INTERNATIONAL JOURNAL OF APPLIED AND EXPERIMENTAL BIOLOGY
(ISSN PRINT: 2790-6523; ISSN ONLINE: 2790-6531)
2025, Vol. ???, NO.???, ???
doi: https://doi.org/10.56612/ijaaeb.v1i1.156
ARK: https://n2t.net/ark:/71142/IJAaEB.v1i1.156
https://eminentscientists.com/

Genomic analysis, evolution and characterization of the heat shock protein-70 gene family in foxtail millet [*Setaria italica* (L.) P.Beauv.] along with expression analysis under saline conditions

Jawad Ali[1], Ali Raza[2], Muhammad Aakif Khan[3], Aqib Zeb[4], Maliha Ghaffar[5], Maqsood Ahmed Khaskheli[6]

[1]Department of Biochemistry, University of Okara, Okara, Pakistan
[2]Anhui Province Key Laboratory of Microbial Pest Management, School of Plant Protection, Anhui Agricultural University, Hefei, 230036, Anhui, P.R. China
[3]Department of Plant Breeding and Genetics, Faculty of Agriculture, University of Agriculture, Faisalabad, Pakistan
[4]State Key Laboratory of Rice Biology, China National Centre for Rice Improvement, China National Rice Research Institute, Hangzhou 310006, P.R. China
[5]Department of Biology, Faculty of Life Sciences, University of Okara, Okara, Pakistan
[6]Department of Plant Pathology, College of Agriculture, Guizhou University, Guiyang, 550001, P.R. China

Abstract

Under stress conditions, heat shock proteins (HSPs) serve as fundamental regulators of cellular homeostasis. The molecular chaperone HSP70 belongs to a group of stress-related proteins which play important roles across growth and developmental events. The production of HSP70 proteins significantly increases during stress events including heat exposure, and salinity and drought conditions to protect proteins and maintain crucial macromolecular structures, thereby improving overall plant stress tolerance. This study identified 28 HSP70 genes in *Setaria italica* through phylogenetic analysis and placed these genes into four separate clades. The preserved structural elements and functional domains and conserved sequence patterns indicated that HSP70 genes maintain evolutionary stability. The HSP70 gene family spanned nine chromosomal scaffolds and most protein products were predicted to function in the cytoplasm. The analysis of synonymous substitutions and non-synonymous substitutions showed that HSP70 genes underwent both purifying and positive selection processes leading to their functional divergence. This study outcomes deliver a significant understanding of HSP70 genes' functional roles together with their association with plant growth and developmental processes.

ARTICLE TYPE
Research Paper (RP)

SECTION
Plant Biology (PB)

HANDLING EDITOR
İbadullayeva, S.J. (PB)

ARTICLE HISTORY
Received: 28 Jan, 2025
Accepted: 04 Mar, 2025
Online: 17 Mar, 2025
Published: ?????????

KEYWORDS
Gene structure;
Genome-wide analysis;
Heat shock proteins;
Hsp70 gene;
Stress tolerance

Introduction

Sudden external conditions represent a major obstacle to normal plant biological processes (He et al., 2022). Immobilized plants employ elaborate control systems to maintain stable physiology in

***CONTACT** Maliha Ghaffar, maliha.ghaffar@uo.edu.pk, Department of Biology, Faculty of Life Sciences, University of Okara, Okara, Pakistan

***CONTACT** Maqsood Ahmed Khaskheli, khaskheli.maqsood89@gmail.com, Department of Plant Pathology, College of Agriculture, Guizhou University, Guiyang, 550001, P.R. China

CITATION (APA): Ali, J., Raza, A., Khan, M.A., Zeb, A., Ghaffar, M., Khaskheli, M.A. (2025). Genomic analysis, evolution and characterization of the heat shock protein-70 gene family in foxtail millet [*Setaria italica* (L.) P.Beauv.] along with expression analysis under saline conditions. *International Journal of Applied and Experimental Biology* ??(??):??????.

changing environmental conditions. Plants experience four main abiotic stresses including salinity and drought along with temperature extremes and cold conditions according to Jiang et al. (2022). During plant growth and development, HSP70 facilitates essential protein folding processes while degrading defective proteins and transporting them between cellular compartments (Berka et al., 2022). Socket proteins function as chaperone molecules that shield thermal stress proteins from both denaturation and aggregation and maintain their stability (Nishad and Nandi, 2021). This stress-related molecular response activates HSP proteins when cells experience heat-stress circumstances (Jacob et al., 2017). Swindell et al. (2007) demonstrated that heat alongside cold and osmotic pressure as well as salinity increase HSP expression levels. Heat shock proteins are classified into five categories based on molecular weight: HSP70, HSP60, HSP90, HSP100, and small HSPS (Sarkar et al., 2009). Among chloroplast heat shock proteins, different families enable specific functions through distinctive operational frameworks (Queitsch et al., 2000).

Plants use the highly conserved HSP70 protein as a key element for developing heat stress tolerance (Usman et al., 2017). Research findings prove that HSP70 expression increases after abiotic stress exposure. Studies have shown that *Arabidopsis thaliana* alongside rice, tobacco, and maize plants exhibit elevated HSP70 expression in stress conditions (Sarkar et al., 2013; Jiang et al., 2021). Additionally, this phenomenon has been documented in *Arabidopsis thaliana,* rice, and tobacco. The interaction between HSP70-3 and phospholipase led to improved stress responses within *Arabidopsis thaliana* (Song et al., 2021). Transfer of herbaceous peony HSP70 genes into *Arabidopsis* plants resulted in improved thermotolerance (Zhao et al., 2019). Through its expression, Fungal HSP70 aids plants in tolerating salt stress as well as osmotic, oxidative and heat stress conditions (Montero-Barrientos et al., 2010). The expression of drought resistance and chemotherapeutic defense systems by NtHSP70-1 in *Nicotiana tabacum* plants has been observed (Cho and Choi, 2009). The proteins from the HSP70 family confer protection against both cold temperatures and dry conditions. Drought-resistant properties have been documented to develop in plants through the actions of PtHSP70 proteins (Yer et al., 2016), although GmHSP70 showed elevated expression levels under PEG treatment which led to improved drought tolerance (Zhang et al., 2015). Under cold stress conditions, potato plants demonstrated increased gene expression of multiple HSP70 proteins including StHSP70-1, StHSP70-10, StHSP70-17 and StHSP70-20 according to Liu et al. (2018).

Human consumption of foxtail millet (*Setaria italica*) started as an antiquated cereal grass thousands of years ago (Lu et al., 2009; Purugganan and Fuller, 2009). Farmers cultivate foxtail millet in India, China and the Philippines. The nutrient composition of this millet includes proteins and fiber as well as minerals and phytochemicals, despite its anti-nutritional factors phytic acid and tannin (Kalsi and Bhasin, 2023). The crop shows antioxidant functions while also behaving as an antihyperlipidemic agent (Sharma and Niranjan, 2018).

In the current study, the principal objective was to perform an all-encompassing examination of heat shock proteins throughout *Setaria Italica* (Foxtail millet) through a genome-wide evaluation. Twenty-eight HSP70 gene members were examined to study phylogenetic relationships while also analyzing gene information through architectural assessments which included motif analysis and evaluation of chromosomal distribution and protein subcellular localization as well as protein-protein interactions and *cis*-regulatory elements.

Material and Methods

HSP70 gene family sequence identification and retrieval

The *Arabidopsis thaliana* HSP70 protein sequence was used as a query sequence for BLAST against the *Setaria italica* database in the Phytozome database (https://phytozome-next.jgi.doe.gov/) to retrieve the *Setaria italica* genome sequences and annotation files, including protein and coding sequences. Relevant sequences were recovered using a hidden Markov model and BLAST, and their reliability was confirmed by ((https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) the Conserved Domain Database NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi), confirmed the domain of the retrieved sequences. Sequences of *Arabidopsis thaliana* and *Oryza sativa* were obtained from the TAIR database (https://www.arabidopsis.org/) and the Rice Genome Annotation Project (https://rice.uga.edu/).

Multiple sequence alignment and phylogenetic analysis

Streams of genetic data were arranged through MUSCLE software available in MEGA 11. The neighbor-joining method based phylogenetic analysis utilized bootstrap values set at 1000 for

reliability testing. Using MEGA 11, we constructed a phylogenetic tree after the analysis was complete. We assigned labels to *Setaria italica* sequences according to their specific chromosomal addresses.

Comparative phylogenetic analysis

Multiple sequence alignment was performed for the sequences of *Setaria italica, Oryza sativa,* and *Arabidopsis thaliana* using MUSCLE, with a bootstrap value of 1000, and a phylogenetic tree was created using the neighbor-joining method.

Domain architecture, gene structure and conserved motif analysis

Conserved motifs were discovered using Meme software (http://meme-suite.org/tools/meme), whereas gene structures were generated using the GFF files of the *Setaria italica* genome in TBtools. The NCBI Conserved Domain Database (Marchler-Bauer et al., 2017) was used to identify and place the domain in the protein sequence architecture (Wang et al., 2023) and TBtools was utilized for visualization.

Chromosomal mapping and duplication analysis

To determine where genes are located on the chromosomes and how many duplicate pairs of genes have developed as a result of environmental stressors, chromosomal mapping and Ka/Ks analysis of the genes were performed using TBtools.

Determination of physiochemical properties

Protein properties, such as transcript name, gene name, gene start and end positions, GRAVY, and strand, sense, or antisense information, were obtained from the Phytozome database. Parameters related to the proteins were calculated using TBtools using protein sequences.

Cis-regulatory elements/promoter analysis

For every relevant gene, we retrieved the 2k nucleotide sequence upstream from the Phytozome database. The acquired sequences were uploaded to the PlantCARE database for *cis*-regulatory analysis (Lescot et al., 2002). TBtools was used to visualize the results.

