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ORIGINAL ARTICLE

Comparative Analysis of Stress Tolerance in Millets

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Background: In the current scenario of a changing environment, climate resilient crops like Millets are promising. Various types of stress limit agricultural crop productivity and threaten food security. A comparative analysis of stress tolerance examines the ability of different millet varieties to withstand various environmental stresses like drought, cold, heat, flooding, rainfall, humidity, wind, light, radiation, elevated carbon-di-oxide, salinity, heavy metal, nutrient stress, etc. There are various morphological, physiological, biochemical and genetic characters (genes, transcription factors & proteins) present in millets contributing to their abiotic tolerance.
Results: Millet crops possess diverse adaptive mechanisms to combat abiotic stresses like elevated carbohydrate level, low Na+/K+ ratio, decreased relative water content (RWC), decreased chlorophyll levels, increased proline, betaine, increase in the phytohormones like ABA & Jasmonic acid. Millets follow the C4 type carbon fixation pathway. Key genes involved in climate resilience are DREB, LEA, HSP, SOD, ABF, CBF. Key proteins like catalases, peroxidases, aquaporins, Zinc finger, PR-proteins & transcription factors like MYB, NAM, NAC, WRKY, bHLH are involved in stress tolerance. The paper reports the study on evolutionary relationship of ABA responsive bZIP, WRKY, DREB and HSP transcription factors in millets *(Eleusine, Setaria, Panicum, Cenchrus, Sorghum*) and cereal grasses (Maize and Rice).
Conclusions: Millets (*Eleusine, Setaria, Panicum*) share strong evolutionary ties across all major stress related TF families. *Cenchrus* aligns with *Panicum/ Setaria*, but shows independent divergence in some TFs. Sorghum and Maize form a distinct cluster. Rice appears as a distant relative but useful as a reference but closely related to stress resilient millets.
Foxtail millet (*Setaria italica*) has excellent abiotic stress tolerance due to its efficient ROS scavenging mechanism. In Pearl millet (*Cenchrus americanus*/*Pennisetum glaucum*) drought responsive genes belong to 8 functional groups -ABA signaling, hormone signaling, ion and osmotic homeostasis, TF mediated regulation, molecular adaptation, signal transduction, physiological adaptations, detoxification. Finger millet (*Eleusine coracana*) is an important climate smart crop with nutraceutical potential grown for creating sustainability in feed and fodder.

Key words: ABA signaling, climate resilience, DREB, ROS scavenging, RWC, signal transduction

Millets are C4 respiratory resource & climate smart highly nutritious small grained cereal food, feed and fodder crops. They are eco-friendly requiring lower water, chemicals and human interventions, so a future crop for sustained agriculture and food security. They are on the basis of area grown and size of grain grouped into major and minor millets. Major millets include Sorghum (jowar) and Pearl millet (bajra). Minor millets include Finger millet (ragi), foxtail millet (kangani), little millet (kutki), kodo millet, barnyard millet (Sawan), proso millet (cheena) and brown top millet (korale) are minor millets. The millets contain 7-12% protein,2-5% fat ,65-75% carbohydrates and 15-20% dietary fibre. Finger millet is the richest source of calcium (300-350 mg/100g). Millets show antioxidant activity due to the presence of phytates, polyphenols, tannins, anthocyanins, phytosterols, and pinacosanols, thus preventing aging and metabolic diseases. Millets possess various morphological, physiological, biochemical and molecular mechanisms imparting them climate resilience. So, millets are suitable for the Earth's changing climate, for the cultivator and the consumer, enriching global biodiversity and our food grain basket. (Bhat, *et al.*, 2018.).

MATERIALS AND METHODS

For *in silico* comparative analysis of stress tolerance in millets firstly stress responsive proteins of *Oryza sativa* retrieved from the UNIPORT database (The UniProt Consortium, 2025) in FASTA format. Each of these retrieved proteins (query sequence) were blasted against various species of millets likewise *Eleusine coracana, Setaria italica, Sorghum bicolor, Paspalum scrobiculatum, Cenchrus americanus* and grasses like *Zea mays via* NCBI protein BLAST using non-redundant protein sequences with a threshold 0.05. BLAST (Basic Local Alignment Search Tool) helped to align & identify homologous sequences to query sequences. Ran the multiple sequence alignment (MSA) algorithm and saved the resulting file. MSA identified conserved regions, variation and phylogenetic relationships. A high percent identity with low E-value suggested a strong evolutionary relationship. COBALT (CONSTRAINT BASED MULTIPLE ALIGNMENT TOOL) was used for constructing phylogenetic trees. For analysing a phylogenetic tree using BLAST results, homologous sequences were aligned using the Neighbor Joining evolutionary model. Also corresponding literature was correlated.

