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AP2 domain structure and protein motif features in Azerbaijan local durum and bread wheat genotypes

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DREB proteins belonging to the superfamily of AP2/ERF plant transcription factors play an important role in the signaling network that modulates many processes, such as stress responses and plant development. In the present study, we have isolated and molecularly characterized partial DREB1 gene from Azerbaijan's local durum and bread wheat genotypes, which are distinguished by tolerance to abiotic stress factors. Analysis of amino acid sequences encoded by the putative DREB genes revealed a strongly conserved AP2/ERF domain with two conserved functional amino acids (14th valine and 19th glutamic acid) which play crucial roles in the recognition of the DNA binding site. Nuclear localization signal and conserved Ser/Thr-rich region were observed in the corresponding amino acid sequences. One α-helix and two β-sheets were detected in the secondary structure of the AP2 domain. In the protein sequence with the AP2 domain, 3 and 11 amino acid substitutions were detected in bread wheat and durum wheat, respectively. The identified sequences of the DREB1 gene from durum and bread wheat are available in the GenBank database (Accession number MZ935737.1, MZ935738.1). Characterization of the DREB genes from the stress-tolerant wheat genotypes is important for further understanding the role of this gene in the plant stress-tolerance mechanisms. Moreover, the identified single nucleotide variations associated with stress tolerance can be used in genome editing for improving crops under stress conditions.

***Keywords**: DREB,* AP2/ERF*, NLS, Ser/Thr-rich region, durum wheat, bread wheat*

INTRODUCTION

According to large-scale transcriptome analyses, protective proteins and regulatory proteins are involved in molecular stress responses (Shahzad et al., 2021). Among them, transcription factors (TFs) were shown to play a crucial role in regulating plant growth and response to abiotic and biotic stresses. TFs are multi-functional proteins that may simultaneously control numerous pathways during stresses in plants-this makes them powerful tools for the manipulation of regulatory and stress-responsive pathways. Defining the structure-function relationships of numerous plant TFs involved in drought and associated stresses allowed the development of practical strategies for engineering plants with enhanced stress tolerance (Hrmova and Hussain, 2021). Recent studies have determined several main superfamilies of transcription factors involved in the stress-response reactions including myeloblastosis (MYB) oncogene, APETALA2/ethylene response factor (AP2/ERF), basic leucine zipper (bZIP), Cys2(C2)His2(H2)-type zinc fingers (ZFs), and transcription factors with a protein domain consisting of the conserved WRKYGQK (WRKY) motif (Lindemosa et al., 2013; Gao et al., 2018).

The AP2/ERF superfamily is characterized by the presence of an AP2/ERF DNA-binding domain of 60–70 amino acids, and it is composed

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of the ERF, AP2, RAV related to ABI3 (abscisic acid incentive 3) and VP1 (viviparous 1) families. ERF and AP2 family proteins consist of one (EREBP) and two (AP2 family) AP2/ERF domains, respectively, whereas RAV family proteins are composed of AP2/ERF domain and DNA binding B3 domain originally named due to its position in the third basic domain of the maize gene Viviparous1, VP1 (McCarty et al., 1991; Zhao et al., 2017; Gao et al., 2018). Results of the genome-wide studies indicate that the AP2/ERF transcription factors were highly conserved during plant evolution (Feng et al., 2020).

DREB, the Dehydration Responsive Element (DRE)-binding proteins family is one of the largest families of TFs that play a significant role in signaling networks modulating many plant processes (Agarwal et al., 2006; Lata and Prasad, 2011; Sarkar et al., 2019). The DREB proteins activate many abiotic stress-responsive genes and maintain water balance in plant systems thus imparting abiotic stress tolerance. The DREB TFs belonging to the AP2/ERF family of transcription factors were divided into two categories, DREB1 and DREB2. DREB1 is affected by low temperatures and DREB2 is induced by high salt and drought stresses (Zhuang et al., 2011; Filiz and Tombuloglu, 2014).

