

In Silico Characterization of DREB and AP2/ERF Domain-Containing Transcription Factors in the important Crop Plants and Their Role in Drought Tolerance in Plants

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Background, the context and purpose of the study: Climate change is having a significant impact on crop plants around the world. As temperatures rise, some crops are becoming more difficult to grow in certain regions and are being replaced by other crops that are more tolerant of heat and drought. Plants have evolved various mechanisms, including physiological adaptations, structural modifications, and biochemical responses to withstand a wide range of environmental stresses, including drought, heat, salinity, and cold. Complex networks of genes play crucial roles in eliciting stress responses in plants. These genes encode a diverse array of proteins that function in various aspects of stress adaptation, from regulating hormone signalling to protecting cellular components from damage.

Transcription factors act as master regulators of gene expression, controlling which genes are turned on or off in response to specific stimuli. Dehydration responsive element binding protein (DREB) and AP2/ERF domain-containing protein are transcription factors in plants that regulate the expression of stress-responsive genes involved in drought tolerance. The purpose of the present study is in silico characterization of DREB and AP2/ERF domain-containing transcription factors, identification of conserved motifs and phylogenetic analysis to understand the evolutionary relationships of these sequences in the important crop plants – *Sorghum bicolor* (Sorghum), *Zea mays* (Maize), *Oryza sativa* subsp. *indica* (Rice), *Hordeum vulgare* (Barley), and *Triticum aestivum* (Wheat).

Results, the main findings: Transcription factors- F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*) have a conserved AP2/ERF domain, all show their subcellular localization in the nucleus and are involved in DNA binding and transcription regulation. There is variation in the position of AP2/ERF domain in the selected transcription factors.

Phylogenetic data indicate that the relative amounts of evolutionary change have occurred since the proteins shared a common ancestor. Data also suggests that these proteins have evolved at different rates, indicating varying degrees of divergence among the proteins.

The grand average of hydropathicity and pI values of DREB - AP2/ERF transcription factors F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*) and A0A1D6FEN0 (*Zea mays*) indicate that these are more stable and have better binding affinity to their target gene, thereby better transcription regulation.

Conclusions, brief summary and potential implications: There is a need to extensively study stress tolerance mechanisms in plants and to increase the adaptability of plants to various abiotic stresses like heat, drought and salinity.

Study reveal that DREB and AP2/ERF domain-containing transcription factors from *Sorghum bicolor* (Sorghum), *Zea mays* (Maize), *Oryza sativa* subsp. *indica* (Rice), *Hordeum vulgare* (Barley), and *Triticum aestivum* (Wheat) share a common ancestor. These proteins have evolved at different rates, indicating varying degrees of divergence among the proteins. Physico-chemical properties of DREB and AP2/ERF domain-containing transcription factors reveal that F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*) are the most stable and with better binding affinity to their target genes thereby better transcriptional regulation.

Overexpression of DREB and AP2/ERF transcription factors in transgenic plants can be a promising strategy for enhancing plant tolerance to abiotic stresses (particularly drought and salinity) and improving crop productivity under challenging environmental conditions.

Key words: Heat and Drought Stress, in silico, DREB (DEHYDRATION RESPONSIVE ELEMENT BINDING) and AP2/ERF (APETALA2, ETHYLENE RESPONSIVE FACTOR), domain containing transcription factors, *Sorghum bicolor* (Sorghum), *Zea mays* (Maize), *Oryza sativa* subsp. *indica* (Rice), *Hordeum vulgare* (Barley), *Triticum aestivum* (Wheat)

Plants are constantly exposed to various environmental stresses, including heat and drought. To cope with these challenges, plants have evolved a complex network of genes that contribute to their ability to tolerate these stresses. Heat shock factors (HSFs), drought-responsive element binding proteins (DREBs), and NAC transcription factors regulate the expression of other genes, playing a crucial role in coordinating the plant's stress response. The specific genes involved in heat and drought stress tolerance vary depending on the plant species and the severity of the stress. However, the general principles of stress response are conserved across plant species.

There are more than 80 transcription factor families in plants, out of which only NAC, MYB, WRKY, bZIP, and ERF/DREB transcription families, with vital roles in abiotic and biotic stress responses have been intensively studied (Baillio *et al.*, 2019).