RESULTS

Foxtail millet (*Setaria italica* L.) has long been used as a model to understand stress responses in plants. The physiological analysis demonstrated that drought stress reduced the RWC and chlorophyll contents of leaves, but increased the REL, while the lengths and surface areas of root systems increased. The comparative proteomic analysis confirmed that the proteins were primarily associated with photosynthesis and leaf development. In contrast, responsive proteins in roots were primarily associated with the metabolic regulation of secondary metabolites and root cell development. The regulatory signaling pathways between leaves and roots in response to drought primarily involved secondary metabolite pathways, hormone regulation, and cell wall synthesis. (Gao, *et al.*, 2023). The transcriptomic studies in Foxtail millet confirmed the upregulation of chloroplastic APX in response to salt stress and selenium supplementation. So, Se act as a mitigant at lower concentrations can alleviate NaCl stress in Foxtail millet. (Saleem, *et al.*, 2023)**.** Foxtail millet has a short growing cycle, lower amount of repetitive DNA, inbreeding nature, small diploid genome, and outstanding abiotic stress-tolerance characteristics. The qRT-PCR revealed the post-transcriptional modification has crucial roles in regulating gene expression. Stress stimulates a regulatory network of different metabolic pathways (Pan, *et al.*, 2018). *S. italica* and *S. viridis* reported stress-specific upregulation of SiαCaH1, SiβCaH5, SiPEPC2, SiPPDK2, SiMDH8, and SiNADP-ME5. Pearl millet (*Pennisetum glaucum* L.) is widely grown in arid and semi-arid regions, being tolerant to drought, due to certain molecular and genetic mechanisms operating within the crop. (Chakraborty, *et al.*, 2022). *Eleusine coracana* (L.) revealed the activation of various genes involved in response to stress, specifically heat, oxidation-reduction process, water deprivation, and changes in heat shock protein (HSP) and transcription factors, calcium signaling, and kinase signaling. The heat stress activates genes such as bZIP involved in basal regulatory processes. Thus, the candidate genes, such as HSPs, are involved in tolerance towards heat stress in ragi (Goyal, *et al.*, 2024).

Multiple sequence alignment of bZIP transcription factors is shown on Figure 1. BLAST result showing description of stress responsive bZip transcription factors. Red blocks signify strong sequence similarity and functional conservation of bZIP family among millets and grasses. The sequences are orthologous, evolved from a common ancestor and involved in ABA mediated drought or stress signalling.

Phylogenetic tree showing the evolutionary relationship of ABA responsive bZIP transcription factors is shown on Figure 2. bZIP protein (PR202_ga02861) of *Eleusine coracana* (finger millet) closely clusters with known ABA-responsive transcription factors such as OsbZIP23 (*Oryza sativa*), ZmABF2 (*Zea mays*), and ABRE-binding factor 2 (*Cynodon dactylon*). This strongly indicates that the *Eleusine* bZIP protein is likely involved in ABA-mediated drought or stress signaling. *Sorghum bicolor* contains bZIP proteins that fall into a separate clade alongside maize's bZIP46 indicating functional diversification. bZIP of *Setaria italica* shows more diversification.

Multiple sequence alignment shows that WRKY transcription factor proteins are involved in stress responses (Figure 3). WRKY sequences of *Oryza sativa* show highly conserved regions (red) among aligned sequences with *Setaria, Paspalum, Eleusine, Sorghum, Zea mays and Panicum.* Highly conserved regions of WRKY transcription factors show shared function across species and that they have evolved from a common ancestor with similar stress regulation pathways.

Phylogenetic tree (Figure 4). WRKY showing common ancestor, collinear relationships and evolutionary conservation. Proso millet (*Panicum miliaceum*), foxtail millet (*Setaria italica*) and Eleusine (*Eleusine coracana*) are placed in the same clade, suggesting a closer evolutionary relationship. Sorghum (*Sorghum bicolor*) is closer to maize and Rice (*Oryza sativa*) is a distant outgroup in the phylogenetic tree.

Multiple alignment: DREB transcription factor proteins shown on Figure 5. *Eleusine coracana, Setaria italica, Sorghum bicolor, Cenchrus americanus,* and *Zea mays* show very close alignment, indicating orthologous relationship. The evolutionary conservation in AP2 domain highlights the importance of DREB protein in drought stress response in millets, maize and other grasses.

Phylogenetic tree of DREB shown on Figure 6. DREB transcription factors play a very important role in abiotic stress responses. Phylogenetic tree of DREB protein of *Eleusine coracana* shows a high sequence similarity with Eragrostis, Maize and Sorghum. *Cenchrus* (Pearl millet), *Paspalum* and *Panicum* show moderate evolutionary relationship with the DREB transcription factor from *Eleusine coracana*. *Setaria* and *Brachypodium* show early divergence and fast evolutionary rate of its DREB protein.

Multiple sequence alignment of Heat Shock Proteins is shown on Figure 7. Heat shock domain is highly conserved in *Cenchrus, Maize, Brachypodium, Oryza sativa japonica group, Eragrostis, Eleusine, Sorghum* and *Setaria.* The high sequence identity supports its functional conservation in stress responses among monocots.

Phylogenetic tree showing the evolutionary relationship of HSP is shown on Figure 8. *Cenchrus americanus, Eleusine coracana, Setaria italica, Zea mays, Sorghum bicolor*, and others share a common evolutionary origin for Heat Shock Protein gene. The conservation of the heat shock protein across these species suggests it has an essential role in stress tolerance (heat, drought). The evolutionary closeness is especially strong between *Eleusine* and *Setaria.*

Table 1. Showing rainfall requirements & climate resilient traits in different millet crops. (Source: B Venkatesh Bhat, B Dayakar Rao & Vilas A. Tonapi. (2018) The Story of Millets, Karnataka state Department of Agriculture, Bengaluru, India with ICAR -IIMR, Hyderabad, India.)

