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Isolation and Characterization of Partial DREB Gene from Four Iranian *Triticum aestivum* Cultivars

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Abstract: Cold acclimation is accompanied by altered gene expression and some of genes are either up or down regulated. Dehydration responsive element binding (DREB) protein is a subfamily of AP2/ERF transcription factors, which control expression of many drought, salinity and cold inducible genes and causing tolerance to environmental stresses in many plants. The main property of DREB gene is conserved AP2 domain that binds to stress responsive elements. We isolated and characterized DREB gene from four Iranian bread wheat cultivars named Alvand, Bayat, Darab1 and Shahi. The sequences were submitted to NCBI GenBank with FC556845, FC556846, FC556847, FC556850 accession numbers. Bioinformatics analysis of the deduced protein showed an AP2 DNA binding domain of 57 amino acids based on the conserved 14th valine and 19th glutamic acid residues.

Key words: Bread wheat, DREB, Cold stress, Transcription factor, AP2 domain

INTRODUCTION

Plants are exposed to environmental stresses such as drought, high salt and low temperature, which cause adverse effects on the growth of plants and quality and quantity of their products. Plants respond to abiotic stresses with a series of physiological and biochemical changes. A Number of genes that respond to abiotic stresses have been identified [1-5]. Many stress-inducible genes are responsive to both osmotic stress and low temperature, suggesting that a common set of signal transduction pathways is triggered during many stress responses [6-9]. Cold stress leads to dehydration via reduction of osmotic potential. Cold acclimation is accompanied by altered gene expression and some of genes are either up or down regulated [8-12]. Promoter analysis of the cold regulated (COR) genes has shown that they contain sequence elements that mediate the stress induction of the genes. The best characterized of these is the dehydration-responsive element (DRE), also known as a C-repeat (CRT) or a low-temperature-responsive element (LTRE). Moreover, some of the COR genes contain ABA-responsive elements (ABREs), that mediate the ABA responsiveness of these genes [10, 13]. The expression of COR genes is regulated by both ABA-independent and ABA-dependent pathways [4,14]. The DREBs belongs to ethylene-responsive element-binding proteins (EREBPs)/AP2 (APETALA2) transcription factor family that is unique to plants. There are an estimated 124 ethylene-responsive element-binding factor (ERF) proteins in Arabidopsis [15]. ERF proteins share a conserved 57–59 amino acid domain (the ERF/AP2 domain) that binds to cis elements, the GCC box, found in many PR (pathogens related) gene promoters conferring ethylene responsiveness [16] and the C-repeat CRT/dehydration responsive element (DRE) motif, involved in the expression of cold and dehydration-responsive genes. DREB1A and DREB2A cDNAs for DRE-binding proteins have been isolated from Arabidopsis. DREB1A and its homologs were induced by low temperature, whereas DREB2A and its single homolog show expression under dehydration and high-salt stress [17]. Five rice DREB homologs, OsDREB1A-D and OsDREB2A that show homology in their ERF/AP2 domains, have been cloned and their transcript analysis indicates their role in abiotic stresses [18]. The over-expression of the OsDREB1A in Arabidopsis showed up-regulation of many important abiotic stress-related genes [18]. Numerous genes have been identified by microarray analysis as potential downstream genes of DREB1 [8, 11, 19, 20].

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To date, many transcriptional activators that bind the DRE have been isolated from *A. thaliana* (CBF/DREBs) [3, 17, 21], rice (OsDREBs) [18], barley (HvCBF) [22], GmDREB in soybean [23], rape (BnCBFs) [24] and wheat (TaDREB1) [25] as well as other plants. In this study isolation and characterization of DREB cDNA sequences in cold stress from four cultivars of Iranian bread wheat named Alvand, Bayat, Darab1 and Shahi are carried out.

MATERIALS AND METHODS

Plant Materials and Cold Stress Treatment: Four bread wheat (*Triticum aestivum* L.) seeds; Alvand, Bayat, Darab1 and Shahi were supplied by Karaj Gene Bank. After germination, seedlings were grown nine days in growth chamber conditions with 16 h light, 500 μmol m^{-2} s^{-1} fluorescent light, 22/15°C day/night temperature and 60% humidity.