DREB1/CBF and DREB2 genes share a sequence similarity at the AP2 domain and bind to the 9 base pair sequence - C-repeat/DRE motif (TACCGACAT) in the promoter region of DREBs. The DRE element was first identified in the rd29A promoter, which contains a DRE core sequence (ACCGAC) (Yamaguchi-Shinozaki and Shinozaki, 1994; Li et al., 2018). All DREB genes have three conservative regions, such as the EREBP/AP2 DNA binding domain, the N-terminal nuclear localization signal, and the Ser/Thr-rich region. The two amino acids in the ERF/AP2 domain, valine (position 14) and glutamic acid (position 19) were found to play an important role in the DNA-binding specificity (Sakuma et al., 2002; Jan et al., 2017). It was an interesting point to find a DRE/CRT motif in a DREB promoter gene since it binds to the same motif in the promoter region of downstream stress-inducible genes. So, it can be predicted that the expression of the DREB gene is regulated by some other transcription factors such as DREB which interact with this unique motif. It was found that the DREB gene is also expressed under non-stress conditions, which may be related to the other functions of stress-inducible genes (Latini et al., 2008). Hence, to explore mechanisms of plants' tolerance to certain stress factors, cloning and sequencing of the DREB genes from different plant varieties with further comparative structural and functional studies seem to be one of the efficient ways to explore the role of DREBs.

The main goal of the study was the isolation and molecular characterization of the DREB gene from the Azerbaijan local wheat cultivars Barakatli 95 and Azamatli 95 which are distinguished by high productivity, quality, and tolerance to extreme factors of the environment.

MATERIALS AND METHODS

Plant materials. Barakatli 95 genotype of durum wheat (*Triticum durum* Desf., AABB, 2n = 4x = 28) and Azamatli 95 genotype of bread wheat (*Triticum aestivum* L., AABBDD, 2n = 6x = 42) were used. Barakatli 95 has been obtained at the Research Institute of Crop Husbandry (Baku, Azerbaijan) by individual selection from intraspecific hybridization of the local folk selection varieties Gyrmyzy Bugda and Garagylchyg due to their high productivity, quality and tolerance to abiotic stress factors. Azamatli 95 has been obtained by individual selection from bread wheat genotypes from the 16th elite variety testing seed plot (16 ESWYT-12) introduced from CIMMYT and adapted to local conditions (Aliyev et al., 2013).

DNA extraction and quantification. The DNA extraction was performed using the modified CTAB method (Murray and Thompson, 1980). The quantity of DNA was evaluated based on the optical density (OD) at λ=260 using the Epoch™ Microplate Spectrophotometer (BioTek, USA). The purity of the genomic DNA was determined by the ratio of absorptions at A260/A280. The quality of the DNA was checked on a 0.8% agarose gel stained with 10 mg/mL of ethidium bromide in 1×TBE (Tris base, Boric acid, EDTA) buffer. The gel was documented using the "Gel Documentation System UVITEK" (UK).

Table 1. Nucleotide sequence of the gene-specific primers

Primer description	Nucleotide sequence (5'- 3')	Product size, bp	Ann. Temp., ℃
PsDREB-F	TATGGATTGCCTTGATGAACA	500	53.3
PsDREB-R	GACTCCGATTCATCCTTCCC		

DNA amplification with gene-specific primers. Gene-specific primer pairs were used for isolation of the DREB gene (Pandey et al., 2014) (Table 1). DNA amplification was performed in a 25 μL reaction mixture volume, containing 10 x buffer, 20 ng of the genomic DNA, 0.2 μM primer, 200 μM of each of the following: dATP, dCTP, dGTP and dTTP, 2.5 mM $MgCl_2$, and 0.2 units of Taq-polymerase in the incubation buffer.

PCR was performed in the "Applied Biosystems 2720 Thermal Cycler" under the following conditions: 1 cycle - 5 min at 94ºC; 35 amplification cycles - 1 min at 94ºC, 1 min at 53.3ºC, 1 min at 72°C; the final elongation was performed at 72°C for 10 min, then kept at 4°C. The amplified products were electrophoresed on a 1.5% agarose gel, stained with ethidium bromide (EtBr), and visualized under ultraviolet light using "Gel Documentation System UVITEK" (UK).

Purification of PCR product and DNA sequencing. The preparative PCR amplification was carried out to amplify the DNA region of interest prior to Sanger sequencing. The amplification products were excised from the agarose gel and purified using ISOLATE II PCR & Gel Kit (BIOLINE) according to the manufacturer's instructions. The purified samples were then sequenced on an ABI 3130xl DNA analyzer (Applied Biosystems, USA).