MILLET CROP	**RAINFALL (Cms.)**	**TRAITS**
PEARL MILLET	80-95	Highly resilient to heat and drought, come up in very poor soils, but responsive to high input management
FINGER MILLET	90-130	Moderately resistant to heat, drought and humidity, adapted to wide altitude range
FOXTAIL MILLET	70-120	Adapted to low rainfall, high altitude
KODO MILLET	100-140	Long duration, but very hardy, needs little rainfall, comes up in very poor soils, good response to improved management
SORGHUM	100-125	Drought tolerant, excellent recovery mechanism from stresses, highly adapted to wide range of soils, altitudes and temperatures, responsive to high input management
BARNYARD MILLET	45-60	Very short duration, not limited by moisture, high altitude adapted
LITTLE MILLET	70-110	Adapted to low rainfall and poor soils- famine food; withstand waterlogging to some extent
FONIO	75-120	Shorter duration, Adapted to poorly fertile sandy and stony soils, low rainfall
TEFF	60-120	Short duration, drought and flood tolerant, high altitude adapted, fit in diverse cropping systems
BROWN TOP MILLET	60-80	Short duration, adapted to poor soils with less rainfall
PROSO MILLET	60-90	Short duration, low rainfall, high altitude adapted

Table 2. Showing varied stress tolerance in millets

MILLET CROP	**STRESS TOLE-RANCE**	**MORPHOLOGICAL/ PHYSIOLOGICAL/ BIOCHEMICAL RESPONSE**	**GENOMIC RESPONSE**	**TRANSCRIPTO-MIC RESPONSE**	**PROTEOMIC RESPONSE**	**REFERENCE**
Setaria italica. L (foxtail millet)	Drought	Root length increased, decrease in RWC, decrease in chlorophyll content, increase in REL.		MPK6, PAL, PER12, CA2, GAPA-2, ADF11	MAPK6, PAL1, PAL2, LOS4, s-APX, SIP2, BGL2, FUC1	Gao, *et al.*, 2023
	Drought	Arginine decarboxylase,spermidine synthase, polyamine oxidase, shikimate o-hydroxycinnamoyl transferase,caffeic acid 3-o-methyl transferase, cinnamoyl -co-A reductase, purple acid phosphatase, GPP,Callose synthase, tubulin α-1 chain, tubulin β-1.		LEA, ASR, nsTLP, Defensin -like protein, SOD, CYSTEINE PROTEINASE INHIBITOR 8-like, POD, PIP, PAP, SiRLK, SiRLK, SiCAT, SiAOS, PPDK, NADP, ME, QUINOR, GAPH, SiFBA, SiFBA, SiPOD, SiTubu	CAT, GST, Thioredoxin, MEG, AQUAPORIN, PIP2, HSP-70, EXPANSIN -B3-LIKE, WD40, PAD	Pan, *et al.*, 2018
	Drought	Lignin metabolism, metabolism of fatty acids				Cui, *et al.*, 2023

		(cutin & wax)				
	Salinity	ROS SCAVENGING		Upregulation of APX		Saleem, *et al.*, 2023
	Low potassium stress			SiMYB3, AP2, NAC,HOMEOBOX,bHLH,WRKY,Bzip,NF,PLATZ,MADS		Cao, *et al.*, 2019
Setaria italica. L. & *S. viridis.* L.	Low potassium stress			SiSnRK2.6 (ABA SIGNALING)		Ma, *et al.*, 2024
	Stress responsive			CaH,PEPC,PPDK,NADP-dependent MDH, NADP-ME		Muthamilorasan *et al.*, 2020
Pennisetum glaucum L. (pearl millet) 2n=14	DROUGHT	Root architecture, stay green property, leaf rolling, stomatal closure, decreased photosynthesis, water use efficiency, transpiration efficiency, accumulation of Osmoprotectants proline, betaine, reduced accumulation of ROS, APX, SOD, CAT, NADH, PROLINE, CAROTENOIDS, GSH, KAT2, HAB1, EDT1, RGS1, SRK2C, TPS1, ERD1, GolS2, P5CS1	AtBG1, GIG1, GIG2, PYL9, AAO3, CYP707A1, CYP707A3, NCED, PIP2, PIP1, OST2, ADAP, FAR1, HSPA1b, AREB1, SNAC1, MYB6			Chakraborty, *et al.*, 2022
Pennisetum glaucum L. (pearl millet) 2n=14	HEAT	upregulation of glycerophospholipid metabolism, down regulation of photosynthesis antenna proteins	HSPs			Sun, *et al.*, 2022
Pennisetum glaucum L. (pearl millet) 2n=14	salinity	C4 photosynthesis Enzymes, efficient enzymatic and non-enzymatic antioxidant system, lower Na+/K+ ratio				Jha, *et al.*, 2022
Pennisetum glaucum L. (pearl millet) 2n=14	Drought	REC increased, ROS scavenging SOD, CAT, APX	Upregulation of NADP-ME, Zinc finger,	bHLH,bZIP,MYB, NAC		Zhang, *et al.*, 2021