DNA Extraction and Primer Design: About 200 mg of leaf tissue were ground to a fine powder in liquid nitrogen and DNA was extracted using modified CTAB method [26]. PCR amplification was carried out with the gene specific primers and genomic DNA as the template. Primers were designed by Oligo5 software and consensus of DREB1 genes were alignment from NCBI GenBank. The PCR was carried out three times for each cultivar in 20 μl volume containing 50 ng of samples DNA, 1X PCR buffer, 1.5 mM $MgCl_2$, 0.25 mM each dNTP, 2 μM of each primer and 1 U *Taq* DNA polymerase. The PCR amplifications were initiated by denaturation at 94°C for 5 min followed by 35 amplification cycles (94°C for 1 min, 58°C for 1 min and 72°C for 1 min) and final extension at 72°C for 10 min. Primers include Forward: 5'-TATGGATTGCCTTGATGAACA-3' and Reverse: 5'-GACTCCGATTCATCCTTCCC-3'. The PCR amplification products were separated in 1% (*w*/*v*) agarose gels and GeneRuler 50bp DNA ladder from Fermentas was used.

Purification of PCR Product and Sequencing: PCR products were purified using High Pure PCR Product Purification Kit (Roche) and sequenced (MWG, Germany).

Sequence Analyses: We make BLAST search to align our isolates with the already exist in the genebank. In addition deduced amino acid sequence analyses were carried out by software's such as PROSITE, Pfam and PRINTS. Alignment was done by multalin program.

RESULTS

The TaDREB DNA sequences were 500 bp in length for Shahi, Alvand, Darab1 and Bayat wheat. Figure 1 shows a fragment of 500 bp that presented DREB gene in four wheat cultivars. Nucleotide BLAST search showed that our sequences are highly similar (99% identity) to *T. aestivum* genome D dehydration-responsive element-

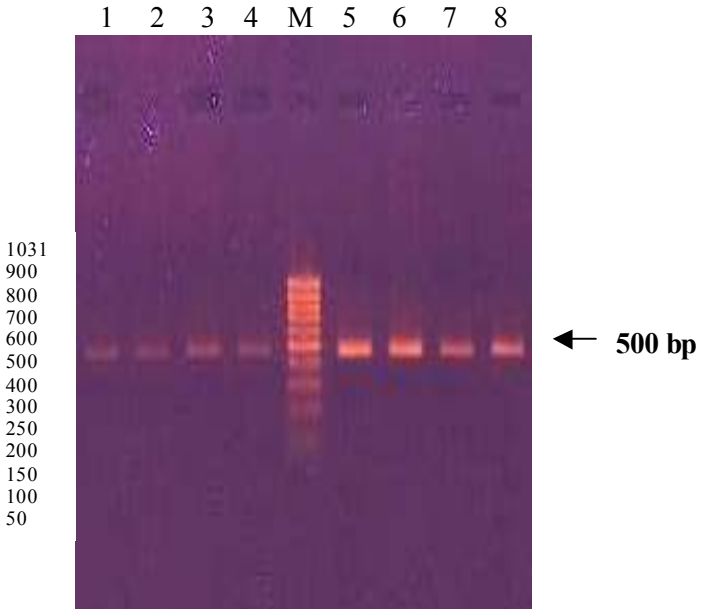

Fig. 1: Gel electrophoresis of PCR product of DREB DNA (1 to 4) and its replication (5 to 8) from Alvand, Bayat, Darab1 and Shahi cultivars, respectively. M = GeneRuler 50bp DNA ladder, sizes of each fragment are shown

A Y R G V R Q R T W G K W *V* A E I R *E* P N R G N R L W L G S F P T
A V E A A R A Y D D A A R A M Y G A K A R V N F

Fig. 2: Deduced amino-acid sequence of AP2 domain of alvand cultivar DREB cDNA. AP2 domain has three ß-sheets and one a-helix, respectively showed by smaller font. The valine$_{14}$ and glutamic acid$_{19}$ amino acids of AP2 domain are showed by italic