Data analyses. To explore the protein-coding capacity of the sequenced DNA fragments and compare them with known genes/proteins, the FGENESH (*http://www.softberry.com/berry.phtml?topic=fgenesh&group=programs& subgroup=gfind*) (Solovyev et al., 2006) and BLAST (https://blast.ncbi.nlm.nih.gov/Blast.cgi) (Altschul et al., 1997) tools were used. To identify the conserved protein domains, the INTERPROSCAN *(http://www.ebi.ac.uk/Tools/pfa/iprscan/)* (Jones et al., 2014) and SMART *(http://smart.embl-heidelberg.de/*) (Letunic et al., 2015) programs were applied. A multiple amino acid sequence alignment was constructed using MAFFT v7.271 *(https://mafft.cbrc.jp/alignment/server/*) (Katoh and Standley, 2014). The protein secondary structure was predicted by PSIPRED method *(http://bioinf.cs.ucl.ac.uk/psipred/)*

RESULTS AND DISCUSSION

Clarification of gene functions and availability of wheat genome sequence information opens up new opportunities for improving crops under stress conditions (Rathan et al., 2021). In our previous studies, using the genome-specific functional markers in Azerbaijan wheat genotypes, a gene encoding DREB1 transcription factor was detected in the A, B, and D genomes (Huseynova et al., 2013). Wheat genotypes of Azerbaijan and German origin with contrasting drought tolerance were used to determine the expression level of the DREB1 transcription factor. In general, the transcript levels of all genotypes exposed to drought stress were found to increase significantly. Further, under drought stress, the expression level of DREB1 in the tolerant genotypes, durum wheat Barakatli 95 and bread wheat Azamatli 95, was increased more than in drought-sensitive ones (Rustamova et al., 2020). In the current study, we isolated a part of the DREB1 gene covering the AP2 domain from these genotypes. Fig.1 shows the electrophoretic profiles of PCR products obtained using gene-specific primer pair PsDREB-F/R. In addition to the expected 500 bp fragments in both genotypes, 300 bp fragments were also amplified. Amplification products were separated in 1.5% (w/v) agarose gel, purified using purification kit ISOLATE II PCR & Gel Kit (BIOLINE) and sequenced.

Further, the nucleotide sequences of the 500 bp and 300 bp DNA fragments were compared

with known DREB genes by the BLAST tool. It was found that the 500 bp fragment is highly similar to the DREB gene of other wheat genotypes from the GenBank (with 95% and 97% similarity levels for Barakatli 95 and Azamatli 95, respectively).

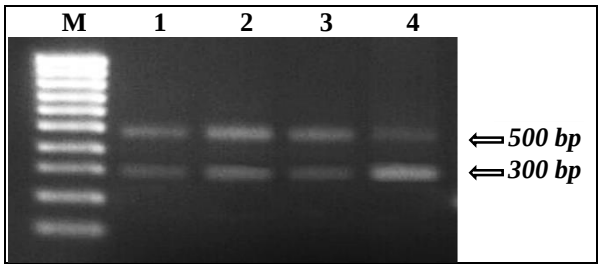

Fig. 1. Gel electrophoresis of PCR products of DREB1 gene in Barakatli 95(1-2) and Azamatli 95 (3-4). Arrows indicate the 300 bp and 500 bp fragments. M-100 bp DNA ladder.

A comparison with the Chinese Spring genome (the Ensembl assembly IWGSC) revealed four hits of significant similarity and three of them belong to the TraesCS3A02G099200, TraesCS3B02G115400, and TraesCS3D02G099500 genes located in the A, B, and D genomes, respectively. The TraesCS3A02G099200 gene has 1 transcript and encodes the DREB protein W73 (UniProtKB/TrEMBL; Q4U0C8) of 278 aa. Two alternative splice variants of the TraesCS3B02G115400 gene (B genome) encode proteins of 1008 aa and 1401 aa. The UniProtKB contains a single protein (Q3LR66) corresponding to this gene. The TraesCS3D02G099500 gene (D genome, chromosome 3) has 2 splice variants and encodes proteins of 1311 aa and 1225 aa. For this gene, the UniProtKB contains 2 proteins (G0YWB9; G0YWC2).