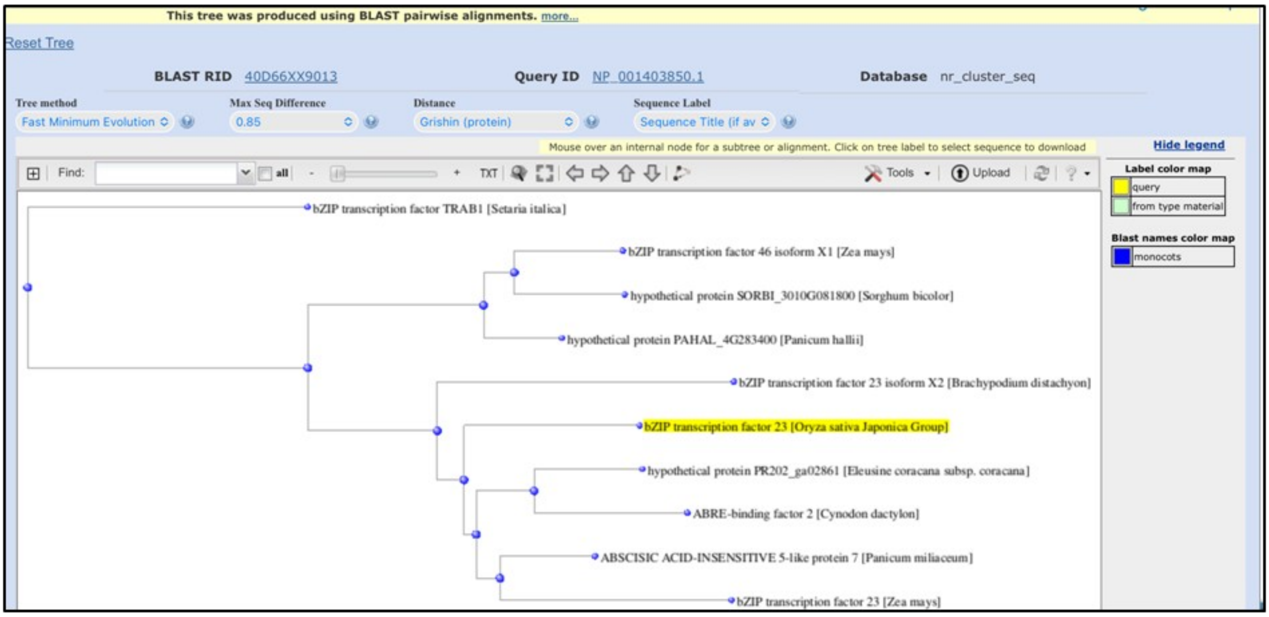
Eleusine coracana (finger millet)	Heat	Decreased electrolyte leakage, ROS Scavenging, SOD, CAT, GR, POD, APX, MDA	EcDREB2A			Singh, *et al.*, 2024
Panicum miliaceum L. (Proso millet)	Drought	POD, SOD, CAT, MDA, JA				Zhang, *et al.*, 2019

Sequence ID	Start	End	Organism
NP_001403850.1	1	357	Oryza sativa Japonica Gr...
GJM86956.1	1	352	Eleusine coracana subsp...
RLM78209.1	1	349	Panicum miliaceum
NP_001359135.1	1	351	Zea mays
UDP26504.1	1	341	Cynodon dactylon
XP_024317370.1	1	354	Brachypodium distachyon
XP_008659130.1	1	351	Zea mays
PAN25199.1	1	305	Panicum hallii
OQU76042.1	1	322	Sorghum bicolor
XP_004957010.1	1	355	Setaria italica

PROTEIN: 1 - 357 (357r shown) - anchor NP_001403850.1

Rows shown: 10/10

Figure 1: Multiple sequence alignment of bZip transcription factors.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BLAST RID 40D66XX9013

Query ID NP_001403850.1

Database nr_cluster_seq

Tree method: Fast Minimum Evolution

Max Seq Difference: 0.85

Distance: Grishin (protein)

Sequence Label: Sequence Title (if av

Mouse over an internal node for a subtree or alignment. Click on tree label to select sequence to download