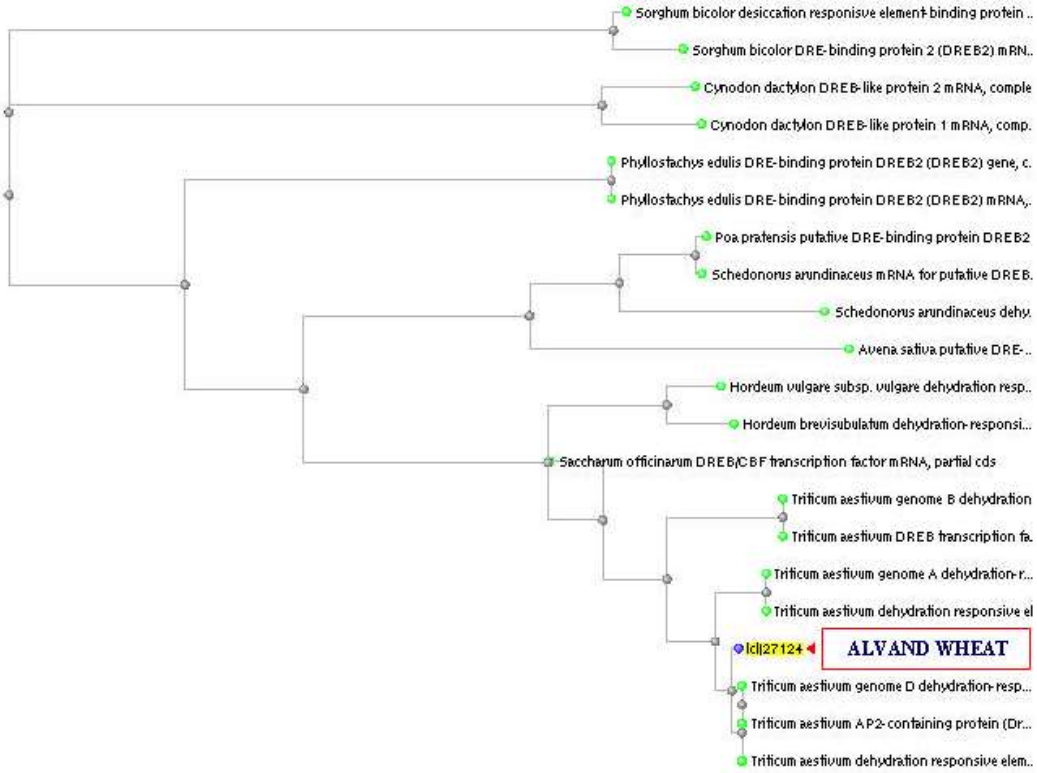

Fig. 3: Phylogenetic tree of Alvand cultivar DNA sequence with some other sequences of gramineae DREB by BLAST software

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           1      10        20        30        40        50        60        70        80        90       100       110       120       130
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Alvand     GGGGTAGCAAGCGGGAAGGAGACTGCCCCGGGCAGGAAAGGAAGAAGAAAGTGCGCAGGAGAAGCACTGGTCCTGATTCGGTTGCTGAAACCATCAAGAAGTGGAAGGAGGAAAACCAGAAGCTCCAGCA
Darab1     GGGGTAGCAAGCGGGAAGGAGACTGCCCCGGGCAGGAAAGGAAGAAGAAAGTGCGCAGGAGAAGCACTGGTCCTGATTCGGTTGCTGAAACCATCAAGAAGTGGAAGGAGGAAAACCAGAAGCTCCAGCA
Bayat      GGGGTAGCAAGCGGGAAGGAGACTGCCCCGGGCAGGAAAGGAAGAAGAAAGTGCGCAGGAGGAGCACTGGTCCTGATTCGGTTGCTGAAACCATCAAGAAGTGGAAGGAGGAAAACCAGAAGCTCCAGCA
Shahi     GGGGTAGCAAGCGGGAAGGAGACTGCCCCGGGCAGGAAAGGAAGAAGAAAGTGCGCAGGAGAAGCACTGGTCCTGATTCGGTTGCTGAAACCATCAAGAAGTGAAAGGAGGAAAACCAGAAGCTCCAGCA
Consensus  GGGGTAGCAAGCGGGAAGGAGACTGCCCCGGGCAGGAAAGGAAGAAGAAAGTGCGCAGGAGaAGCACTGGTCCTGATTCGGTTGCTGAAACCATCAAGAAGTGgAAGGAGGAAAACCAGAAGCTCCAGCA