DREBs are a subfamily of the AP2/ERF transcription factor family (Feng *et al.*, 2020) which are characterised by the presence of a conserved DNA-binding domain, the AP2/ERF domain. AP2/ERF domain allows them to bind to specific DNA sequences called dehydration-responsive elements (DREs) in the promoters of target genes. DREB proteins are induced in response to dehydration stress and activate the expression of genes involved in drought tolerance, such as genes for osmotic protectants, stress-responsive proteins, and detoxification enzymes. AP2/ERF domain-containing proteins are a large and diverse family of transcription factors that are found in plants, animals, and fungi. They are characterised by the presence of the AP2/ERF domain, which allows them to bind to a wide variety of DNA sequences. AP2/ERF proteins are involved in a wide range of biological processes, including development, metabolism, and stress response.

Both DREBs and AP2/ERF domain-containing proteins play important roles in plant responses to abiotic stresses, including dehydration, salinity, cold, and heat stress. They activate the expression of genes involved in stress tolerance, such as genes for osmotic protectants, stress-responsive proteins, and

detoxification enzymes. These proteins help plants to withstand stress conditions and maintain their growth and productivity.

DREBs and AP2/ERF domain-containing proteins () are essential components of the plant stress response machinery. They play a critical role in helping plants to adapt to and survive abiotic stress conditions. Understanding the functions of these proteins is important for developing strategies to improve crop stress tolerance and ensure food security in a changing climate.

MATERIALS AND METHODS

The aim of the present study was to undertake *in silico* comparative study of DREB and AP2/ERF domain-containing transcription factors from selected species - *Oryza sativa* subsp. *indica* (Rice), *Triticum aestivum* (Wheat), *Zea mays* (Maize), *Sorghum bicolor* (Sorghum) and *Hordeum vulgare* (Barley). The objective was to identify conserved regulatory motifs and divergent regions to shed light on the evolutionary history and functional diversification of these sequences. Physico-chemical properties of these transcription factors were compared to assess their stability and binding efficiency with the target gene.

The UniProt database (UniProt Consortium, 2023) was used to select DREB and AP2/ERF domain-containing transcription factors (Table 1). Amino acid sequence of F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*) of DREB and AP2/ERF domain-containing transcription factors were subjected to multiple sequence alignment using clustal omega database (Sievers *et al.*, 2011). Percentage sequence similarity, conserved motifs, evolutionary distances between the sequences were used for phylogenetic analysis to understand the evolutionary relationships of the selected DREB and AP2/ERF domain-containing transcription factors (Madeira *et al.*, 2022; Pieper *et al.*, 2014). The ExPASy ProtParam tool was used to calculate various physico-chemical properties of DREB and AP2/ERF domain-containing transcription factors.

RESULTS

Multiple sequence alignment was carried out using clustal omega database for the selected DREB and AP2/ERF domain-containing transcription factors- F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*). The sequence alignment data is presented in table 2 and percent identity matrix in table 3.

In a phylogenetic tree (Table 4), branch lengths represent the estimated evolutionary distances between the sequences of selected DREB and AP2/ERF domain-containing transcription factors - F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*).

In the given phylogenetic tree, branch lengths range from 0.00186 to 0.12735. These values indicate the relative amount of evolutionary change has occurred since the proteins shared a common ancestor. Data also suggests that the proteins included in the tree have evolved at different rates, indicating varying degrees of divergence among the proteins.

In general, longer branch lengths suggest greater evolutionary divergence, while shorter branch lengths suggest less evolutionary divergence. The branch lengths of C5XNL0 and F8V180 of *Sorghum bicolor* are relatively short, with values of 0.00186 and 0.00578 and high sequence similarity of 99.24% supporting the close evolutionary relationship between the two sequences. This means that the two sequences share a very similar genetic makeup, indicating that they have diverged from each other relatively recently. This means that they share a relatively recent common ancestor and have undergone similar evolutionary changes since they diverged.

A2WL19 of *Oryza sativa* has the longest branch length (0.12735), indicating that it has diverged the most from the common ancestor. This means that it has undergone more evolutionary changes since it diverged from the common ancestor than the other proteins.