Comparison of putative DREB1 gene fragments from Barakatli 95 and Azamatli 95 genotypes with the reference DREB1 gene (DQ195068; https:// .ncbi.nlm.nih.gov/nuccore/DQ195068.1) by the MAFT tool revealed 88.42% similarity between them (Fig. 2).

Further, a search for possible genes/open reading frames (ORF) in the 500 bp DNA sequence of the durum wheat genotype Barakatli 95 by the FGENESH program predicted a gene fragment with one exon of 396 bp length (positions 16-412 positions). This fragment might encode a polypeptide of 132 amino acids (aa) in length. In the 500 bp sequence of bread wheat Azamatli 95, one exon of 381 bp length (positions 12-393) was predicted. This exon might encode a polypeptide of 127 aa length (Fig.3).

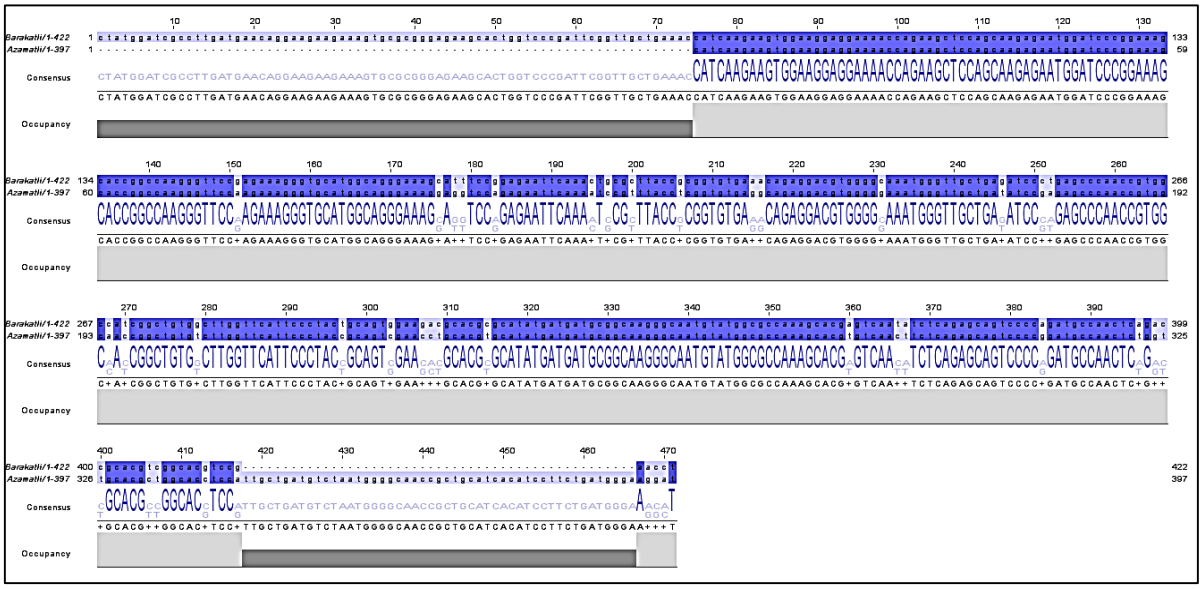

Fig. 2. Sequence alignment of the partial DREB1 gene isolated from Barakatli 95 and Azamatli 95 *using* MAFFT tool. Conservations of nucleotides are distinguished by various shades of blue color.