Protein sub-cellular localization analysis

WoLF PSORT (https://wolfpsort.hgc.jp/) was used to predict the subcellular localization of HSP70 proteins (Horton et al., 2007), whereas TBtools was used to visualize the subcellular localization of the HSP70 protein.

Protein-protein interactome, gene ontology, KEGG, and Reactome Pathway enrichment analysis

Protein-protein interaction analysis of HSP70 was performed using the String Database (https://string-db.org/). Gene ontology, KEGG, and Reactome Pathway analysis were performed using the String database. Gene ontology, including cellular component, molecular function, and cellular process, was analyzed using the String database.

Synteny analysis

Syntenic regions among the HSP70 proteins were visualized using the Circoletto webtool (https://bat.infspire.org/circoletto/), while MCscanX in TBtools was used to perform and construct the dual synteny among the *Setaria italica* and *Arabidopsis thaliana* genomes using their genome and GFF files.

Gene expression analysis

For the expression profiles of the SIHSP70 proteins, previously published transcriptome data (GSE278652) were used to evaluate the expression pattern after saline stress treatment during the seedling stage in the two varieties of foxtail millet. Eighteen samples were used for the collection of RNA-seq data from the salt-tolerant variety JK3 and the salt-sensitive variety B175 at three different time periods, with three repeats at each time point. Expression profiling was performed using high-throughput sequencing.

Results

Identification of the HSP70 gene family

The domain sequence of the HSP70 *Arabidopsis thaliana* protein sequence was used as a query sequence for BLAST against the *Setaria italica* genome database to retrieve the protein, nucleotide sequences, CDS, and protein properties of *Setaria italica* to identify the HSP70 gene family. The identities of the recovered sequences were confirmed using the Hidden Markov Model (Marchler-Bauer et al., 2017; Lu et al., 2020) and the results were displayed using TBtools.

Comparative phylogenetic analysis

We analyzed the evolutionary history of HSP70 genes by downloading protein sequences from the *Arabidopsis thaliana, Oryza Sativa*, and *Setaria italica* databases. Eighteen sequences of *Arabidopsis* proteins, 32 of *Oryza sativa*, and 28 of *Setaria italica* were obtained. The MUSCLE algorithm was used to align the retrieved sequences (**Figure 1**).

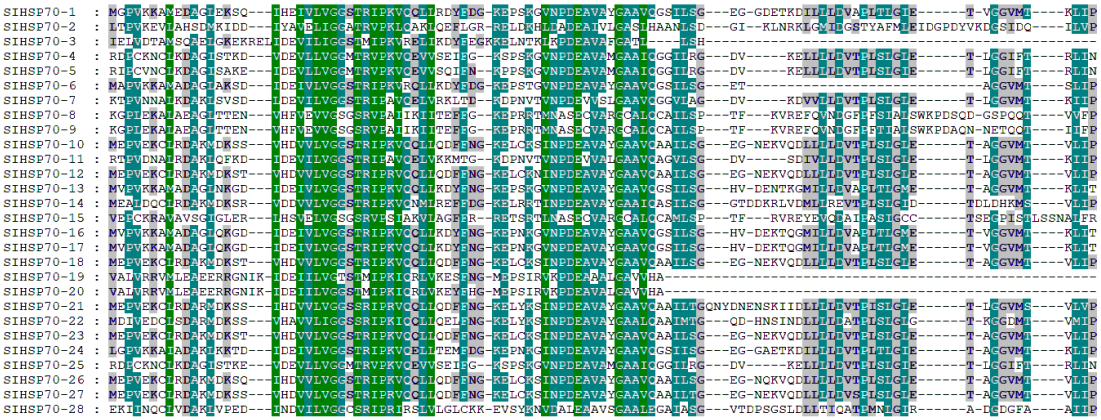

Figure 1: Multiple sequence alignment of the SIHSP70 proteins. Various shades represent the sequence identity among the multiple sequences.

The neighbor-joining method was used to construct the phylogenetic tree, with a bootstrap value of 1000 (Tamura et al., 2021). The *Setaria italica* genes were clustered with their homologs into different clades, showing that SIHSP70 genes are well conserved. All 78 genes were classified into four clades; Clades A, B, C, and D contained 28, 14, 14, and 22 genes, respectively. Clade A was the largest, and clades B and C were the smallest. Clade A is cytoplasmic/nuclear HSP70s, whereas clade B is (Bip) endoplasmic reticular HSP70. Clade C and D members are chloroplastic/mitochondrial and ER/cytoplasmic HSP70s, respectively (**Figure 2**).

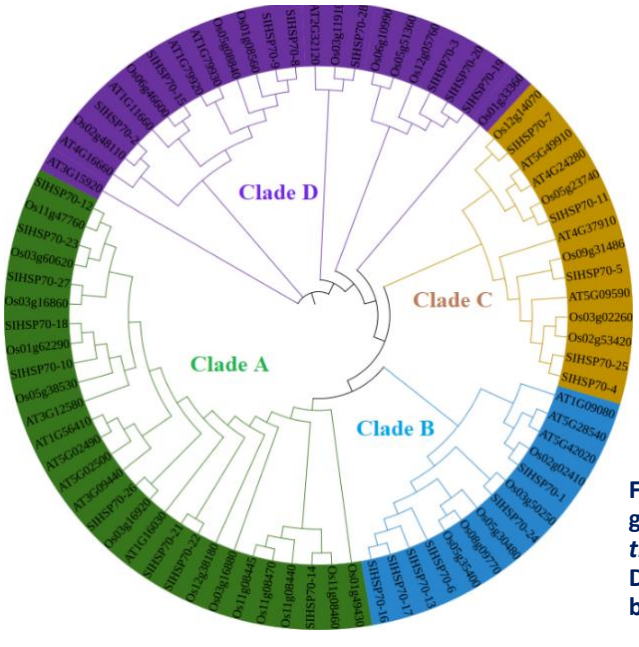

Figure 2: Comparative phylogenetic tree of HSP70 gene family members of *Setaria italica*, *Arabidopsis thaliana and Oryza sativa*. Four clades (A, B, C and D) are shaded with four different colors, green, blue, yellow, and purple, respectively.

Phylogeny, conserved motif and gene structure analysis of HSP70

Phylogenetic analysis revealed that SIHSP70 genes were classified into four clades. Clade A had the highest number of genes (9), while Clade C contained the fewest genes (5). Clades D and B contained 8 and 6 genes, respectively.

Conserved Motif Analysis can highlight the diversity and functionalities of gene family members. We utilized the Meme Suite wrapper to locate and visualize the motifs in the protein sequences. Proteins grouped in a similar clade had a similar composition and arrangement of motifs. Clade A members had eight motifs in common, with most members having 10 motifs, whereas Clade B had 10 conserved motifs in common, except SIHSP70-6. Clade C members shared 10 motifs, while clade D members shared seven conserved motifs. These findings confirm the conservation of protein composition, which lays the foundation for their function.

Gene exon and intron structures indicate the evolution of a gene family. The structure of the HSP70 gene family was studied using TBtools (Chen et al., 2023). The gene structure differed within the family. The range of exon count was 1–14. The majority of Clade A members had two CDS along with one intron, while Clade B members had diversity in the intron and exon structures, where three members were intronless with just one CDS. Clades C and D were highly diverse in terms of intron and exon structures, having multiple introns and exons, with an average of two untranslated regions. Gene structure conservation demonstrated that their functionality was preserved (**Figure 3**).

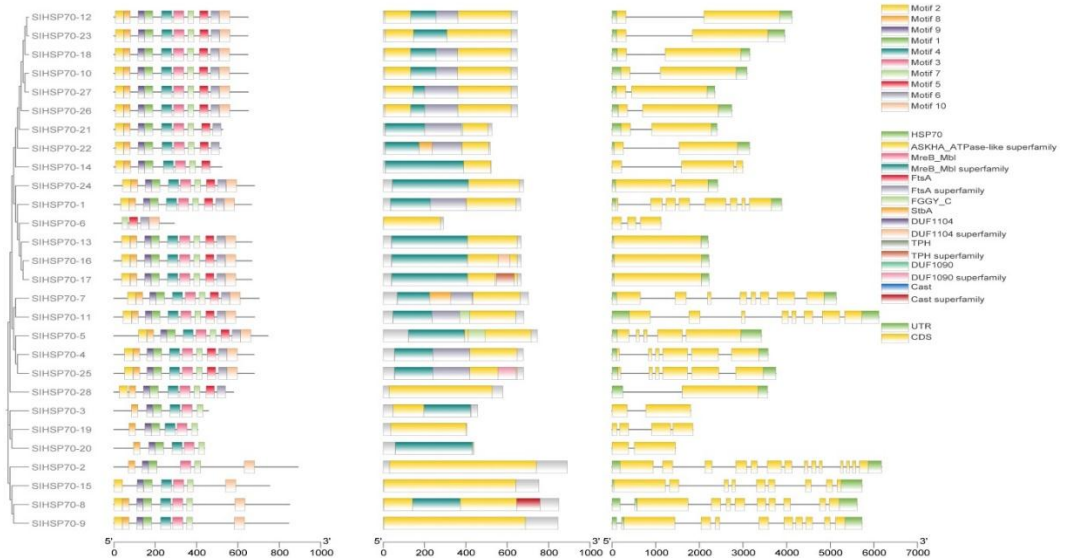

Figure 3: (Left to right) Phylogenetic diagram based upon Multiple Sequence Alignment, conserved motifs of SIHSP70 proteins, domain architecture, and exon and intron organization of SIHSP70s.