Q4U0C8 of *Triticum aestivum* and Q4ZGK0 of *Hordeum vulgare* have relatively short branch lengths of 0.03587 and 0.03607 and sequence similarity of 92.81%, respectively, suggesting that they are closely related.

A0A1D6FEN0 of *Zea mays* has an intermediate branch length (0.05897), indicating that it diverged from the common ancestor more recently than A2WL19 but earlier than C5XNL0, F8V1800, Q4U0C8, and Q4ZGK0. A0A1D6FEN0 of *Zea mays* shows 89.84% sequence similarity with C5XNL0 and 89.45% with F8V180 of *Sorghum bicolor* and share a common ancestor.

All the DREB and AP2/ERF domain-containing transcription factors - F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*) under study have a conserved AP2/ERF domain, all show their subcellular localization in the nucleus and are involved in DNA binding and transcription regulation. There is variation in id position of AP2/ERF domain in the selected transcription factors and branch lengths in the phylogenetic tree are also variable showing evolutionary divergence (Table 5.).

The AP2/ERF domain is the key domain in DREBs and AP2/ERF transcription factors that mediates their binding with target genes. This domain consists of approximately 60-70 amino acids and is characterised by a conserved structure that allows it to recognize and bind to specific DNA sequences called cis-acting elements (CAEs). The AP2/ERF domain contains two conserved subdomains, AP2 and ERF, which contribute to its DNA-binding ability.

The AP2 subdomain is responsible for sequence-specific recognition of CAEs, while the ERF subdomain is involved in stabilising the DNA-protein interaction.

When a DREB or AP2/ERF transcription factor binds to a target gene promoter, the AP2/ERF domain interacts with the major groove of the DNA helix, forming hydrogen bonds and stabilising the complex. The AP2/ERF domain is the essential structural element that enables DREBs and AP2/ERF transcription factors to bind with target genes and regulate their expression. This domain plays a crucial role in plant development

and stress response by mediating the interaction of transcription factors with specific DNA sequences and controlling gene activity.

The id position of AP2/ERF domain of F8V180 Dehydration responsive element binding protein 1001 and C5XNL0 AP2/ERF domain-containing protein of *Sorghum bicolor* are identical (82-139) with branch lengths of 0.00186 and 0.00578 in phylogenetic tree and high sequence similarity of 99.24%. The data supports the close evolutionary relationship between these two sequences.

Q4U0C8 of *Triticum aestivum* and Q4ZGK0 of *Hordeum vulgare* have similar id position of AP2/ERF domain at 76-133, relatively short branch lengths of 0.03587 and 0.03607 in phylogenetic tree and sequence similarity of 92.81%, respectively, suggesting that they are closely related.

A0A1D6FEN0 of *Zea mays* has the id position of AP2/ERF domain at 80-137 and has an intermediate branch length 0.05897 in phylogenetic tree indicating that it diverged from the common ancestor more recently than A2WL19.

A2WL19 Dehydration-responsive element-binding protein 2A of *Oryza sativa* subsp. *indica* has the id position of AP2/ERF domain at 75-132, longest branch length (0.12735) in a phylogenetic tree indicating that it has diverged the most from the common ancestor.

The ExPASy ProtParam tool was used to calculate various physico-chemical properties of DREB and AP2/ERF domain-containing transcription factors - F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*). These properties were compared to gain insights into the structure, function, and behaviour of proteins.

The hydropathicity of a protein plays an important role in its structure, stability and function. Hydrophobic amino acids tend to cluster together to form the interior of a protein, while hydrophilic amino acids tend to be located on the surface of a protein where they can interact with water. This arrangement of amino acids is important for the proper function of many proteins.

During evolution, the hydropathicity of proteins has changed in response to changes in the environment. Changes in hydropathicity can have a significant impact on the evolution of proteins.

The grand average of hydropathicity of F8V180 (*Sorghum bicolor*) and C5XNL0 (*Sorghum bicolor*) and A0A1D6FEN0 (*Zea mays*) is -0.626, -0.639 and -0.658. These have higher amounts of hydrophobic amino acids resulting in more hydrophobic interactions stabilizing the protein's folded structure. These protein transcription factors are less likely to be unfolded by water, as the hydrophobic amino acids in the protein's interior will tend to repel water molecules.