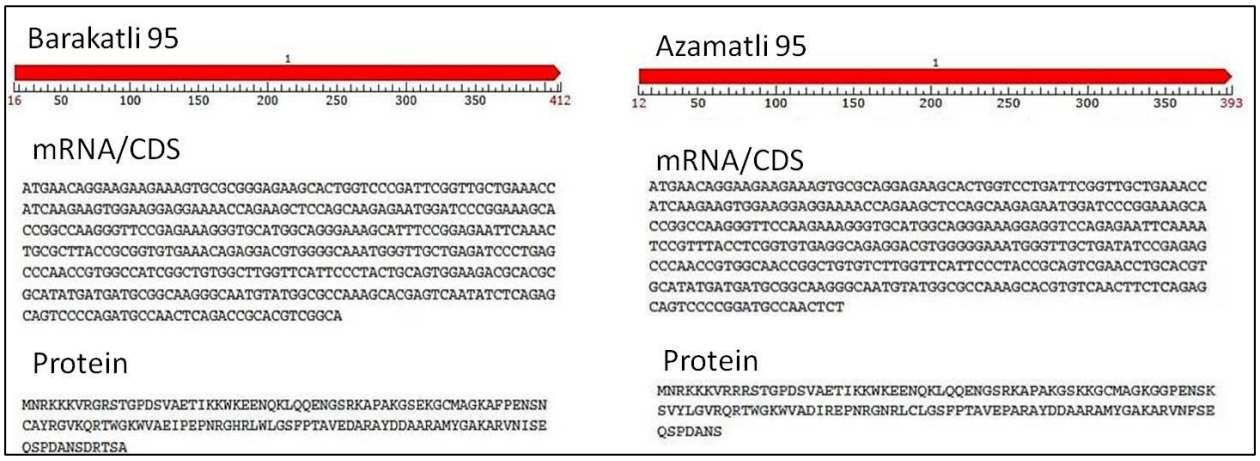

Fig. 3. Putative gene fragment (exon), CDS and protein in the 500 bp DNA sequence from Baraktli and Azamatli wheat genotypes predicted by the FGENESH.

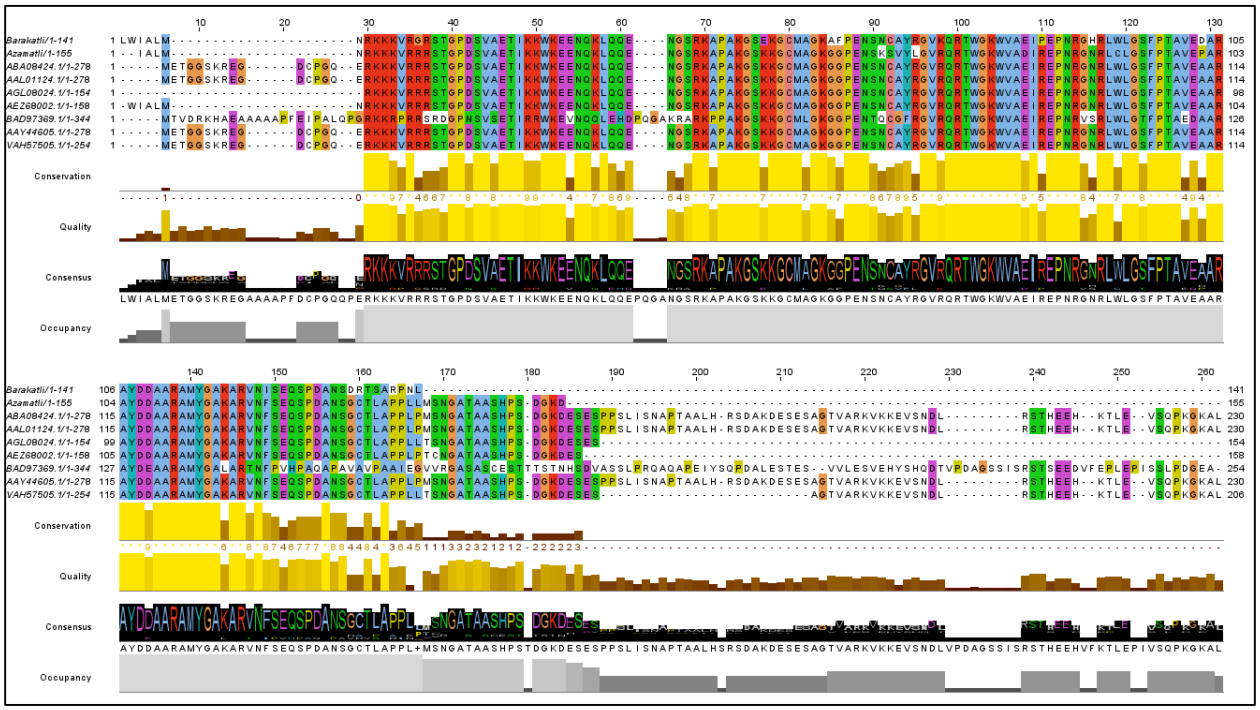

Fig. 4. Multiple alignment of DREB protein sequences by MAFFT tool.

