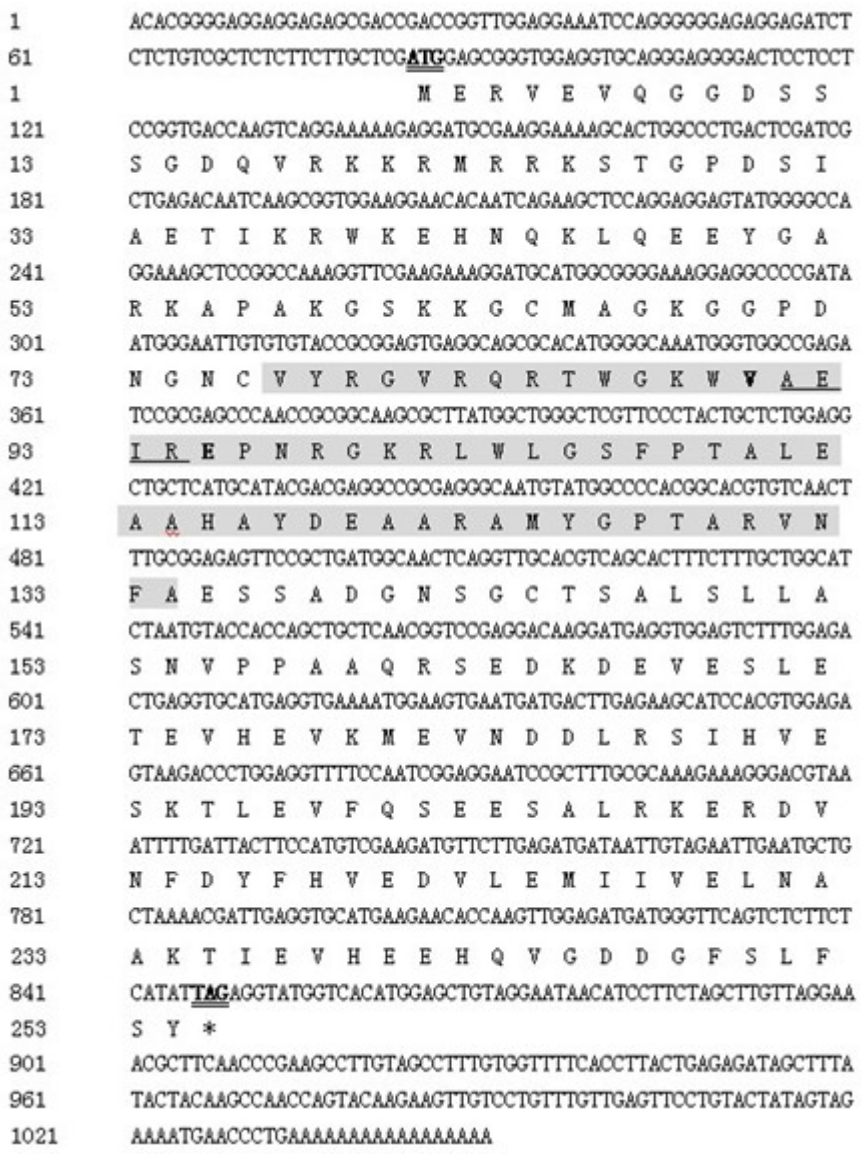

Figure 2. Full length coding sequence of the BdDREB2 gene and deduced amino acid sequence
AP2/EREBP conserved motifs are shaded

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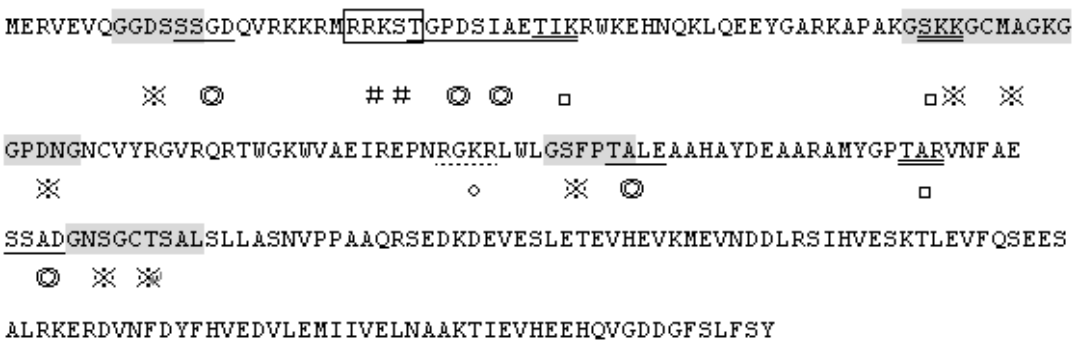

Figure 3. The analysis of BdDREB2 motifs

"※", the N-myristoylation sites; "◎", the Casein kinase II phosphorylation sites; " # ", the cAMP- and cGMP-dependent protein kinase phosphorylation sites; "□", the Protein kinase C phosphorylation sites; "○", the Amidation site