The grand average of hydropathicity of A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*) is -0.878, -0.983 and -0.835 are less stable.

The pI of a transcription factor can have a significant impact on its binding affinity to its target gene. This is because the DNA of the target gene is negatively charged at physiological pH. A transcription factor with a low pI (more positive) will have a stronger electrostatic attraction to the DNA of the target gene than a transcription factor with a high pI (more negative).

As a result, transcription factors F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*) and A2WL19 (*Oryza sativa* subsp. *indica*) with pI of 5.52, 5.52, 5.18 and 5.77 are expected to have stronger binding affinity to its target gene than the transcription factor Q4U0C8 (*Triticum aestivum*) with a pI of 7.65.

DISCUSSION

These transcription factors play critical roles in regulating gene expression in response to stress signals, and their overexpression can lead to activation of stress-responsive genes that promote adaptation and survival. The grand average of hydropathicity and pI values of F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*) and A0A1D6FEN0 (*Zea mays*) indicate that these DREB and AP2/ERF domain-containing transcription factors are more stable and have better binding affinity to their target gene, thereby better transcription regulation. The results of the *in silico*

study suggest that sorghum and maize are very resilient parts of the world. crops that are well-adapted to challenging environmental conditions and therefore ensuring food security in many

Table 1: Selected DREB and AP2/ERF domain-containing transcription factors

S. No	Entry	Entry Name	Protein Names	Gene Names	Organism	Length
1.	F8V180	F8V180_SORBI	Dehydration responsive element binding protein 1001	DREB1001	<i>Sorghum bicolor</i> (Sorghum)	262 AA
2.	C5XNL0	C5XNL0_SORBI	AP2/ERF domain-containing protein	SORBI_3003G058200	<i>Sorghum bicolor</i> (Sorghum)	262 AA
3.	A0A1D6FEN0	A0A1D6FEN0_MAIIZE	Dehydration responsive element binding protein 2A	ZEAMMB73_Zm0001d008665	<i>Zea mays</i> (Maize)	261 AA
4.	A2WL19	DRE2A_ORYSI	Dehydration responsive element binding protein 2A, Protein DREB2A	DREB2A, ERF40, Osl_000512	<i>Oryza sativa indica</i> (Rice)	274AA
5.	Q4ZGK0	Q4ZGK0_HORVV	AP2/EREBP like protein, Dehydration responsive element binding protein 1, Predicted protein	DREB1	<i>Hordeum vulgare</i> subsp. <i>vulgare</i> (Domesticated barley)	278AA
6.	Q4U0C8	Q4U0C8_WHEAT	Dehydration responsive element binding protein W73	W73, CFC21_034241, CFC21_034242, CFC21_072571	<i>Triticum aestivum</i> (Wheat)	278AA

Table 2. CLUSTAL O(1.2.4) multiple sequence alignment of DREB and AP2/ERF domain-containing transcription factors - F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*).