           131    140       150       160       170       180       190       200       210       220       230       240       250       260
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Alvand     AGAGAATGGATCCCGGAAAGCACCGGCCAAGGGTTCCAAGAAAGGGTGCATGGCAGGGAAAGGAGGTCCAGAGAATTCAAACTGCGCTTACCGCGGTGTGAGGCAGAGGACGTGGGGGAAATGGGTTGCT
Darab1     AGAGAATGGATCCCGGAAAGCACCGGCCAAGGGTTCCAAGAAAGGGTGCATGGCAGGGAAAGGAGGTCCAGAGAATTCAAACTGCGCTTACCGCGGTGTGAGGCAGAGGACGTGGGGGAAATGGGTTGCT
Bayat      AGAGAATGGATCCCGGAAAGCACCGGCCAAGGGTTCCAAGAAAGGGTGCATGGCAGGGAAAGGAGGTCCAGAGAATTCAAACTGCGCTTACCGCGGTGTGAGGCAGAGGACGTGGGGGAAATGGGTTGCT
Shahi     AGAGAATGGATCCCGGAAAGCACCGGCCAAGGGTTCCAAGAAAGGGTGCATGGCAGGGAAAGGAGGTCCAGAGAATTCAAACTGCGCTTACCGCGGTGTGAGGCAGAGGACGTGGGGGAAATGGGTTGCT
Consensus  AGAGAATGGATCCCGGAAAGCACCGGCCAAGGGTTCCAAGAAAGGGTGCATGGCAGGGAAAGGAGGTCCAGAGAATTCAAACTGCGCTTACCGCGGTGTGAGGCAGAGGACGTGGGGGAAATGGGTTGCT

           261    270       280       290       300       310       320       330       340       350       360       370       380       390
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Alvand     GAGATCCGTGAGCCCAACCGTGGCAATCGGCTGTGGCTTGGTTCATTCCCTACCGCAGTCGAAGCTGCACGTGCATATGATGATGCGGCAAGGGCAATGTATGGCGCCAAAGCACGTGTCAACTTCTCAG
Darab1     GAGATCCGTGAGCCCAACCGTGGCAATCGGCTGTGGCTTGGTTCATTCCCTACCGCAGTCGAAGCTGCACGTGCATATGATGATGCGGCAAGGGCAATGTATGGCGCCAAAGCACGTGTCAACTTCTCAG
Bayat      GAGATCCGTGAGCCCAACCGTGGCAATCGGCTGTGGCTTGGTTCATTCCCTACTGCAGTCGAAGCTGCACGTGCATATGATGATGCGGCAAGGGCAATGTATGGCGCCAAAGCACGTGTCAACTTCTCAG
Shahi     GAGATCCGTGAGCCCAACCGTGGCAATCGGCTGTGGCTTGGTTCATTCCCTACCGCAGTCGAAGCTGCACGTGCATATGATGATGCGGCAAGGGCAATGTATGGCGCCAAAGCACGTGTCAACTTCTCAG
Consensus  GAGATCCGTGAGCCCAACCGTGGCAATCGGCTGTGGCTTGGTTCATTCCCTACcGCAGTCGAAGCTGCACGTGCATATGATGATGCGGCAAGGGCAATGTATGGCGCCAAAGCACGTGTCAACTTCTCAG