Chromosomal mapping, duplication and dual synteny analysis

Using chromosomal mapping, we identified the locations of various gene members on the chromosomes. It was found that different chromosomes had different numbers of genes distributed over them, and the length of the chromosomes had no correlation with the distribution of genes on the chromosomes. Twenty-eight (28) genes of the HSP70 family in *Setaria italica* were distributed on nine scaffolds. Scaffolds 1, 6, and 7 had the lowest number of genes, whereas Scaffolds 3 and 9 had the highest number of genes, 8 and 6, on a single chromosome. Scaffolds 2 and 5 shared a similar number of genes, whereas scaffolds 4 and 8 had similar numbers of genes as 3 and 2, respectively (**Figure 4**). We also investigated gene pairs that duplicated over time. Eighteen (18) gene pairs with duplications were found, i.e., SIHSP70-3-SIHSP70-6, SIHSP70-3-SIHSP70-14, SIHSP70-3-SIHSP70-17, SIHSP70-3-SIHSP70-19, SIHSP70-3-SIHSP70-20, SIHSP70-6-SIHSP70-14, SIHSP70-6-SIHSP70-17, SIHSP70-6-SIHSP70-18, SIHSP70-14-SIHSP70-15, SIHSP70-14-SIHSP70-17, SIHSP70-14-SIHSP70-19, SIHSP70-14-SIHSP70-20, SIHSP70-15-SIHSP70-17, SIHSP70-15-SIHSP70-19, SIHSP70-15-SIHSP70-20, SIHSP70-17-SIHSP70-19, SIHSP70-17-SIHSP70-20, and SIHSP70-19-SIHSP70-20 (**Figure 5**). Gene or gene region duplication was revealed using the Ka/Ks value (Hurst, 2002). Neutral and positive selections were confirmed by a Ka/Ks ratio of 1 or above, whereas purifying selection was confirmed by a Ka/Ks ratio of less than 1**.**

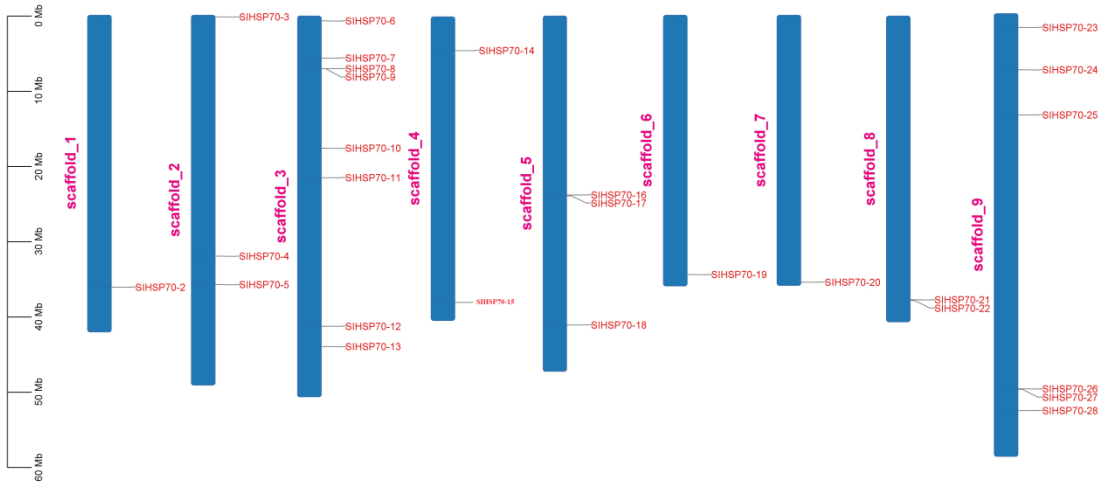

Figure 4: SIHSP70 gene family members exist within *Setaria italica* chromosome regions. Coded as bars display chromosome representations while showing their numbers on the left side. Each chromosome has SIHSP70 gene family members labelled to their right side. A scale on the left indicates the lengths of the chromosomes.

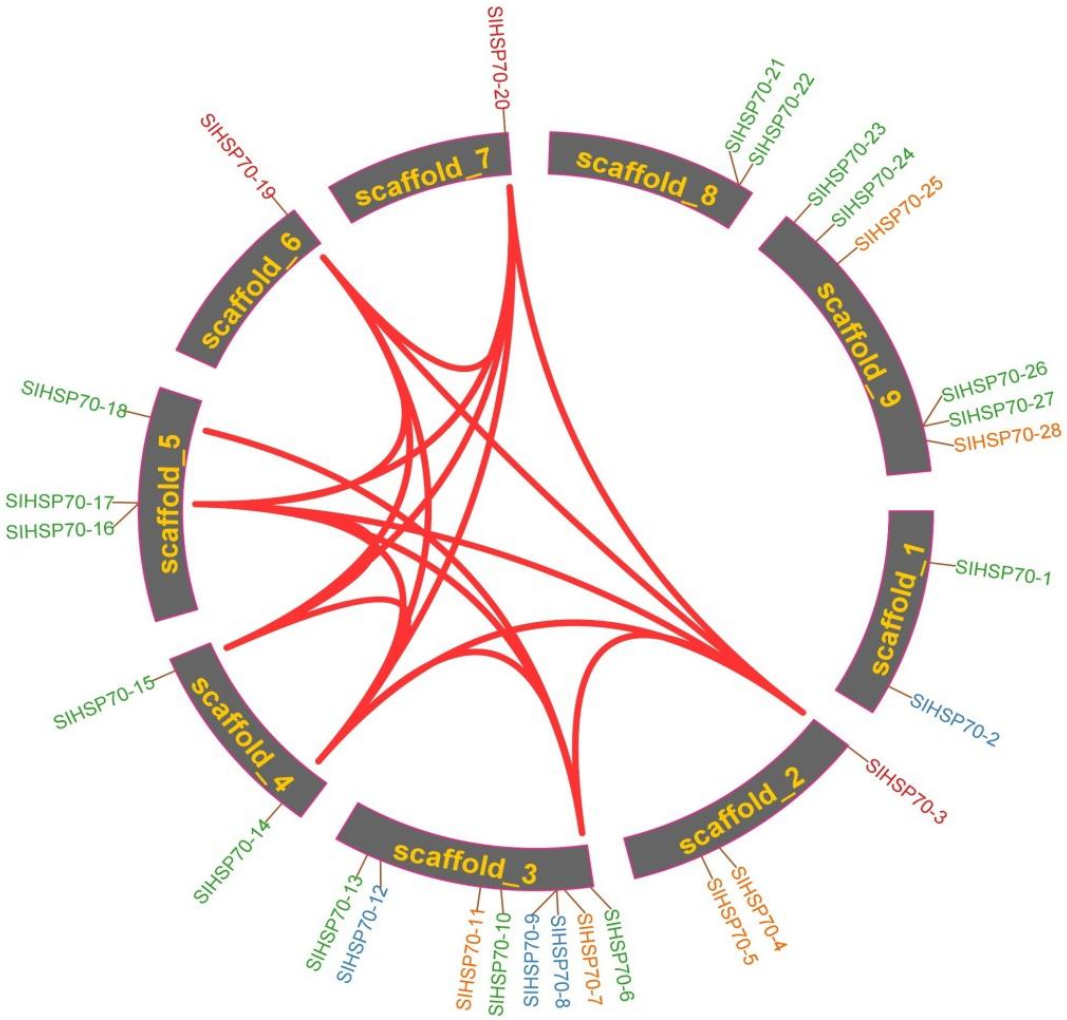

Figure 5: A CIRCOS diagram used to illustrate the distribution of SIHSP70 genes. Each chromosome is uniquely labelled, with the approximate positions of the SIHSP70 genes marked by short black lines along the circular layout. Red curves indicate gene duplication events among the SIHSP70 genes.

Synteny analysis was performed to identify the identity and similarity of the HSP70 proteins among *Setaria italica*, *Arabidopsis thaliana*, and *Oryza sativa*, while Dual Synteny analysis was performed to identify the co-linear regions among *Arabidopsis thaliana* and *Setaria italica* genomes (**Figures 6 and 7**).

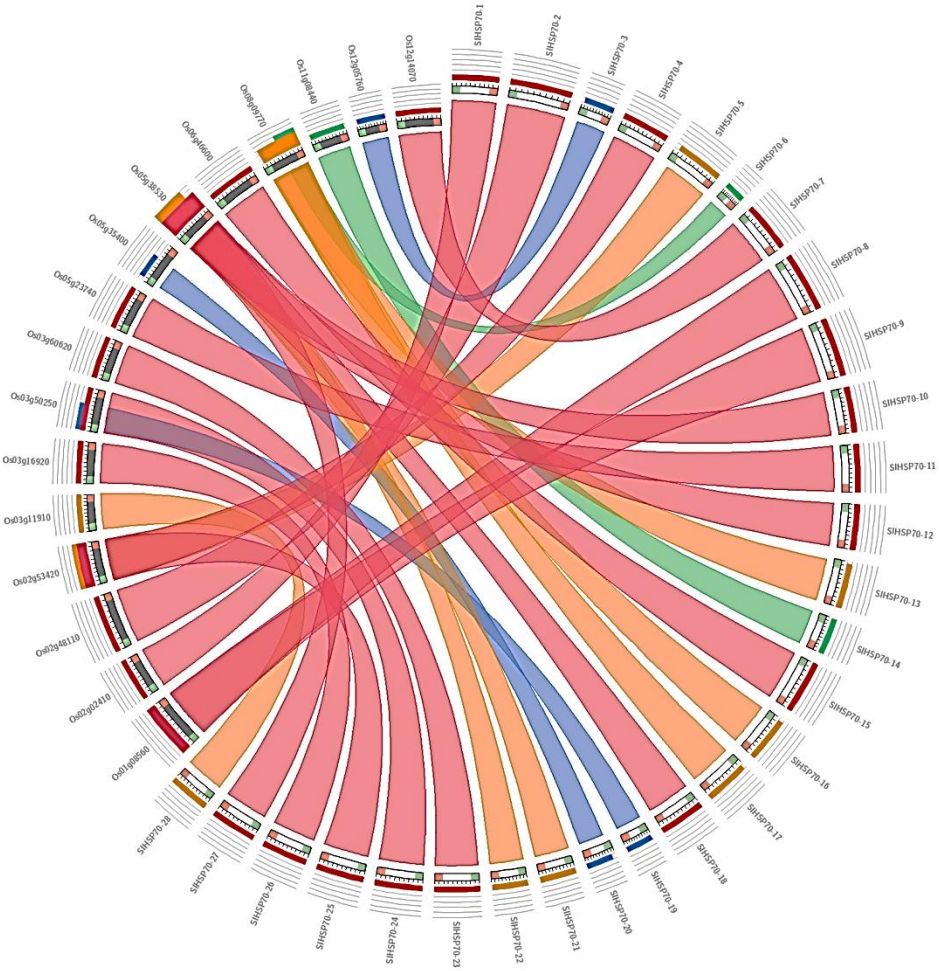

Figure 6: Synteny showing the identity and similarity among the selected HSP70 proteins of *Setaria italica* and *Oryza sativa* using Circoletto webtool. Blue represents the lowest with less than 25% of identity, while red represents the identity higher than 75%.