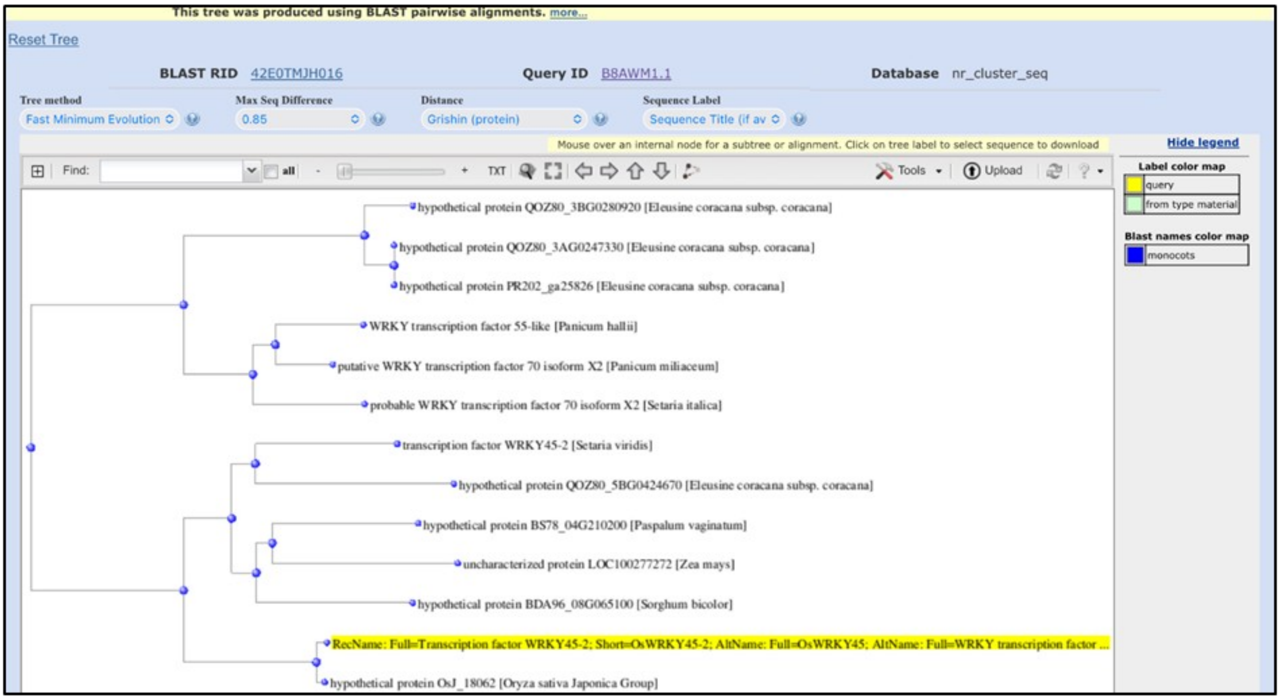
Figure 2: Phylogenetic tree showing the evolutionary relationship of ABA responsive bZIP transcription factors.

Sequence ID	Start	End	Organism
B8AWM1.1	1	322	Oryza sativa Indica Group
EEE63252.1	1	260	Oryza sativa Japonica Gr…
XP_034583912.1	1	292	Setaria viridis
KAJ1280158.1	2	306	Paspalum vaginatum
KAK3135881.1	3	294	Eleusine coracana subsp…
KAG0520330.1	2	319	Sorghum bicolor
NP_001144361.2	66	368	Zea mays
KAK3147321.1	10	218	Eleusine coracana subsp…
XP_025796867.1	44	240	Panicum hallii
KAK3151548.1	11	202	Eleusine coracana subsp…
XP_004984468.1	10	191	Setaria italica
RLN40061.1	6	203	Panicum miliaceum
GJN07949.1	11	202	Eleusine coracana subsp…

PROTEIN: 1 - 322 (322r shown) - anchor B8AWM1.1

Rows shown: 13/13

Figure 3: Multiple sequence alignment: WRKY transcription factor proteins are involved in stress responses.


This tree was produced using BLAST pairwise alignments. more...

Reset Tree

BLAST RID 42E0TMJH016 **Query ID** B8AWM1.1 **Database** nr_cluster_seq

Tree method: Fast Minimum Evolution
Max Seq Difference: 0.85
Distance: Grishin (protein)
Sequence Label: Sequence Title (if av

Mouse over an internal node for a subtree or alignment. Click on tree label to select sequence to download

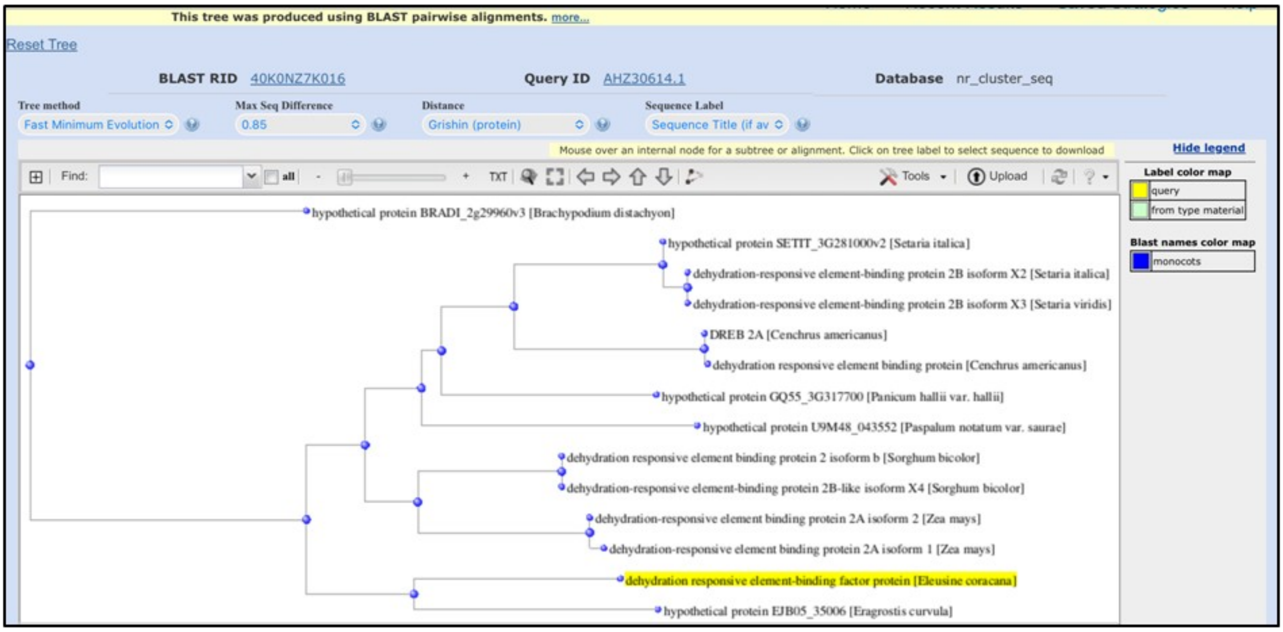
Figure 4: Phylogenetic tree of WRKY proteins.