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tr|A0A1D6FEN0|A0A1D6FEN0_MAIIZE      MEELGD--AGQGPQGD-ASGALVRKKRMRKSTGPDZIAETIRWVKEQNQLQDESGSRK      57
tr|F8V180|F8V180_SORBI                -MELGDATAGQGAQGDAAASGALVRKKRMRKSTGPDZIAETIKWVKEQNQLQDESGSRK      59
tr|C5XNL0|C5XNL0_SORBI                -MELGDATAGQGAQGDAAASGALVRKKRMRKSTGPDZIAETIKWVKEQNQLQDESGSRK      59
sp|A2WL19|DRE2A_ORYSI                 -ME-----RGEGRRGD--CSVQVRKKRTRRKSDGPDZIAETIKWVKEQNQLQDESGSRK      52
tr|Q4ZGK0|Q4ZGK0_HORVV                MET-----GGSKREGD--CPGQERTKKVRRRTTGPDSVAETIKWVKEQNQLQDESGSRK      53
tr|Q4U0C8|Q4U0C8_WHEAT                MET-----GGSKREGD--CPGQERKKVRRRSTGPDZIAETIKWVKEQNQLQDESGSRK      53
                                         *  .**  .      *:* **:: *****: *****:*****
tr|A0A1D6FEN0|A0A1D6FEN0_MAIIZE      APAKGSKKGCMMGKGGPENVCVYRGRVQRTWGWVVAEIREPNRGRRLWLSFPPTAVEAA      117
tr|F8V180|F8V180_SORBI                APAKGSKKGCMTGKGGPENVCVYRGRVQRTWGWVVAEIREPNRGRRLWLSFPPTAVEAA      119
tr|C5XNL0|C5XNL0_SORBI                APAKGSKKGCMTGKGGPENVCVYRGRVQRTWGWVVAEIREPNRGRRLWLSFPPTAVEAA      119
sp|A2WL19|DRE2A_ORYSI                 APAKGSKKGCMAKGGPENSNCAIRGRVQRTWGWVVAEIREPNRGRRLWLSFPPTAVEAA      112
tr|Q4ZGK0|Q4ZGK0_HORVV                APAKGSKKGCMAKGGPENSNCAIRGRVQRTWGWVVAEIREPNRGRRLWLSFPPTAVEAA      113
tr|Q4U0C8|Q4U0C8_WHEAT                APAKGSKKGCMAKGGPENSNCAIRGRVQRTWGWVVAEIREPNRGRRLWLSFPPTAVEAA      113
                                         ***** ** ***** ** ***** ** ***** ** ***** **
tr|A0A1D6FEN0|A0A1D6FEN0_MAIIZE      HAYDEAAKAMYGPKARVNFSESSADANSNGCTSALLASSVPAAALQQRSDKQVETEVE--      176
tr|F8V180|F8V180_SORBI                HAYDEAAKAMYGPKARVNFSDNSADANSNGCTSALLASSVPVATLQ-RSDEKQVETEVE--      177
tr|C5XNL0|C5XNL0_SORBI                HAYDEAAKAMYGPKARVNFSDNSADANSNGCTSALLASSVPVATLQ-RSDEKQVETEVE--      177
sp|A2WL19|DRE2A_ORYSI                 HAYDEAARAMYGPTARVNFADNSTDANSNGCTSAPSLMMSNGPAT--I-PSDEKDELESPP      169
tr|Q4ZGK0|Q4ZGK0_HORVV                RAYDDAARAMYGATARVNFPEHS PDANSNGCTMAPSLLTSNGATAVSH-PSDGKDESESP      172
tr|Q4U0C8|Q4U0C8_WHEAT                RAYDDAARAMYGAKARVNFSEQSPDANSNGCTLAPLPLTSNGATAASH-PSDGKDESESP      172
                                         :***:***:***:***:***:***:***:***:***:***:***:***:***:***:***:***
tr|A0A1D6FEN0|A0A1D6FEN0_MAIIZE      -----SVETEEVVEVKTEAKNGDLGSVKVTCVI---QSAEETVLH      214
tr|F8V180|F8V180_SORBI                -----SVETE--VHEVKTE--GNDDLGSVHVACTVDVIQSEKSVLH      215
tr|C5XNL0|C5XNL0_SORBI                -----SVETE--VHEVKTE--GNDDLGSVHVACTVDVIQSEKSVLH      215
sp|A2WL19|DRE2A_ORYSI                 FIVANGPAVL--YQPKDKDLERVVPEVDVKTE--GSNGLKRVQERKTEVCESEGLVLH      227
tr|Q4ZGK0|Q4ZGK0_HORVV                SLVSNAPTAALHRSDAKDEFESAGTVAHVKVTE--VSNDLGSTHEEHKALEVFQPKGKALH      231
tr|Q4U0C8|Q4U0C8_WHEAT                SLTSNGPTAALRRSDAKDESEAGTVARKVKKE--VSNDLRSTHEEHKLEVSQPKGKALH      231
                                         *  .:***.  .**  .      *  .:***.  .**  .
tr|A0A1D6FEN0|A0A1D6FEN0_MAIIZE      KEGDVSYDYFNVEDVVEMIIVELNADKKFEAHEEYLDGDDGFSLFAY      261
tr|F8V180|F8V180_SORBI                KVGVEVSYDYFNVEEVVEMIIIELNADKKIEAHEEYHDGDDGFSLFAY      262
tr|C5XNL0|C5XNL0_SORBI                KAGEVSYDYFNVEEVVEMIIIELNADKKIEAHEEYHDGDDGFSLFAY      262
sp|A2WL19|DRE2A_ORYSI                 KEVNI SYDYFNVVEVEMIIIVELSDQKTEVHEEYQEGDDGFSLSFY      274
tr|Q4ZGK0|Q4ZGK0_HORVV                KEANVSYDYFNVEEVDMIIIVELSDVVKMEAEHEEYQEGDDGFSLSFY      278
tr|Q4U0C8|Q4U0C8_WHEAT                KEANVSYDYFNVEEVLDMIIIVELSDVVKMEAEHEEYQEGDDGFSLSFY      278
                                         *  :*****:***:***:***:***:***:***:***:***:***:***:***:***:***:***:***
    