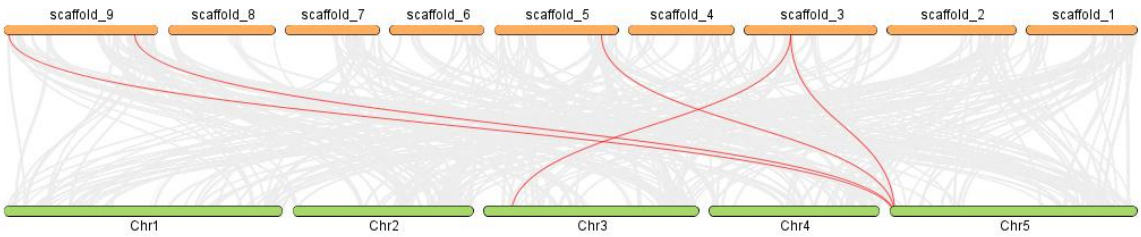

Figure 7: Dual Synteny among the whole genome of *Setaria italica* and *Oryza sativa* using TBtool. *Arabidopsis thaliana* chromosome bars are represented by the green bars, while brown chromosomal scaffolds represent the *Setaria italica*. Red lines between these bars represent the syntenic regions among the genome of *Arabidopsis thaliana* and *Setaria italica*.

Physiochemical properties

Physicochemical characteristics of the genes and their proteins were investigated. Genetic diversity affects the length of the coding sequences and proteins. SIHSP70 genes had exon lengths ranging from 105 to 2222 (bp), CDS lengths ranging from 0 to 200 (bp), and protein lengths from 292 to 890 amino acids. Proteins with an instability value of less than 40 were stable, whereas proteins with an instability index of more than 40 were unstable. Molecular weight ranged from 31.8 to 98.2 kDa, with a theoretical PI of 4.72-8.89. GRAVY ranged from (-0.645 to +0.013), indicating that the majority of the proteins were hydrophobic, with the exception of three proteins, i.e., SIHSP70-26, SIHSP70-28, and SIHSP70-28 were found to be hydrophilic (**Table 1**).

Table 1: Physiochemical information about SIHSP70 gene family of *Setaria italica*

Gene Name	Gene ID	Chr name	Strand	Gene start (bp)	Gene end (bp)	Gene length (bp)	AA length
SIHSP70-1	Seita.1G117600	scaffold_1	-1	10246407	10250295	3888	665
SIHSP70-2	Seita.1G293400	scaffold_1	1	36152184	36158357	6173	890
SIHSP70-3	Seita.2G003700	scaffold_2	-1	227872	229668	1796	457
SIHSP70-4	Seita.2G218100	scaffold_2	1	32024184	32027760	3576	677
SIHSP70-5	Seita.2G255900	scaffold_2	-1	35804612	35808034	3422	745
SIHSP70-6	Seita.3G012300	scaffold_3	-1	650939	652060	1121	292
SIHSP70-7	Seita.3G086700	scaffold_3	-1	5573789	5578929	5140	702
SIHSP70-8	Seita.3G106000	scaffold_3	-1	6988816	6994433	5617	850
SIHSP70-9	Seita.3G106100	scaffold_3	1	7000051	7005780	5729	845
SIHSP70-10	Seita.3G216900	scaffold_3	1	17574140	17577230	3090	649
SIHSP70-11	Seita.3G250300	scaffold_3	-1	21467152	21473268	6116	680
SIHSP70-12	Seita.3G327900	scaffold_3	-1	41234723	41238843	4120	649
SIHSP70-13	Seita.3G341900	scaffold_3	-1	43942947	43945147	2200	667
SIHSP70-14	Seita.4G060500	scaffold_4	-1	4474303	4477299	2996	522
SIHSP70-15	Seita.4G261100	scaffold_4	-1	37951109	37956838	5729	753
SIHSP70-16	Seita.5G187000	scaffold_5	1	23768449	23770667	2218	667
SIHSP70-17	Seita.5G187500	scaffold_5	1	23871870	23874092	2222	667
SIHSP70-18	Seita.5G376100	scaffold_5	1	41050578	41053735	3157	648
SIHSP70-19	Seita.6G232400	scaffold_6	-1	34467838	34469692	1854	405
SIHSP70-20	Seita.7G325600	scaffold_7	-1	35472298	35473748	1450	439
SIHSP70-21	Seita.8G225000	scaffold_8	1	37720473	37722880	2407	526
SIHSP70-22	Seita.8G225900	scaffold_8	1	37819233	37822386	3153	518
SIHSP70-23	Seita.9G033500	scaffold_9	-1	1843821	1847778	3957	648
SIHSP70-24	Seita.9G120300	scaffold_9	-1	7468166	7470588	2422	679
SIHSP70-25	Seita.9G191200	scaffold_9	1	13463319	13467072	3753	678
SIHSP70-26	Seita.9G451500	scaffold_9	1	49893762	49896508	2746	650
SIHSP70-27	Seita.9G451900	scaffold_9	-1	49940009	49942361	2352	649
SIHSP70-28	Seita.9G488400	scaffold_9	1	52764426	52767986	3560	578

Gene Name	Exons	Introns	Molecular weight (Da)	Clade	Theoretical pI	Instability Index	Aliphatic Index	GRAVY
SIHSP70-1	8	2	73331.99	A	5.05	27.78	86.21	-0.479
SIHSP70-2	14	2	98235.27	C	5.5	42.2	86	-0.422
SIHSP70-3	2	0	49934.9	D	5.88	30.52	92.23	-0.11
SIHSP70-4	6	2	72824.48	B	5.69	35.21	84.77	-0.336
SIHSP70-5	5	2	79756.22	B	8.89	38.98	84.77	-0.306
SIHSP70-6	3	0	31886.49	A	4.72	26.55	73.73	-0.645
SIHSP70-7	8	2	74370.18	B	5.12	30.14	86.14	-0.262
SIHSP70-8	9	3	93885.22	C	5.1	42.48	77	-0.487
SIHSP70-9	9	3	93220.5	C	4.98	38.6	79.35	-0.451
SIHSP70-10	2	2	71012.45	A	5.13	33.49	81.79	-0.409
SIHSP70-11	8	2	73029.41	B	5.11	31.24	85.75	-0.331
SIHSP70-12	2	2	71120.57	A	5.13	34.98	84.19	-0.398
SIHSP70-13	2	2	73448.8	A	5.08	26.06	83.9	-0.44
SIHSP70-14	3	0	57339.43	A	5.79	36.65	89.46	-0.22
SIHSP70-15	9	2	82902.48	C	5.46	46.27	85.55	-0.332
SIHSP70-16	2	2	73534.97	A	5.08	27.14	84.48	-0.444
SIHSP70-17	2	2	73446.82	A	5.08	27.12	83.75	-0.442
SIHSP70-18	2	2	70933.46	A	5.1	33.41	82.65	-0.395
SIHSP70-19	4	0	44073.79	D	7.16	34.34	94.91	0.013
SIHSP70-20	2	0	48070.37	D	5.82	31.05	94.44	0.013
SIHSP70-21	2	2	57971.38	A	7.01	39.3	91.18	-0.223
SIHSP70-22	2	2	57201.39	A	5.77	32.18	87.92	-0.214
SIHSP70-23	2	2	71284.87	A	5.06	35.09	83.84	-0.415
SIHSP70-24	2	2	74126.98	A	-0.405	5.34	29.76	84.33
SIHSP70-25	6	2	72642.31	B	-0.304	5.62	40.19	86.37
SIHSP70-26	2	2	71411.94	A	-0.442	5.23	34.2	81.8
SIHSP70-27	2	2	71122.47	A	-0.432	5.1	35.47	81.48
SIHSP70-28	1	2	62445.76	B	0.008	5.54	36.77	97.87

Cis-Elements Analysis of HSP70 Family

To investigate the significance of the HSP70 family in growth, development, and stress responses, we submitted a 2kb promoter sequence to the PLANTCARE Database, revealing the *cis*-elements (Rombauts et al. 1999) (**Figure 8**)**.** Along with the very basic *cis*-elements, such as TATA-box

and CAAT-box elements, many other *cis*-elements related to growth and development were present. Hormone responsive (GARE-Motif, AuxRe, ERE, TCA-Element, TGA-Element, ABRE) and stress responsive (MBS, ARE, LTR) genes were also present in the upstream promoter sequence from the start codon. As per current assumptions, SIHSP70 functions as an important factor in multiple growth-associated developmental processes of *Setaria italica* starting from light response through photosynthesis to seed germination and ending with leaf development and rhizome lengthening. Multiple *cis*-elements active under stress and hormone conditions indicate environmental factors control on HSP70 protein synthesis and activity.