Sequence ID	Start	End	Organism
AHZ30614.1	1	268	Eleusine coracana
TVU18888.1	130	399	Eragrostis curvula
RCV18201.1	1	262	Setaria italica
PUZ66520.1	1	261	Panicum hallii var. hallii
XP_004962409.1	78	332	Setaria italica
XP_034583800.1	126	380	Setaria viridis
AFI71293.1	1	261	Sorghum bicolor
XP_021302434.1	71	331	Sorghum bicolor
NP_001292874.1	1	244	Zea mays
AAV90624.1	72	332	Cenchrus americanus
WVZ98071.1	79	337	Paspalum notatum var. s...
NP_001292873.1	127	363	Zea mays
KQK06813.1	1	263	Brachypodium distachyon
AEY70512.1	30	231	Cenchrus americanus

PROTEIN: 1 - 268 (268r shown) - anchor AHZ30614.1

Rows shown: 14/14

Figure 5: Multiple alignment: DREB transcription factor proteins.


This tree was produced using BLAST pairwise alignments. more...

Reset Tree

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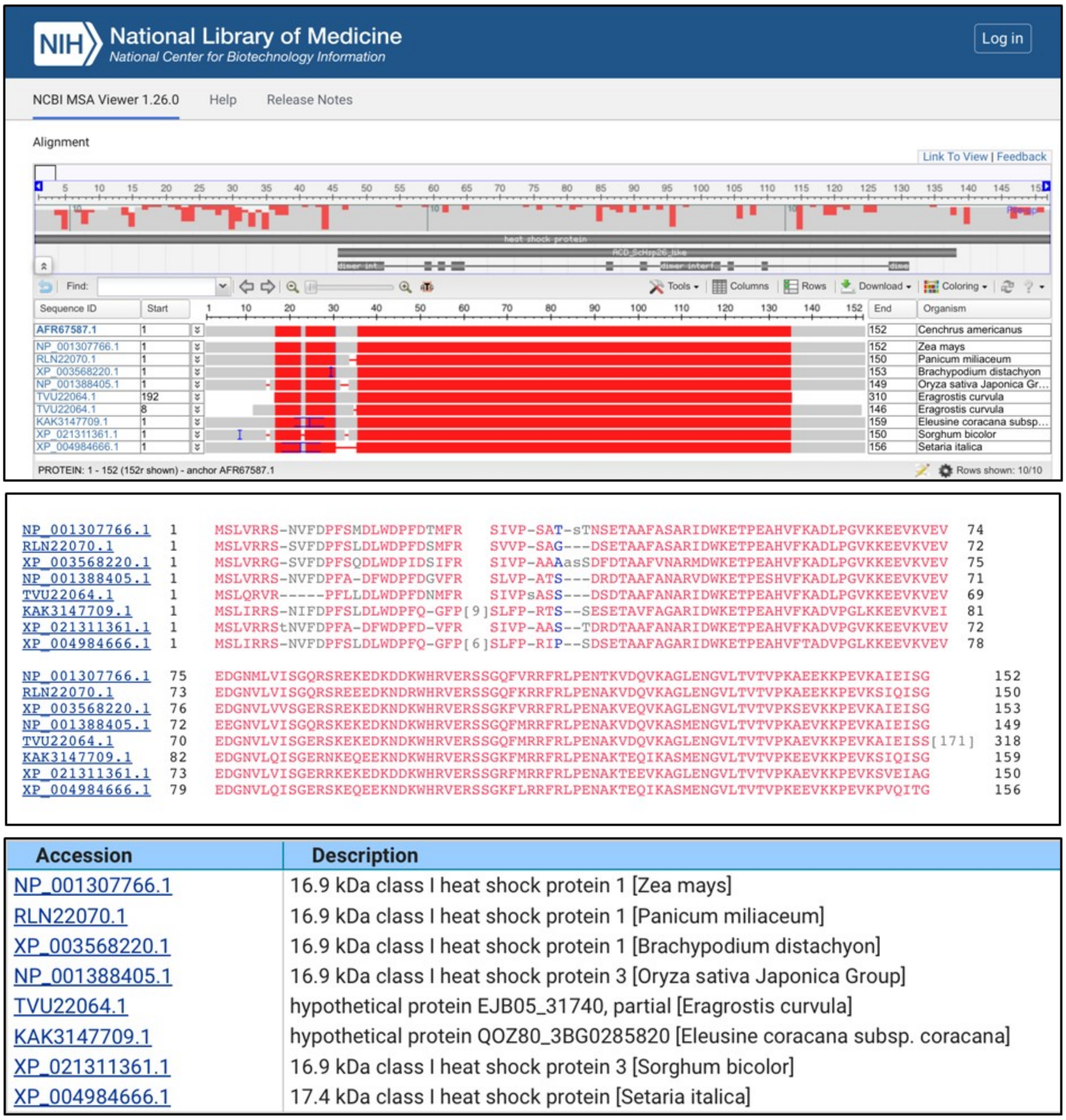
Tree method: Fast Minimum Evolution
Max Seq Difference: 0.85
Distance: Grishin (protein)
Sequence Label: Sequence Title (if av

Mouse over an internal node for a subtree or alignment. Click on tree label to select sequence to download

Figure 6: Phylogenetic tree DREB.