To identify conserved regions, deduced amino-acid sequences of partial DREB genes and some AP2-containing proteins deposited in the NCBI GenBank, a multiple alignment of these sequences by MAFFT software was performed. Figure 4 describes the alignment of dehydration-responsive element binding proteins of *Triticum aestivum* L. (Azamatli 95, Accession Number AAL01124.1, ABA08424.1, AEZ68002.1, BAD97369.1), *Triticum durum* (Barakatli 95), *Triticum dicoccoides* (Accession Number AGL08024.1) and *Triticum turgidum subsp. Durum* (Accession number VAH57505.1) samples. As seen in the figure, single nucleotide variations (SNVs) available in the Barakatli 95 genotype result in amino acid substitutions in 11 points (positions 36, 77, 85, 86, 110, 116, 129, 159, 160, 164, and 166). In Azamatli 95 genotype amino acids were replaced in only 3 positions (91, 95, and 129).

Analysis of amino acid sequences encoded by the putative DREB1 genes from Barakatli 95 and Azamatli 95 by INTERPROSCAN and SMART computer programs revealed the AP2 domain in the 500 bp fragment with the two conserved functional amino acids (valine (V) and glutamic acid (E)) at the 14th and 19th residues which play crucial roles in recognition of the DNA-binding sequence (Fig. 5). However, some studies demonstrated that E19 might not be as necessary as V14 for this case (Sakuma et al., 2002; Rana et al., 2013). DREB proteins demonstrated a high level of identity, especially in the conserved regions. KKK and KKWK in the N-terminal region function as a nuclear localization signal (NLS). An entry of the nucleus-targeted transcription factors into the nucleus is regulated by the NLS (Akhtar et al., 2012; Pandey et al., 2014). A region of 56 amino acid residues, underlined with a solid line, is strongly conserved among DREB proteins. This region is referred to as the AP2/ERF DNA-binding domain. Two highly conserved functional amino acids at the 14th and 19th positions were also observed in the AP2 domain. These amino acids distinguish the DREB (valine and glutamic acid) from the ERF (alanine and aspartic acid) (Agarwal et al., 2007). Besides, tryptophan was found in the AP2 domain, followed by serine and threonine amino acids in polypeptides corresponding to the sequenced partial DREB gene. The results of our study are in line with data obtained by previous studies. The role of tryptophan rings in the recognition of GCC-box and determination of the geometry GCC-box binding domain was reported by Mondini et al. (2014).

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Barakatli       LWIALM----------------------NRKKKVRGRSTGPDSVAETIKKWKEENQKLQQ
Azamatli        --IALM----------------------NRKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
ABA08424.1      -----METGGSKREG------DCPGQ--ERKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
AAL01124.1      -----METGGSKREG------DCPGQ--ERKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
AGL08024.1      ----------------------------RKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
AEZ68002.1      -WIALM----------------------NRKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
BAD97369.1      -----MTVDRKHAEAAAAAPFEIPALQPGRKKRPRRSRDGPNSVSETIRRWKEVNQQLEH
AAY44605.1      -----METGGSKREG------DCPGQ--ERKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
VAH57505.1      -----METGGSKREG------DCPGQ--ERKKKVRRRSTGPDSVAETIKKWKEENQKLQQ
                                        ***: *    **:**:***::*** **:*::

Barakatli       E----NGSRKAPAKGSEKGCMAGKAFPENSNCAYRGVKQRTWGKWVAEIREPNRGHRLWL
Azamatli        E----NGSRKAPAKGSKKGCMAGKGGPENSKSVYLGVRQRTWGKWVADIREPNRGNRLCL
ABA08424.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
AAL01124.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
AGL08024.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
AEZ68002.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
BAD97369.1      DPQGAKRARKPPAKGSKKGCMLGKGGPENTQCGFRGVRQRTWGKWVAEIREPNRVSRLWL
AAY44605.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
VAH57505.1      E----NGSRKAPAKGSKKGCMAGKGGPENSNCAYRGVRQRTWGKWVAEIREPNRGNRLWL
                :    : :**.*****:**** **. ***::. : **:*********:* ****  ** *