Figure 8: Putative *cis*-elements of SIHSP70s

Protein Sub-cellular location

Subcellular localization studies provide information on the structural or functional placement of proteins inside the cell. The majority of SIHSP70 proteins were detected in the cytoplasm, with a few detected in the chloroplast, endoplasmic reticulum, mitochondria, and nucleus. Clade A members were present in the nucleus and cytoplasm, while Clade B members were located in the endoplasmic reticulum. Clade C members are chlorogenic and mitochondrial HSP70; thus, they exist in these cell compartments. Clade D members are similar to the HSP110 class of the HSP70 superfamily, which performs their functions in the cytoplasm and ER. This information indicates that most HSP70 proteins function in the cytoplasm, while other proteins function in different compartments based on their compositional and structural subclasses. Proteins function in the groups so they can work in various compartments at a time (**Figure 7**).

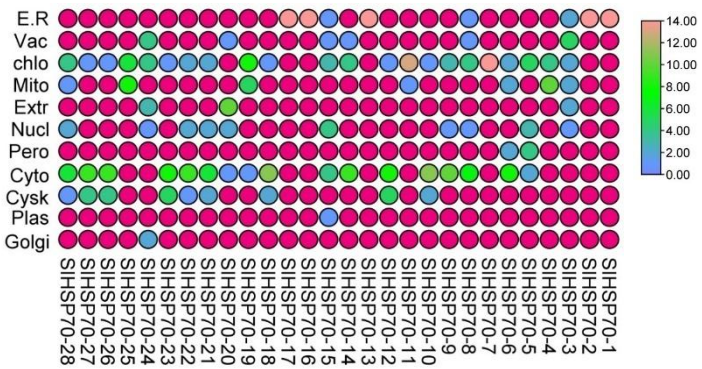

Figure 7: Sub-cellular localization of the SIHSP70 gene family members in various compartments of the cells. Most of the proteins were localized in the cytoplasm, while other proteins were located in the chloroplast, ER, nucleus, and mitochondria probabilistically.

Protein-protein interactome analysis

An interactive display of all HSP70 genes was acquired from the STRING database. Different color schemes showed the division areas among clusters in the network. The examination produced 28 network nodes and 42 connections for an average three-way link between nodes. Network analysis indicated that the observed local clustering coefficient reached 0.775 while maintaining a theoretical average number of 0 edges. The PPI enrichment analysis produced a *p*-value less than 1.0e-16 which confirms substantial enrichment in the network. Protein clustering analysis included k-means clustering that grouped proteins into three distinct clusters. The proteins within each cluster displayed strong interaction patterns according to **Figure 8.** Testing of protein-protein interaction (PPI) showed that SIHSP70 proteins bind together for executing biological functions.

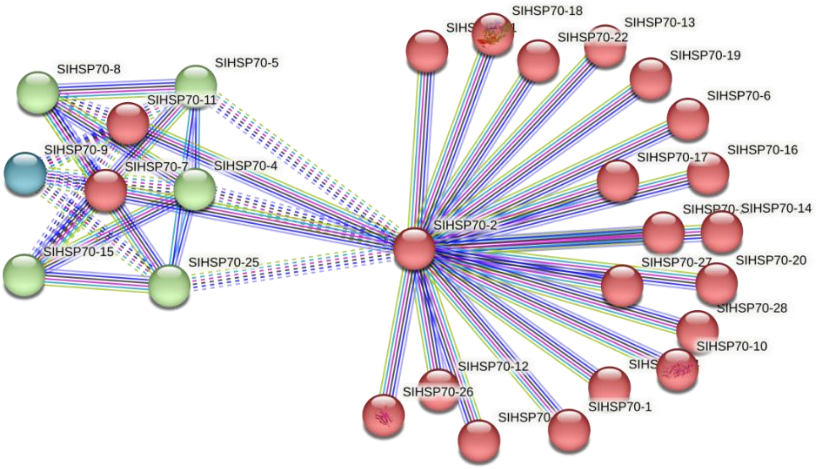

Figure 8: Protein-protein interaction of various SIHSP70 protein members

Gene ontology, KEGG pathways and Reactome pathways enrichment

Gene ontology, KEGG, and Reactome pathway enrichment analyses were used to determine the biological and functional relevance of the genes. GO results of the gene ontology are divided into three categories: molecular function (MF), biological process (BP), and cellular component (CC). ATP-dependent protein folding chaperones, ATP binding, and protein folding were highly enriched terms in molecular function. Protein folding, protein refolding, and response to chemicals were the most enriched terms for biological processes, and most of the proteins were predicted to be working in the cytoplasm and intracellular membrane-bound organelles (**Figure 11 A, B and C**)**.** Molecular and biological functions were depicted using KEGG and Reactome pathway analyses. Enrichment analysis predicted the involvement of SIHSP70 genes in five KEGG (**Figure 10**) and nine Reactome pathways (**Figure 9**). Most of the proteins were involved in protein processing in the endoplasmic reticulum, whereas relatively similar numbers of genes were involved in protein export, endocytosis, and spliceosome. Regulation of HSF-1 mediated heat shock response and cellular response to stress, heat stress, and stimuli.

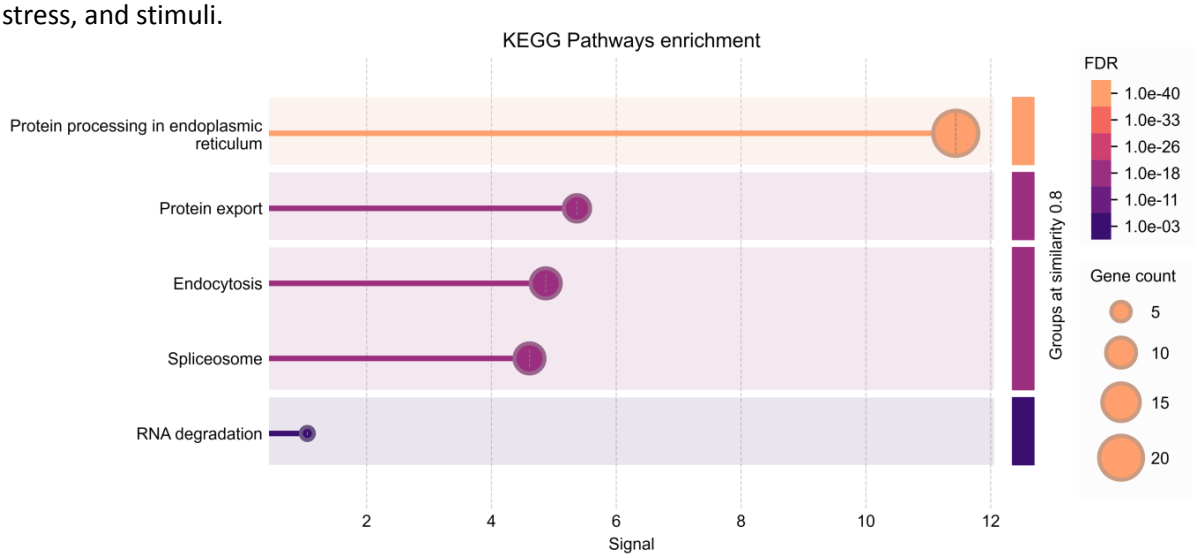

Figure 9: KEGG pathway HSP70 proteins of *Setaria italica*

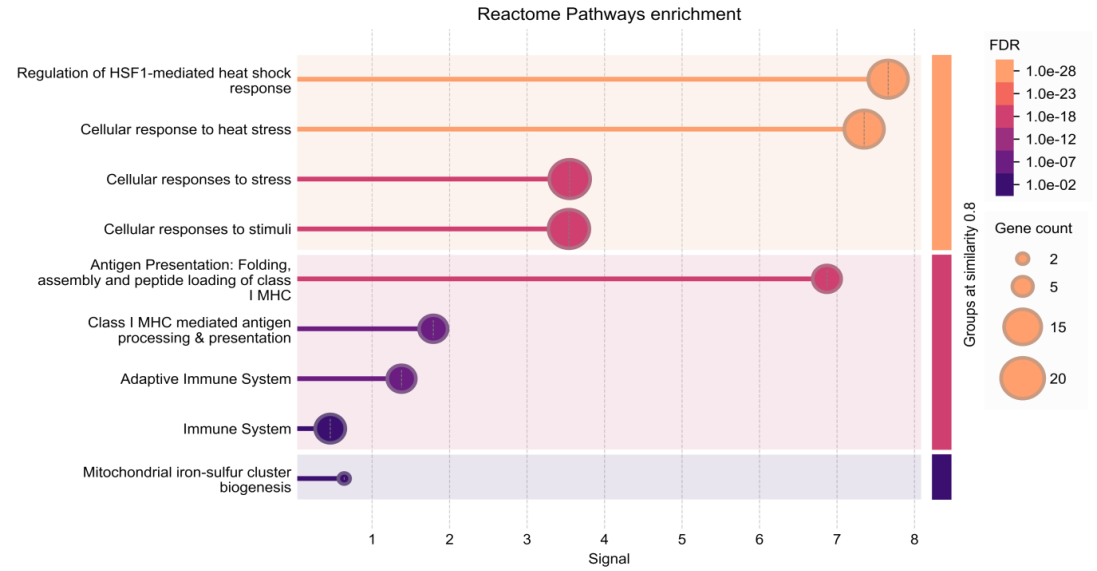

Figure 10: Reactome pathway enrichment of HSP70 proteins of *Setaria italica*

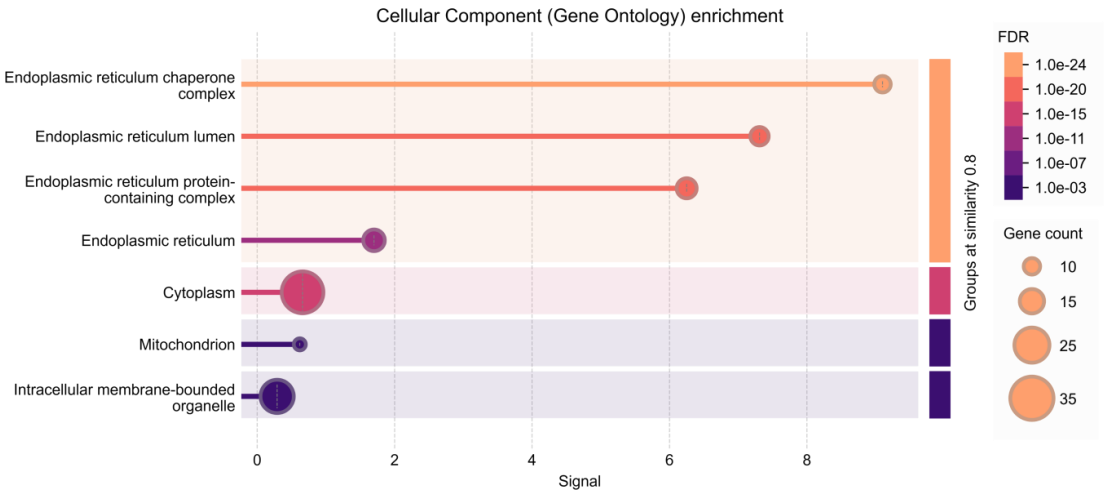

Figure 11A: Cellular component (Gene ontology) of HSP70 proteins in *Setaria italica*