Sequence ID	Start	End	Organism
AFR67587.1	1	152	Cenchrus americanus
NP_001307766.1	1	152	Zea mays
RLN22070.1	1	150	Panicum miliaceum
XP_003568220.1	1	153	Brachypodium distachyon
NP_001388405.1	1	149	Oryza sativa Japonica Gr…
TVU22064.1	192	310	Eragrostis curvula
TVU22064.1	8	146	Eragrostis curvula
KAK3147709.1	1	159	Eleusine coracana subsp…
XP_021311361.1	1	150	Sorghum bicolor
XP_004984666.1	1	156	Setaria italica

PROTEIN: 1 - 152 (152r shown) - anchor AFR67587.1

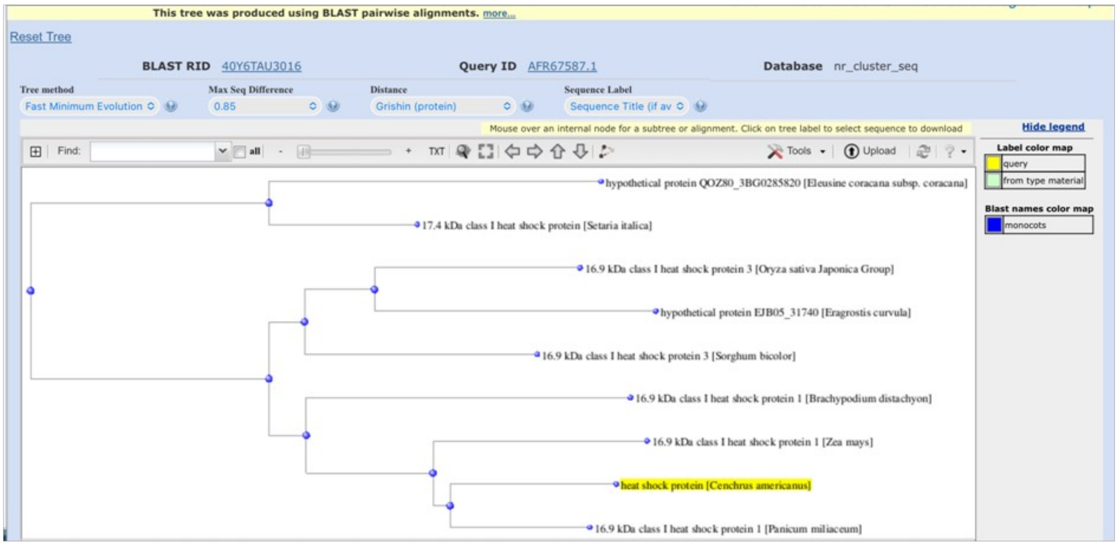
Rows shown: 10/10

```
NP_001307766.1  1     MSLVRRS-NVFDPFSMDLWDPFDTMFR    SIVP-SAT-sTNSETAAFASARIDWKETPEAHVFKADLPGVKKEEVKVEV  74
RLN22070.1      1     MSLVRRS-SVFDPFSLDLWDPFDSMFR    SVVP-SAG---DSETAAFASARIDWKETPEAHVFKADLPGVKKEEVKVEV  72
XP_003568220.1  1     MSLVRRG-SVFDPFSQDLWDPIDSIFR    SIVP-AAAasSDFDTAAFVNARMDWKETPEAHVFKADLPGVKKEEVKVEV  75
NP_001388405.1  1     MSLVRRS-NVFDPFA-DFWDPFDGVFR    SLVP-ATS---DRDTAAFANARVDWKETPESHVFKADLPGVKKEEVKVEV  71
TVU22064.1      1     MSLQRVR-----PFLLDLWDPFDNMFR    SIVPsASS---DSDTAAFANARIDWKETPEAHVFKADLPGVKKEEVKVEV  69
KAK3147709.1    1     MSLIRRS-NIFDPFSLDLWDPFQ-GFP[9]SLFP-RTS--SESETAVFAGARIDWKETPEAHVFKADVPGLKKEEVKVEI  81
XP_021311361.1  1     MSLVRRStNVFDPFA-DFWDPFD-VFR    SIVP-AAS--TDRDTAAFANARIDWKETPEAHVFKADVPGVKKEEVKVEV  72
XP_004984666.1  1     MSLIRRS-NVFDPFSLDLWDPFQ-GFP[6]SLFP-RIP--SDSETAAFAGARIDWKETPEAHVFTADVPGLKKEEVKVEV  78

NP_001307766.1  75    EDGNMLVISGQRSREKEDKDDKWHRVERSSGQFVRRFRLPENTKVDQVKAGLENGVLTVTVPKAEEKKPEVKAIEISG       152
RLN22070.1      73    EDGNVLVISGQRSREEEDKNDRWHRVERSSGQFKRRFRLPENAKVDQVKAGLENGVLTVTVPKAEEKKPEVKSIQISG       150
XP_003568220.1  76    EDGNVLVVSGERSREKEDKNDKWHRVERSSGKFVRRFRLPENAKVEQVKAGLENGVLTVTVPKSEVKKPEVKAIEISG       153
NP_001388405.1  72    EEGNVLVISGQRSKEKEDKNDKWHRVERSSGQFMRRFRLPENAKVDQVKASMENGVLTVTVPKAEVKKPEVKAIEISG       149
TVU22064.1      70    EDGNVLVISGERSKEKEDKNDKWHRVERSSGQFMRRFRLPENAKVDQVKAGLENGVLTVTVPKAEVKKPEVKAIEISS[171] 318
KAK3147709.1    82    EDGNVLQISGERNKEQEEKNDKWHRVERSSGKFMRRFRLPENAKTEQIKASMENGVLTVTVPKEEVKKPEVKSIQISG       159
XP_021311361.1  73    EDGNVLVISGERRKEKEDKDDKWHRVERSSGRFMRRFRLPENAKTEEVKAGLENGVLTVTVPKAEVKKPEVKSVEIAG       150
XP_004984666.1  79    EDGNVLQISGERSKEQEEKNDKWHRVERSSGKFLRRFRLPENAKTEQIKASMENGVLTVTVPKEEVKKPEVKPVQITG       156
```