           391    400       410       420       430       440       450       460       470       480       490       500
           |--------+---------+---------+---------+---------+---------+---------+---------+---------+---------+---------|
Alvand     AGCAGTCCCCGGATGCCAACTCTGGTTGCACGCTGGCACCTCCATTGCCGACGTCTAATGGGGCAACCGCTGCGTCACATCCTTCTGATGGGAAGGATGAATCGGAGTCA
Darab1     AGCAGTCCCCGGATGCCAACTCTGGTTGCACGCTGGCACCTCCATTGCTGACGTCTAATGGGGCAACCGCTGCGTCACATCCTTCTGATGGGAAGGATGAATCGGAGTCA
Bayat      AGCAGTCCCCGGATGCCAACTCTGGTTGCACGCTGGCACCTCCATTGCCGACGTCTAATGGGGCAACCGCTGCGTCACATCCTTCTGATGGGAAGGATGAATCGGAGTCA
Shahi      AGCAGTCCCCGGATGCCAACTCTGGTTGCACGCTGGCACCTCCATTGCCGATGTCTAATGGGGCAACCGCTGCATCACATCCTTCTGATGGGAAGGATGAATCGGAGTCA
Consensus  AGCAGTCCCCGGATGCCAACTCTGGTTGCACGCTGGCACCTCCATTGCcGAcGTCTAATGGGGCAACCGCTGCgTCACATCCTTCTGATGGGAAGGATGAATCGGAGTCA
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Fig. 4. Sequence alignment of four wheat DREB DNA sequences

binding protein (Dreb1) gene and to *T. aestivum* AP2-containing protein (Dreb1) mRNA. In addition, sequences analysis indicated that deduced protein belonged to DREB transcription factor subfamily and contained a conserved AP2 domain with three β-sheets and one α-helix and the valine$_{14}$ and glutamic acid$_{19}$ amino acids (Fig. 2). Figure 3 shows the position of Alvand cultivar DNA sequence in Phylogenetic tree as a sample with some other sequences of gramineae DREB by BLAST software. Alignment of four wheat cultivars DNA sequences by multalin software is shown in Figure 4. The DNA sequences have been submitted to NCBI GenBank with FC556845, FC556846, FC556847, FC556850 accession numbers.

DISCUSSION

Low temperature as a key environmental factor represents one of the principal limitations affecting plant species distribution and crop productivity [27]. Cold induces the expression of a number of plant genes that encode proteins to enhance tolerance of plants to low temperatures. One group of these genes is to regulate gene expression and signal transduction. They include transcription factors for example CBF/DRE-binding protein or CBF/DREB [25]. We analyzed the TaDREB1 gene in *T. aestivum* and found that its deduced protein has a conserved AP2 DNA binding domain of 57 amino acids, suggesting that the TaDREB1 protein might function as a transcriptional activator in these four bread wheat cultivars.

Comparison of the DNA sequences of four wheat cultivars which are grown in Iran by bioinformatics tools indicates that there are very similar and this part of DREB sequence had no intron. As mentioned in the Results section, AP2 domain of the sequences have three β-sheets and one α-helix and the valine$_{14}$ and glutamic acid$_{19}$ amino acids, which are typical characteristics of AP2/EREBP protein [17, 21, 28]. Previous researches indicated that in DREB proteins, AP2 domain is characterized by a conserved valine and glutamic acid in the 14th and 19th positions, respectively, which may play important roles in recognition of the DNA-binding sequence [17, 28], whereas further research showed that E_{19} might not be as important as V_{14} for the recognition of the DNA-binding sequence [16]. According to Figure 2, Alvand cultivar sequence as a sample of four isolated sequences has high similarity to other wheat DREB sequences which have been deposited to NCBI GenBank. Alignment of four wheat DREB DNA sequences (Figure 4) and BLAST search indicates that our sequences have 99 % homology to each other but have differences in some nucleotides that might be critical for amino acid changes. Alvand and Shahi cultivars are cold tolerant wheat, while Bayat and Darab1 cultivars are sensitive to cold, but results showed that all of them have DREB gene in their genome. Of course gene expression maybe occurred differentially in both cold resistant and sensitive cultivars. DREB1 gene is unique in that it is specifically induced by cold stress [29], so the level of gene expression may be higher in cold resistant cultivars that can be determined by quantitative PCR. DREB gene expresses in non-stress condition may be related to the other roles of stress-inducible genes as mentioned by Latini and co-workers [30]. Some researchers noted that the elevated constitutive expression levels for stress-responsive genes with key roles in cellular metabolism are affected under stress in tolerant plants [31, 32]. Sequence-function relationship did not seen by genomic survey but mRNA analysis under different cold-stress intensity can show different level of DREB gene expression in one cultivar and between resistant and sensitive cultivars.

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