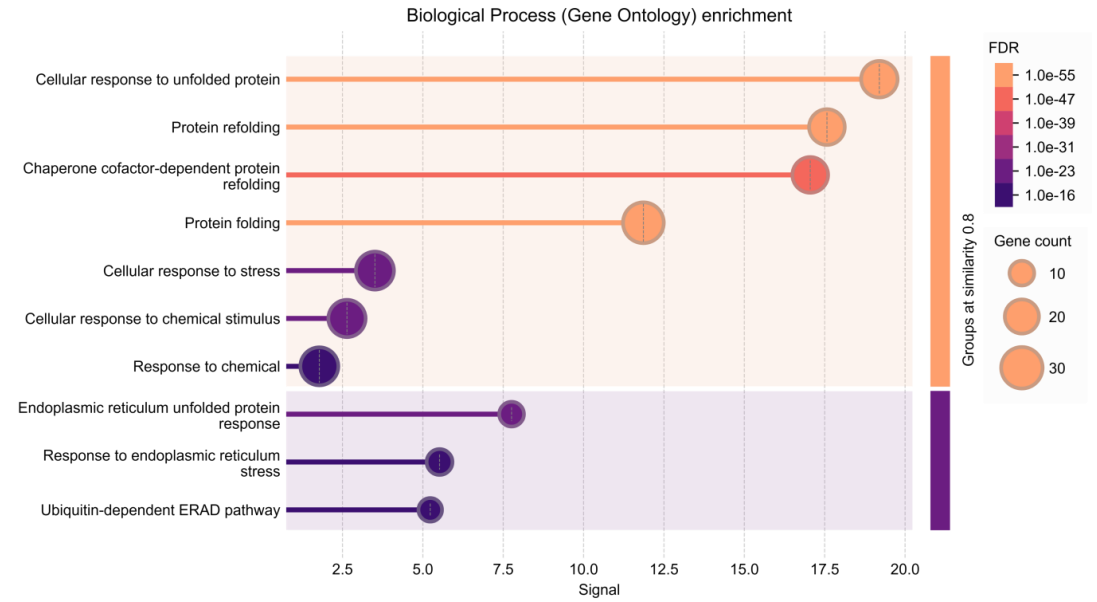

Figure 11B: Biological process (Gene ontology) of HSP70 proteins in *Setaria italica*

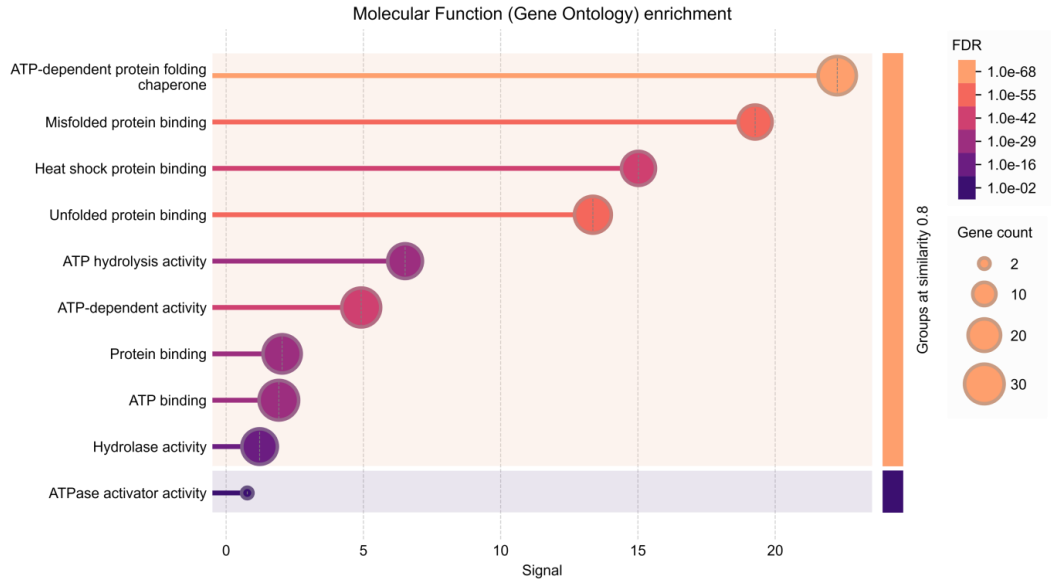

Figure 11C: Molecular function (Gene ontology) of HSP70 proteins in *Setaria italica*

Temporal expression analysis of foxtail millet genotypes

To understand the expression pattern of foxtail millet genotypes in a saline environment, previously published data were used, and a heatmap was created, as shown in **Figure 12**. Expression level of the various genes varied greatly under saline stress condition. A few genes in the saline-tolerant variety JK3 were highly expressed, whereas they were also expressed in the saline-sensitive variety B175, but with relatively low intensity. SIHSP70-1/9/17/18 was highly expressed in both genotypes. SIHSP70-7/12/15/19/20/22/27 was moderately expressed in both genotypes. SIHSP70-26/23/2116/14/8 was expressed at higher levels in the saline-tolerant genotype JK3 than those in the saline-sensitive variety B175.

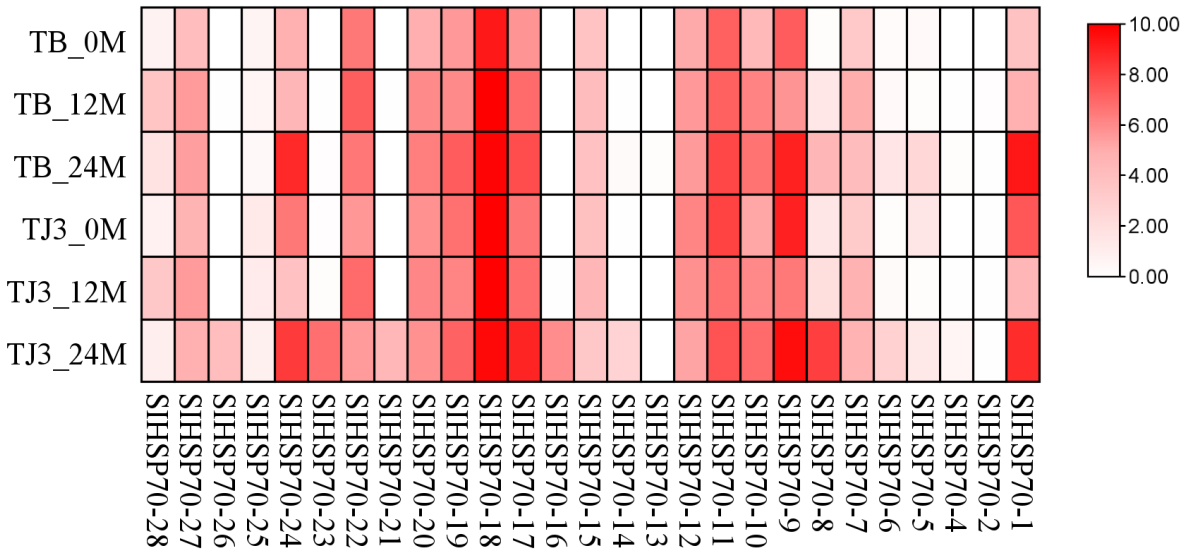

Figure 12: Temporal expression analysis of the SIHSP70 proteins under salt stress in seed during the seedling stage of plant development. Expression analysis results are shown in the log scale having 2 base. T_0 represents the time before the treatment, while T_12 and T_24 represent the expression pattern after 12 and 24 hours of the imposition of saline treatment.

Discussion

Elevated temperatures affect plant growth parameters, which can affect *Setaria italica* production worldwide (Panneerselvam et al., 2019). Heat shock proteins are crucial in homeostatic and responder roles under stress conditions (Timperio et al., 2008). The presence of heat shock proteins (HSPs) in plants protects them from environmental stress by preventing cellular damage. For

example, HSP70 was reported to greatly increase tolerance to drought (Cho and Hong, 2006), heat (Zhang et al., 2023), and other abiotic stress tolerance in tobacco. Research on HSP70 in *Setaria italica* is lacking; therefore, in this study, we studied the SIHSP70s genes to identify and characterize the associated gene family members. Preliminary analysis of HSP70s has been performed in rice and *Arabidopsis* (Lin et al., 2001; Jung et al., 2013; Sarkar et al., 2013). In this study, we identified 28 HSPs genes in *Setaria italica* more than those in *Arabidopsis thaliana* (18), but less than those in *Oryza sativa* (32 HSPs). Owing to evolutionary selection pressures, the number of the same gene family in various plants varies (Zhang et al., 2015). In addition, we analyzed their physicochemical properties to identify their physical properties and various stability revealing factors. The HSP70 family comprises multiple genes that are distributed in various subcellular compartments (Wei et al., 2017). In rice Clade A and B genes are located in the cytoplasm, nucleus, and endoplasmic reticulum. Similarly, most the genes of the SIHSP70 are located in the cytoplasm, nucleus, mitochondria, and chloroplasts. Gene members of Clade C were localized in the mitochondria and chloroplasts (Sarkar et al., 2013). Different subcellular locations can be attributed to the specification of the functions (Lin et al., 2001).