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Table 3. Percent identity matrix of DREB and AP2/ERF domain-containing transcription factors - F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*).

<input type="checkbox"/> tr A0A1D6FEN0 A0A1D6FEN0_MAIZE	100.00%	89.45%	89.84%	69.84%	66.67%	67.06%
<input type="checkbox"/> tr F8V180 F8V180_SORBI	89.45%	100.00%	99.24%	72.33%	66.67%	67.06%
<input type="checkbox"/> tr C5XNL0 C5XNL0_SORBI	89.84%	99.24%	100.00%	72.73%	67.06%	67.45%
<input type="checkbox"/> sp A2WL19 DRE2A_ORYSI	69.84%	72.33%	72.73%	100.00%	70.44%	69.34%
<input type="checkbox"/> tr Q4ZGK0 Q4ZGK0_HORVV	66.67%	66.67%	67.06%	70.44%	100.00%	92.81%
<input type="checkbox"/> tr Q4U0C8 Q4U0C8_WHEAT	67.06%	67.06%	67.45%	69.34%	92.81%	100.00%

Table 4. Phylogenetic tree of DREB and AP2/ERF domain-containing transcription factors - F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*).

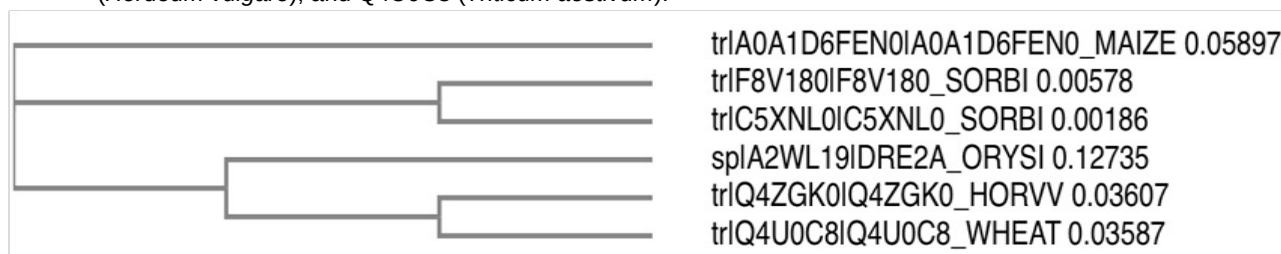


Table 5. Description and characteristics of selected DREB and AP2/ERF domain-containing transcription factors - F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*).