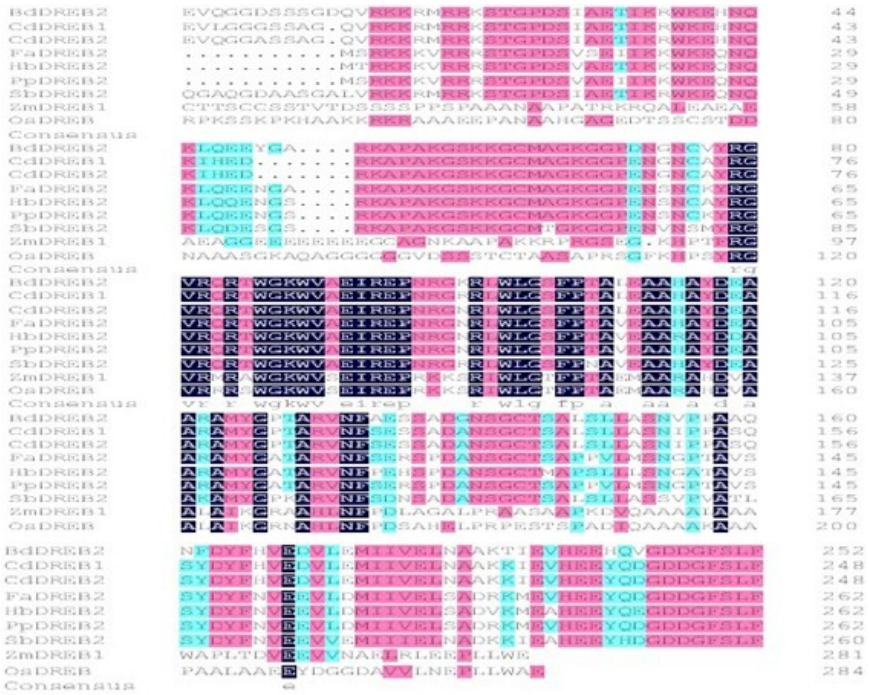

Figure 4. The alignment of BdDREB2 with DREB gene in other plants

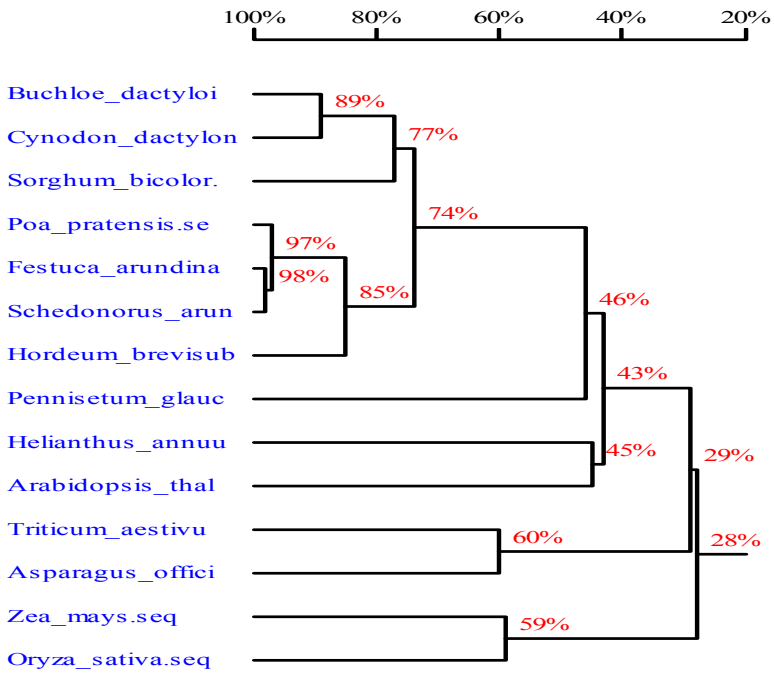

Figure 5. Homology analysis of BdDREB2 with other DREB released in GenBank

Genes and corresponding accession numbers are AAS46285，ABD66654，AAS59530，AAR11157，AAU29412，AAV90624，AAS82861，NP_001031837， AAX13274，ABB89754，AAN76733，AAO39764

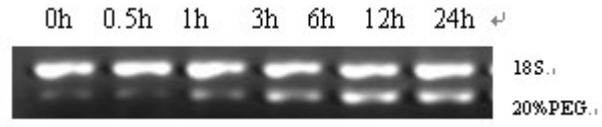

Figure 6. Expression of BdDREB2 in different periods of 20% PEG treatment

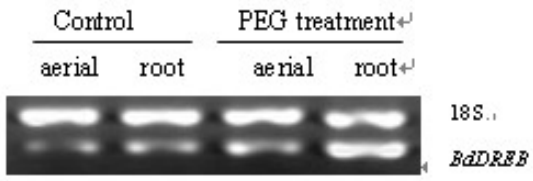

Figure 7. Expression of *BdDREB2* in different tissues under 20% PEG stress for 6h

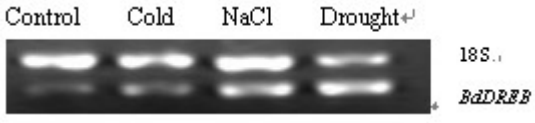

Figure 8. Expression of *BdDREB2* under different abiotic stress

Cold, 4℃ for 6h; NaCl, 3% for 6h; Drought, 20%PEG for 6h