Phylogenetic analysis revealed that the gene family members were divided into four clades, similar to rice and *Arabidopsis thaliana* (Sarkar et al., 2013). Phylogenetic findings were further supported by gene structure, conserved motifs, and subcellular localization analyses. Clade members had similar gene structures. Clade A in *Setaria italica* contained up to 1-3 CDS. Clade B has a CDS of up to 8, Clade C contains a maximum of eight CDS, and Clade D has the highest number of CDS, up to 14. In soybean, gene members clustered together have a similar intron and exon structure (Zhang et al., 2015). SIHSP70-12 and SIHSP70-23 have similar phylogenetic clades, gene structures, and gene localization in the cytoplasm; presumably, they perform similar functions. SIHSP70-20 and SIHP70-19 share a similar clade and perform similar functions in the similar location in the cytoplasm; chloroplast SIHSP70-16 and SIHSP-17 are also located in the endoplasmic reticulum. SIHSP70-4 and SIHSP70-25 also have a similar clade and share a similar gene structure and location in the cytoplasm; therefore, it is highly likely that they will perform similar functions. These findings are in agreement with the findings of HSP70 genes in soybean, rice and *Arabidopsis*, where genes with similar gene structures and locations share similar locations in the cell and perform similar functions (Zhang et al., 2015).

The SIHSP70 gene family members are distributed on the nine chromosomal scaffolds, while most of the genes are located on scaffold 3 with eight genes. Clade B has up to 10 conserved motifs, whereas clade C also has 10 conserved motifs. Clade D has a varied number of conserved motifs, whereas Clade A contains 10 motifs, with the exception of a few members that contain fewer motifs. In StHSP70, a similar order, and number and type of motifs were found, but they were different from other subfamilies (Liu et al., 2018). It is possible that the number and distribution of motifs are associated with the diversity in their genetic functions. We performed *cis*-element analysis to evaluate *cis*-regulatory elements related to hormones and environmental stress. Most of *cis*-elements are associated with methyl jasmonate (MeJA) and abscisic acid (ABA) responsiveness (Kaur et al., 2017). Abscisic acid (ABA) is a stress-response hormone that improves stress response (Davoudi et al., 2022.). Methyl jasmonate (MeJA) and jasmonic acid are hormones that play roles in stress response. Duan et al. (2011) confirmed that MeJA induced TaHSC70 expression after 2 h of spraying. This amount reached a maximum, indicating that TaHSC70 may participate in the basic defense of wheat through the JA signaling pathway. Along with these *cis*-elements, many other *cis*-elements were also present, indicating the role of SIHSP70 in various environmental stress responses.

Based on phylogenetic, comparative phylogenetic, gene structure, motif analysis, and protein subcellular localization, the *Oryza sativa* HSP70 protein family has been divided into four classes. The *Oryza sativa* HSP70 superfamily family has four subclasses, HSP70 protein, cytoplasmic/nuclear HSP70, endoplasmic reticular HSP70 named as BiP, mitochondrial/chlorolastic HSP70 and HSP110/SSE family. Similarly, SIHSP70 also has four subclasses based on their phylogenetic, gene structure, motif analysis, and subcellular location analysis. These analyses confirm their division into four classes. Clade A and D *Oryza sativa* members are localized in the nucleus and cytoplasm, and most of the *Setaria italica* HSP70 protein members are also localized in the same cellular compartments.

All the Clade B SIHSP70 members are grouped with the BiP proteins that are located in the endoplasmic reticulum (ER), and comparative phylogenetically and sub-cellular location-wise

members of Clade B exist in the endoplasmic reticulum. BiP protein inactivation in rice has been found to be associated with the accumulation of various substances in the ER, which affects the expression of genes related to starch synthesis, making rice grains chalky (Li et al., 1993). The absence of BiP increases the rate of chalky grains at high temperatures (Shimoyanagi et al., 2021). Therefore, BiP is involved in the activation of the unfolded protein response (UPR) in the ER and maintains the internal environment of the ER constant, thus ensuring normal rice endosperm development under high temperatures for sustainable growth and development of plants (Wang et al., 2024). Thus, Clade B SIHSP70-1/6/13/16/17/24 members are involved in ER homeostasis. BiPs are expressed during the early and middle stages of seed development, and aid in quality control during seed maturation (Hatano et al., 1997). Clade C was divided into two subclasses based on their relationship with the *Oryza sativa* mitochondrial and chlorolastic HSP70s. Chlorolastic and mitochondrial HSP70 (cHSP70-6, mtHSP70-1, mtHSP70-3) are involved in the positive regulation of seed setting, thus controlling plant yield, while cHSP70-7 negatively regulates seed setting (Wang et al., 2024). Therefore, SIHSP70-12 negatively regulates seed setting, while SIHSP70-4/5/25 is involved in the positive regulation of seed setting; collectively, grain size and plant yield are controlled.

Genes are involved in multiple pathways, as depicted by KEGG and Reactome pathways analysis. Strong and reliable physical and functional relationships were depicted in the KEGG as compared to the Reactome enrichment analysis, which is why KEGG pathways were fewer in number than those in the Reactome.

The findings presented here will aid in the identification and description of the functions of HSP70 genes derived from *Setaria italica*, potentially improving our understanding of their roles in plant development and growth under stressful environments.

Conclusion

In this study, 28 genes were found to be related to the HSP70 gene family in *Setaria italica*. The recovered genes were then subjected to phylogenetic analysis, gene structure analysis, motif analysis, promoter analysis, protein-protein interaction, chromosomal analysis, and subcellular location determination. A systematic study has revealed that gene and protein characteristics are relatively conserved. The expansion of the HSP gene family is caused by large-scale purification and positive selection. These findings contribute to a better understanding of the structural and functional characteristics of the *HSP* gene family in plant species.

Author(s), Editor(s) and Publisher's declarations

Source of funding
None declared.

Conflict of interest
The authors declare no conflict of interest.

Contribution of authors
Conceptualization and designing of the study: JA, AR, MG. Conduction of experiments: JA, AR, AZ. Data collection, visualization, and interpretation: JA, MAK, AZ, MG, MAK. Formal statistical analysis: JA, AR, MAK, AZ, MG, MAK. Writing of first draft: JA, MAK, AZ, MG, MAK. Proof reading and approval of the final version: JA, AR, MAK, AZ, MG, MAK.

Ethical approval
This study does not involve human/animal subjects, and thus no ethical approval is required.

Handling of bio-hazardous materials
The authors certify that all experimental materials were handled with great care during collection and experimental procedures. After completion of the study, all materials were properly discarded to minimize/eliminate any types of bio-contamination(s).

Supplementary material
No supplementary material is included with this manuscript.

Availability of primary data and materials
As per editorial policy, experimental materials, primary data, or software codes are not submitted to the publisher/Journal management. These are available with the corresponding author (s) and/or with other author(s) as declared by the corresponding author (s) of this manuscript.

Authors' consent
All authors have critically read this manuscript and agreed to publish in IJAaEB.

Disclaimer/editors'/publisher's declaration
All claims/results/prototypes included in this manuscript are exclusively those of the authors and do not inevitably express those of their affiliated organizations/enterprises, or those of the publisher/journal management, and the editors/reviewers. Any product mentioned in this manuscript, or claim rendered by its manufacturer, is not certified by the publisher/Journal management. The journal management disowns responsibility for any injury to organisms including humans, animals and plants or property resulting from any ideas/opinions, protocols/methods, guidelines or products included in the publication. The IJAaEB publisher/Management stays impartial/neutral pertaining to institutional affiliations and jurisdictional claims in maps included in the manuscript.

Declaration of generative AI and AI-assisted technologies in the writing process
It is declared that the authors did not use any AI tools or AI-assisted services in the preparation, analysis, or creation of this manuscript submitted for publication in the International Journal of Applied and Experimental Biology (IJAaEB).

References

Berka, M., Kopecká, R., Berková, V., Brzobohatý, B., Černý, M. (2022). Regulation of heat shock proteins 70 and their role in plant immunity. *Journal of Experimental Botany* 73(7):1894–1909.

Chen, C., Wu, Y., Li, J., Wang, X., Zeng, Z. et al. (2023). TBtools-II: A "one for all, all for one" bioinformatics platform for biological big-data mining. *Molecular Plant* 16(11):1733-1742.

Cho, E.K., Choi, Y.J. (2009). A nuclear-localized HSP70 confers thermoprotective activity and drought-stress tolerance on plants. *Biotechnology Letters* 31(4):597–606.

Cho, E.K., Hong, C.B. (2006). Over-expression of tobacco NtHSP70-1 contributes to drought-stress tolerance in plants. *Plant Cell Reports* 25(4):349–358.

Davoudi, M., Chen, J., Lou, Q. (2022). Genome-wide identification and expression analysis of heat shock protein 70 (*HSP70*) gene family in pumpkin (*Cucurbita moschata*) rootstock under drought stress suggested the potential role of these chaperones in stress tolerance. *International Journal of Molecular Sciences* 23(3):1918. https://doi.org/10.3390/ijms23031918

Duan, Y.H., Guo, J., Ding, K., Wang, S.J., Zhang, H. et al. (2011). Characterization of a wheat HSP70 gene and its expression in response to stripe rust infection and abiotic stresses. *Molecular Biology Reports* 38(1):301–307.