S. No.	Plant	Gene	DREB Transcription factor	Subcellular localization	Molecular function	Biological process	AP2/ERF domain (ID position)	Branch lengths in phylogenetic tree
1.	<i>Sorghum bicolor</i> -	DREB1001	F8V180 Dehydration responsive element binding protein 1001 (262AA)	Nucleus	DNA binding	Transcription regulation	82-139	0.00578
2.	<i>Sorghum bicolor</i> -	ORF names SORBI_3003G058200	C5XNL0 AP2/ERF domain-containing protein (262AA)	Nucleus	DNA binding	Transcription regulation	82-139	0.00186
3.	<i>Zea mays</i> -	ORF names ZEAMMB73_Zm00001d008665	A0A1D6FEN0 Dehydration-responsive element-binding protein 2A (261 AA)	Nucleus	DNA binding	Transcription regulation	80-137	0.05897
4.	<i>Oryza sativa</i> subsp. <i>indica</i> -	DREB2A	A2WL19 Dehydration-responsive element-binding protein 2A (274 AA)	Nucleus	Activator, DNA binding	Stress response, Transcription regulation	75-132	0.12735
5.	<i>Hordeum vulgare</i> -	DREB1	Q4ZGK0 AP2/EREBP-like protein (278 AA)	Nucleus	DNA binding	Transcription regulation	76-133	0.03607
6.	<i>Triticum aestivum</i> -	W73	Q4U0C8 Dehydration responsive element binding protein W73 (278 AA)	Nucleus	DNA binding	Transcription regulation	76-133	0.03587

Table 6. Physico-chemical properties of DREB and AP2/ERF domain-containing transcription factors - F8V180 (*Sorghum bicolor*), C5XNL0 (*Sorghum bicolor*), A0A1D6FEN0 (*Zea mays*), A2WL19 (*Oryza sativa* subsp. *indica*), Q4ZGK0 (*Hordeum vulgare*), and Q4U0C8 (*Triticum aestivum*).

<i>Sorghum bicolor</i>	<i>Sorghum bicolor</i>	<i>Zea mays</i>	<i>Hordeum vulgare</i>	<i>Triticum aestivum</i>	<i>Oryza sativa</i>
F8V180_SORBI	C5XNL0_SORBI	A0A1D6FEN0_MAIZE	Q4ZGK0_HORVV	Q4U0C8_WHEAT	DRE2A_ORYSI
262 AA	262 AA	261 AA	278 AA	278 AA	274 AA
Theoretical pI: 5.52	Theoretical pI: 5.52	Theoretical pI: 5.18	Theoretical pI: 6.11	Theoretical pI: 7.65	Theoretical pI: 5.77
The instability index 39.32	The instability index 39.72	The instability index 43.56	The instability index 42.95	The instability index 51.66	The instability index 43.37
This classifies the protein as stable.	This classifies the protein as stable.	This classifies the protein as unstable.	This classifies the protein as unstable.	This classifies the protein as unstable.	This classifies the protein as unstable.
Grand average of hydropathicity - 0.626	Grand average of hydropathicity - 0.639	Grand average of hydropathicity - 0.658	Grand average of hydropathicity - 0.878	Grand average of hydropathicity - 0.983	Grand average of hydropathicity - 0.835

Transgenic line of soybean with overexpression of AtDREB1A exhibited enhanced tolerance to drought stress, displaying improved stomatal conductance and water use efficiency, reduced stress-induced leaf senescence, and increased photosynthetic rates under water-deficit conditions (Polizel *et al.*, 2011). PgDREB2A transcription factor from *Pennisetum glaucum* was engineered for multiple stress tolerance in tobacco plants. The transgenic tobacco plants exhibited enhanced tolerance to both hyper-ionic and hyperosmotic stresses. Transgenic lines showed better germination percentage, better growth like leaf area, root number, root length and fresh weight compared to wild type for both the NaCl and mannitol stress (Agarwal *et al.*, 2010). Transgenic lines of indica cultivar of rice were developed by introducing cDNA encoding the transcription factors DREB1A and DREB1B under the control of the stress inducible rd29 promoter. Transgenic lines of Rice showed enhanced tolerance to dehydration as observed in the transgenic lines of rice transformed with DREB1A isolated from *Arabidopsis* while DREB1B was found to be more effective for salt tolerance (Datta *et al.*, 2012). These examples illustrate the potential of overexpressing DREB and AP2/ERF transcription factors in transgenic plants to enhance abiotic stress tolerance and improve crop productivity under challenging environmental conditions. Overexpression of DREB and AP2/ERF factors of sorghum in transgenic plants can emerge as a promising strategy for enhancing plant tolerance to abiotic stresses, particularly drought and salinity.

CONFLICTS OF INTEREST

The authors declare that they have no potential conflicts of interest.

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