Accession	Description
NP_001307766.1	16.9 kDa class I heat shock protein 1 [Zea mays]
RLN22070.1	16.9 kDa class I heat shock protein 1 [Panicum miliaceum]
XP_003568220.1	16.9 kDa class I heat shock protein 1 [Brachypodium distachyon]
NP_001388405.1	16.9 kDa class I heat shock protein 3 [Oryza sativa Japonica Group]
TVU22064.1	hypothetical protein EJB05_31740, partial [Eragrostis curvula]
KAK3147709.1	hypothetical protein QOZ80_3BG0285820 [Eleusine coracana subsp. coracana]
XP_021311361.1	16.9 kDa class I heat shock protein 3 [Sorghum bicolor]
XP_004984666.1	17.4 kDa class I heat shock protein [Setaria italica]

Figure 7: Multiple sequence alignment: Heat Shock Proteins.


This tree was produced using BLAST pairwise alignments. more...

Reset Tree

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Tree method: Fast Minimum Evolution

Max Seq Difference: 0.85

Distance: Grishin (protein)

Sequence Label: Sequence Title (if av

Mouse over an internal node for a subtree or alignment. Click on tree label to select sequence to download

Figure 8 Phylogenetic tree showing the evolutionary relationship of HSP.

DISCUSSION

The paper reports the study on evolutionary relationship of ABA responsive bZIP, WRKY, DREB and HSP transcription factors in millets (*Eleusine, Setaria, Panicum, Cenchrus, Sorghum*) and cereal grasses (Maize and Rice). ABA bZIP TFs are functionally conserved among millets, suggesting shared drought adaptation strategies. WRKY TFs in millets evolved under similar stress response selection pressures, while Sorghum and Maize show divergence. High sequence similarity across millets and cereal grasses show the conserved role of WRKY TFs in drought responses. DREB TFs evolved early in monocots but diversified functionally in stress prone millets and provided adaptive advantage in arid climates. High sequence similarity in HSP TFs shows evolutionary conservation and their role in heat stress resilience.

Millets (*Eleusine, Setaria, Panicum*) share strong evolutionary ties across all major stress related TF families. *Cenchrus* aligns with *Panicum/ Setaria*, but shows independent divergence in some TFs. Sorghum and Maize form a distinct cluster. Rice appears as a distant relative but useful as a reference but closely related to stress resilient millets.

Millet crops possess diverse adaptive mechanisms to combat abiotic stresses like elevated carbohydrate level, low Na+/K+ ratio, decreased relative water content (RWC), decreased chlorophyll levels, increased proline, betaine, increase in the phytohormones like ABA & Jasmonic acid. Millets follow the C4 type carbon fixation pathway. Key genes involved in climate resilience are DREB, LEA, HSP, SOD, ABF, CBF. Key proteins like catalases, peroxidases, aquaporins, Zinc finger, PR-proteins & transcription factors like MYB, NAM, NAC, WRKY, bHLH are involved in stress tolerance.

Stress responsive proteins in millets have future scope to be incorporated into contemporary crops for improving their abiotic stress tolerance.

CONCLUSIONS

Millets (*Eleusine, Setaria, Panicum*) share strong evolutionary ties across all major stress related TF families. *Cenchrus* aligns with *Panicum/ Setaria*, but shows independent divergence in some TFs. Sorghum and Maize form a distinct cluster. Rice appears as a distant relative but useful as a reference and is closely related to stress resilient millets.

Foxtail millet (*Setaria italica*) has excellent abiotic stress tolerance due to its efficient ROS scavenging mechanism. In Pearl millet (*Cenchrus americanus*/*Pennisetum glaucum*) drought responsive genes belong to 8 functional groups – ABA signaling, hormone signaling, ion and osmotic homeostasis, TF mediated regulation, molecular adaptation, signal transduction, physiological adaptations, detoxification. Finger millet (*Eleusine coracana*) is an important climate smart crop with nutraceutical potential grown for creating sustainability in feed and fodder.

CONFLICTS OF INTEREST

All authors declare that they have no conflicts of interest.

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