Hatano, K., Shimada, T., Hiraiwa, N., Nishimura, M., Hara-Nishimura, I. (1997). A rapid increase in the level of binding protein (BiP) is accompanied by synthesis and degradation of storage proteins in pumpkin cotyledons. *Plant and Cell Physiology* 38(3):344–351.

He, Z., Webster, S., He, S.Y. (2022). Growth-defense trade-offs in plants. *Current Biology* 32(12):634-639.

Horton, P., Park, K.J., Obayashi, T., Fujita, N., Harada, H. et al. (2007). WoLF PSORT: protein localization predictor. *Nucleic Acids Research* 35(Web Server issue):W585–W587. https://doi.org/10.1093/nar/gkm259

Hurst, L.D. (2002). The Ka/Ks ratio: Diagnosing the form of sequence evolution. *Trends in Genetics* 18(9):486–487.

Jacob, P., Hirt, H., Bendahmane, A. (2017). The heat-shock protein/chaperone network and multiple stress resistance. *Plant Biotechnology Journal* 15(4):405–414.

Jiang, L., Hu, W., Qian, Y., Ren, Q., Zhang, J. (2021). Genome-wide identification, classification and expression analysis of the *Hsf* and *Hsp70* gene families in maize. *Gene* 770:145348. https://doi.org/10.1016/j.gene.2020.145348.

Jung, K.H., Gho, H.J., Nguyen, M.X., Kim, S.R., An, G. (2013). Genome-wide expression analysis of *HSP70* family genes in rice and identification of a cytosolic *HSP70* gene highly induced under heat stress. *Functional & Integrative Genomics* 13(3):391–402. https://doi.org/10.1007/S10142-013-0331-6

Kalsi, R., Bhasin, J.K. (2023). Nutritional exploration of foxtail millet (*Setaria italica*) in addressing food security and its utilization trends in food system. *eFood* 4(5):e111. https://doi.org/10.1002/efd2.111

Kaur, A., Pati, P.K., Pati, A.M., Nagpal, A.K. (2017). *In-silico* analysis of *cis*-acting regulatory elements of pathogenesis-related proteins of *Arabidopsis thaliana* and *Oryza sativa*. *PloS One* 12(9):e0184523. https://doi.org/10.1371/journal.pone.0184523

Lescot, M., Déhais, P., Thijs, G., Marchal, K., Moreau, Y. et al. (2002). PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Research* 30(1):325–327. https://doi.org/10.1093/NAR/30.1.325

Li, X., Wu, Y., Zhang, D.Z., Gillikin, J.W., Boston, R.S. et al. (1993). Rice prolamine protein body biogenesis: a BiP-mediated process. *Science* 262:1054–1056.

Lin, B.L., Wang, J.S., Liu, H.C., Chen, R.W., Meyer, Y. et al. (2001). Genomic analysis of the Hsp70 superfamily in *Arabidopsis thaliana*. *Cell Stress & Chaperones* 6(3):201–208.

Liu, J., Pang, X., Cheng, Y., Yin, Y., Zhang, Q. et al. (2018). The *Hsp70* gene family in *Solanum tuberosum*: Genome-wide identification, phylogeny, and expression patterns. *Scientific Reports* 8(1): https://doi.org/10.1038/S41598-018-34878-7

Lu, H., Zhang, J., Liu, K.B., Wu, N., Li, Y. et al. (2009). Earliest domestication of common millet (*Panicum miliaceum*) in East Asia extended to 10,000 years ago. *Proceedings of the National Academy of Sciences of the United States of America* 106(18):7367–7372.

Lu, S., Wang, J., Chitsaz, F., Derbyshire, M.K., Geer, R.C. et al. (2020). CDD/SPARCLE: the conserved domain database in 2020. *Nucleic Acids Research* 48(D1):D265–D268. https://doi.org/10.1093/nar/gkz991

Marchler-Bauer, A., Bo, Y., Han, L., He, J., Lanczycki, C.J. et al. (2017). CDD/SPARCLE: functional classification of proteins via subfamily domain architectures. *Nucleic Acids Research* 45(D1):D200–D203. https://doi.org/10.1093/nar/gkw1129

Montero-Barrientos, M., Hermosa, R., Cardoza, R.E., Gutiérrez, S., Nicolás, C. et al. (2010). Transgenic expression of the *Trichoderma harzianum hsp70* gene increases *Arabidopsis* resistance to heat and other abiotic stresses. *Journal of Plant Physiology* 167(8):659–665. https://doi.org/10.1016/j.jplph.2009.11.012

Nishad, A., Nandi, A.K. (2021). Recent advances in plant thermomemory. *Plant Cell Reports* 40(1):19–27. https://doi.org/10.1007/S00299-020-02604-1

Panneerselvam, S., Dheebakaran, G., Kokilavani. S. (2019). Effect of elevated temperature on growth parameters of foxtail millet (*Setaria italica*). *Madras Agricultural Journal* 106:349. https://doi.org/10.29321/MAJ.2019.000272.

Purugganan, M.D., Fuller, D.Q. (2009). The nature of selection during plant domestication. *Nature* 457(7231):843–848. https://doi.org/10.1038/NATURE07895

Queitsch, C., Hong, S.W., Vierling, E., Lindquist, S. (2000). Heat shock protein 101 plays a crucial role in thermotolerance in *Arabidopsis*. *Plant Cell* 12(4):479–492. https://doi.org/10.1105/TPC.12.4.479

Rombauts, S., Déhais, P., Van Montagu, M., Rouzé, P. (1999). PlantCARE, a plant cis-acting regulatory element database. *Nucleic Acids Research* 27(1):295–296. https://doi.org/10.1093/NAR/27.1.295

Sarkar, N. K., Kim, Y. K., & Grover, A. (2009). Rice *sHsp* genes: Genomic organization and expression profiling under stress and development. *BMC Genomics* 10:393. https://doi.org/10.1186/1471-2164-10-393

Sarkar, N.K., Kundnani, P., Grover, A. (2013). Functional analysis of Hsp70 superfamily proteins of rice (*Oryza sativa*). *Cell Stress and Chaperones* 18(4):427–437. https://doi.org/10.1007/S12192-012-0395-6

Sharma, N., Niranjan, K. (2018). Foxtail millet: Properties, processing, health benefits, and uses. *Food Reviews International* 34(4):329–363.

Shimoyanagi, R., Abo, M., Shiotsu, F. (2021). Higher temperatures during grain filling affect grain chalkiness and rice nutrient contents. *Agronomy* 11:1360. https://doi.org/10.3390/agronomy11071360

Song, P., Jia, Q., Xiao, X., Tang, Y., Liu, C. et al. (2021). HSP70-3 interacts with phospholipase Dδ and participates in heat stress defense. *Plant Physiology* 185(3):1148–1165.

Swindell, W. R., Huebner, M., Weber, A.P. (2007). Transcriptional profiling of *Arabidopsis* heat shock proteins and transcription factors reveals extensive overlap between heat and non-heat stress response pathways. *BMC Genomics* 8: https://doi.org/10.1186/1471-2164-8-125

Tamura, K., Stecher, G., Kumar, S. (2021). MEGA11: molecular evolutionary genetics analysis version 11. *Molecular Biology and Evolution* 38(7):3022–3027.

Timperio, A.M., Egidi, M.G., Zolla, L. (2008). Proteomics applied on plant abiotic stresses: Role of heat shock proteins (HSP). *Journal of Proteomics* 71(4):391–411.

Usman, M.G., Rafii, M.Y., Martini, M.Y., Yusuff, O.A., Ismail, M.R. et al. (2017). Molecular analysis of Hsp70 mechanisms in plants and their function in response to stress. *Biotechnology and Genetic Engineering Reviews* 33(1):26–39. https://doi.org/10.1080/02648725.2017.1340546

Wang, H., Charagh, S., Dong, N., Lu, F., Wang, Y. et al. (2024). Genome-wide analysis of heat shock protein family and identification of their functions in rice quality and yield. *International Journal of Molecular Sciences* 25(22): https://doi.org/10.3390/ijms252211931

Wang, J., Chitsaz, F., Derbyshire, M.K., Gonzales, N.R., Gwadz, M. et al. (2023). The conserved domain database in 2023. *Nucleic Acids Research* 51(D1):D384–D388.

Wei, Q., Ma, C., Xu, Y., Wang, T., Chen, Y. et al. (2017). Control of chrysanthemum flowering through integration with an aging pathway. *Nature Communications* 8(1):829. https://doi.org/10.1038/s41467-017-00812-0

Yer, E.N., Baloglu, M.C., Ziplar, U.T., Ayan, S., Unver, T. (2016). Drought-responsive hsp70 gene analysis in *Populus* at genome-wide level. *Plant Molecular Biology Reporter* 34(2):483–500.

Zhang, L., Zhao, H.K., Dong, Q.L., Zhang, Y.Y., Wang, Y.M. et al. (2015). Genome-wide analysis and expression profiling under heat and drought treatments of *HSP70* gene family in soybean (*Glycine max* L.). *Frontiers in Plant Science* 6:773. https://doi.org/10.3389/fpls.2015.00773

Zhang, X., Li, J., Li, M., Zhang, S., Song, S. et al. (2023). NtHSP70-8b positively regulates heat tolerance and seed size in *Nicotiana tabacum. Plant Physiology and Biochemistry* 201:107901. https://doi.org/10.1016/j.plaphy.2023.107901.

Zhao, D., Xia, X., Su, J., Wei, M., Wu, Y. et al. (2019). Overexpression of herbaceous peony HSP70 confers high temperature tolerance. *BMC Genomics* 20(1):70. https://doi.org/10.1186/s12864-019-5448-0