Barakatli       GSFPTAVEDARAYDDAARAMYGAKARVNISEQSPDANSDRTSARPNL-------------
Azamatli        GSFPTAVEPARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLLMSNGATAASHPS-
ABA08424.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPMSNGATAASHPS-
AAL01124.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPMSNGATAASHPS-
AGL08024.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLLTSNGATAASHPS-
AEZ68002.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPTCNGATAASHPS-
BAD97369.1      GTFPTAEDAARAYDEAARAMYGALARTNFPVHPAQAPAVAVPAAIEGVVRGASASCESTT
AAY44605.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLPMSNGATAASHPS-
VAH57505.1      GSFPTAVEAARAYDDAARAMYGAKARVNFSEQSPDANSGCTLAPPLLTSNGATAASHPS-
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Fig. 5. AP2/ERF DNA-binding domain of DREB gene. The area underlined with a solid line shows the DNA-binding domain. The specific signal peptide sequence area is highlighted in red. The valine 14t^{h} and glutamic acid 19^{h} amino acids of the AP2 domain are shown in bold and italics. Black boxes are the aromatic rings of Trp. The specific sequence for α-helix is yellow. Green colors are the T and S residues that distinguished the Ser/Thr-rich region.

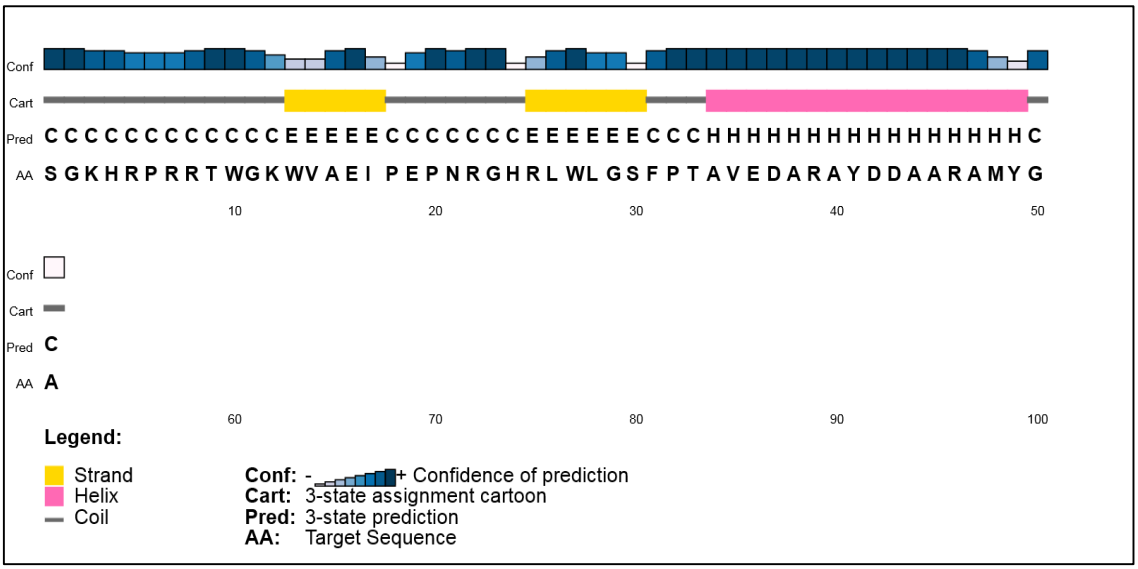

Fig. 6. Information about the secondary structure of the AP2 domain, as predicted by PSIPRED analysis.

Liu et al. (1998) have demonstrated the presence of a conserved Ser/Thr-rich region adjacent to the EREBP/AP2 binding domain containing the phosphorylation site for the regulation of gene activity. Using the protein structure prediction software PSIPRED 4.0 (Predict Secondary Structure), analysis of deduced amino acid sequences of the AP2 domain was performed in putative DREB1 gene fragments from Barakatli 95 and Azamatli 95, and one α-helix and two β-sheets (Fig 5, Fig.6) were found in the secondary structure. The 3D structure of the complex of the Arabidopsis AtERF1-DNA-binding domain and its target DNA was determined by NMR (Allen et al., 1998) and used for molecular modeling of ParCBF1. To understand the structure-function relationships, for the first time, Pandey et al. (2014) built the tertiary structure of the DREB2 protein from wheat by homology modeling based on the crystal structure of the GCC-box binding domain of *Arabidopsis thaliana*.

Protein docking with the DNA containing GCC-box revealed more similarities in the AP2/EREBP protein between *A. thailana* and *T. aestivum*. It was found that proteins interact through their β-sheet, with the major DNA groove by hydrogen and hydrophobic bond providing structural stability to the molecule (Fig. 6).

The second 300 bp DNA fragments were compared with all nucleotide sequences of plant origin collected in GenBank. This fragment shows 99% identity with certain areas on the 3B chromosome of the bread wheat (*T. aestivum* L.) genotype, 'Chinese Spring' ('CS'). Besides, it has a 46% similarity with the *gag* polyprotein of retroviruses.

To date, many studies for understanding the mechanisms and functions of DREB transcription factors have been conducted. The first DREB transcription factor, CBF1 was isolated from Arabidopsis (Liu et al., 1998). Since then, several homologs of DREB1 and DREB2 have been identified in different plants, such as barley (Choi et al., 2002, Skinner et al., 2005), canola (Jaglo-Ottosen et al., 2001), Bell pepper (Hong and Kim, 2005), soybean (Li et al., 2005), tobacco (Park et al., 2001), tomato (Jaglo-Ottosen et al., 2001) and wheat. The DREB1 gene, primarily isolated from *T. aestivum* (Shen et al., 2003), was strongly induced by drought, salinity, and low temperature. DREB2 was isolated from wheat seedlings and its expression was activated by cold, drought, salt, and exogenous ABA treatment (Egawa et al., 2006). 500 bp TaDREB DNA sequences were detected in the Iranian wheat genotypes (Andeani et al., 2009). A new DREB family member classified as TaDREB3 transcription factor was isolated by Morran et al.

(2011), who further developed some transgenic populations of wheat and barley over-expressing both TaDREB2 and TaDREB3 factors. The elevated expression in the transgenic of other CBF/DREB genes and a large number of stress-responsive LEA/COR/DHN genes, which are responsible for the protection of cells from damage and desiccation under stresses, is due to the increased expression of TaDREB2 and TaDREB3. Two isoforms of WDREB2 were isolated and molecularly characterized in wheat and WDREB2 was shown to have 3 alternative splice forms or isoforms. β isoform that lacks a transcription activation domain is inactive while α is an active isoform (Sazegari and Niazi, 2012). SNPs in the EREBP/AP2 domain of DREB1, DREB2, DREB3, DREB4, and DREB5 genes were identified and characterized in some durum wheat (*T. turgidum L. var durum*) cultivars with contrasting salt and drought tolerance (Mondini et al., 2015). The DREB gene was strongly expressed in roots followed by stem, leaf, and inflorescence (Khan et al., 2017). Interestingly, both dehydration-tolerant and dehydration-sensitive wheat varieties explored contain the DREB gene in their genome. It is supposed that DREB gene expression under normal conditions may be related to other functions in a cell (Latini et al., 2008; Khan. 2011). Identification of wheat DREB genes was performed by Niu et al. (2020) at the genome level. Functions of TaDREB genes were characterized and in total, 210 TaDREB genes, which can be divided into 6 subgroups were detected. Among them, the expression of three TaDREB3 homoeologous genes was induced by abiotic stresses. Using sequence-based phylogenetic analyses, Hassan et al. (2021) identified 32 new DREB subfamily members, not belonging to any known sub-group.

CONCLUSION

DREB transcription factor is one of the most promising candidate genes, involved in plant tolerance to multiple abiotic stresses. From this point of view, molecular and computational characterization of the DREB gene from the different wheat genotypes is important for developing new tolerant ones. We have isolated and sequenced the partial DREB1 gene from Azerbaijan's local wheat genotypes. To identify conserved regions, deduced amino-acid sequences of partial DREB1 genes and some AP2-containing proteins deposited in the GenBank, were multi-aligned. These proteins demonstrated a high level of amino acid identity, especially in the conserved regions. The gene was shown to contain a highly conserved AP2 domain, a nuclear localization signal, and a conserved Ser/Thr-rich region. The studies of transcription factors will provide important bases for plant molecular breeding. Since tolerance to abiotic stress is polygenic in nature, the transfer of any single-acting gene is likely to be insufficient to induce the desired level of tolerance. Transcription factors regulate regulon expression as a single whole gene and, therefore, can be used to simultaneously activate several downstream genes induced by stress.

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CONFLICT OF INTEREST

There is no conflict of interest in the present study.

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