

GENOME-WIDE DISTRIBUTION AND CHARACTERIZATION OF HIGH TEMPERATURE SHOCK TRANSCRIPTION FACTOR GENES FOR DROUGHT AND HIGH TEMPERATURE STRESS IN *SOLANUM TUBEROSUM* L.

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ABSTRACT: Abiotic stresses, mainly drought and high temperature affect potato production greatly. Molecular mechanisms associated with high temperature and drought stresses are yet to be deciphered fully. In many crops "high temperature shock transcription factors" (*hsf*) play important role in providing tolerance to plants under these stresses however, the role of *hsf* family in potato (*Solanum tuberosum*) in relation to abiotic stress is not fully explored yet. We performed detailed genome-wide analysis of *hsf* genes in potato. Fifteen *hsf* genes were identified in the potato genome which were found to be scattered on all chromosomes except chromosomes 1, 3 and 5. The structural analysis of predicted potato *hsf* genes indicated presence of only one intron in potato *hsf* genes. The lengths of deduced *hsf* proteins were found to be ranging from 55 to 536 amino acids, having molecular weights in the range of 12.20 kDa to 60.25 kDa with theoretical pI values from 4.71 to 9.58. The grand average of hydropathy (GRAVY) values was found to be negative for all the fifteen potato *hsfs*, suggesting their hydrophilic nature. Of the fifteen *hsf* genes identified in the potato genome, five were cloned and sequenced (partially) and their sequences were submitted to NCBI GenBank and were provided NCBI accession numbers. In response to drought, higher expression of all the five cloned genes was observed in drought tolerant cultivar- Kufri Jyoti (in leaf as well as root tissues) as compared to that in drought susceptible cultivar- Kufri Pukhraj. In response to high temperature stress, although the expression of most of the cloned *hsf* genes decreased in both high temperature tolerant (Kufri Surya) as well as susceptible cultivar (Kufri Chandramukhi), however, the decrease was comparatively more in case of K. Chandramukhi (high temperature susceptible cultivar) as compared to that in Kufri Surya (high temperature tolerant cultivar). The study indicated involvement of these *hsf* genes in imparting tolerance to drought and high temperature stress and thus, can be utilized for developing more climate resilient potato cultivars.

KEYWORDS chromosome location, phylogenetic, expression analysis, potato genome, abiotic stress.

INTRODUCTION

Potato (*Solanum tuberosum* L.) is the world's third most important food crop in terms of human consumption (FAOSTAT

2026, Dutt *et al*, 2017). Potato production in India has been increasing over the years, with an increase of almost 21 times in production between 1961 and 2022 (FAOSTAT 2026).

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In the year 2025-26, the volume of potato produced across India has been estimated to be around 60 million metric tons making India second largest producer after China (Area and Production of Horticulture crops for 2025-26, 2nd Advance Estimates; FAOSTAT 2026; <https://agriwelfare.gov.in/en/StatHortEst>). The impact of climate change on potato production in India is significant (Jatav *et al.*, 2017), with the Indo-Gangetic Plains (IGP) being the main potato growing region. Climate change is projected to reduce potato yields by approximately 2.5%, 6%, and 11% in the IGP region in 2020, 2050, and 2080, respectively (Muthoni and Shimelis, 2020). High temperature and drought are major factors contributing to these yield reductions.

Potato is a cool-season crop that is highly sensitive to drought and high-temperature stress, both of which substantially impair plant growth, tuberization, yield and tuber quality. Water deficit causes stomatal closure, reduced leaf expansion and photosynthetic activity, altered assimilate partitioning and accelerated senescence, ultimately restricting tuber initiation and bulking (Obidiegwu *et al.*, 2015; Hill *et al.*, 2021). High temperature, similarly affects photosynthesis, respiration, hormonal balance and source-sink relationships; although vegetative growth is favored at approximately 20–25°C, optimum tuber growth occurs at about 15–20°C, and temperatures above 25°C can markedly delay or inhibit tuberization (Dahal *et al.*, 2019). Night temperature is a particularly important environmental determinant of potato (*Solanum tuberosum* L.) growth and tuberization. Relatively cool nights favour tuber induction and subsequent tuber growth, whereas elevated night temperatures, particularly above about 18–22°C, can markedly inhibit or delay tuber formation and favour the allocation of assimilates towards vegetative growth rather than tubers (Menzel,

1980; Levy and Veilleux, 2007). High night temperature can prolong stolon growth and tuber setting, delay the onset of rapid tuber bulking, and reduce carbohydrate partitioning to developing tubers. Consequently, plants may produce greater above-ground biomass but exhibit a lower harvest index, fewer large tubers and reduced tuber yield. In a field study, increasing night temperature by approximately 4°C during tuber initiation reduced tuber yield by 17.2% and the proportion of large tubers (>100 g) by 53.7%, primarily through delayed tuber development rather than inhibition of photosynthesis (Kim and Lee, 2019). Thus, increasing night temperatures associated with climate warming may pose a substantial constraint to potato productivity, particularly in tropical, subtropical and warmer potato-growing regions. High temperature and drought also promote oxidative stress through excessive accumulation of reactive oxygen species, requiring activation of antioxidant and other protective mechanisms. When drought and high temperature occur simultaneously, their effects may be particularly damaging because elevated temperature increases evaporative demand and intensifies plant water deficit. Consequently, improving drought and heat tolerance through physiological, biochemical, molecular and genetic approaches is an important priority for sustaining potato productivity under changing climatic conditions (Dahal *et al.*, 2019; Gervais *et al.*, 2021). To mitigate the effects of climate change on potato production, adaptation strategies are essential.

The response to high temperatures and drought in a plant is a complex adaptive, biochemical, and physiological phenomenon that gets triggered by a cascade of events usually governed by transcription factors (TFs), which starts with stress perception and ends with an expression of a set of

target genes. These genes produce important enzymes and metabolic proteins, thereby protecting cells from stress. They also have a role in regulation of signal transduction and gene expression by synthesizing regulatory proteins (Lata and Prasad, 2011; Nakashima *et al.*, 2012). TFs are regulatory proteins which play a critical role in association with cis-acting elements of the promoter region of various stress-responsive genes to convert stress signal to stress-responsive gene expression in the signal transduction processes. As a result of which signalling cascades activate the whole network of genes which enhances plant tolerance to adverse environmental conditions (Akhtar *et al.*, 2012). In case of plant genomes, ~7 % of the coding sequences code for TFs (Udvardi *et al.*, 2007). These TFs often belong to large gene families as compared to animals and yeasts, like high temperature shock transcription factors (*hsf*) family (Baniwal *et al.*, 2004; Udvardi *et al.*, 2007). In plants, *hsfs*, also known as heat shock transcription factors, are the terminal components of signal transduction chain and studies have indicated the roles of *hsfs* in abiotic stresses including high temperature stress (HS) (Scharf *et al.*, 2012). Though *hsfs* play significant roles in stress signal transduction, gaps are still there in the proper understanding of their role. Present study was envisaged with this objective to have better insights into potato *hsfs* in relation to drought and high temperature stress.

MATERIALS AND METHODS

Genome-wide Identification and phylogenetic analysis

To identify *hsf* gene family members, we searched genes annotated as *hsf* using the keyword “High temperature Shock Factor” in the Plaza database (<https://www.vandepoelelab.be/plaza/>) to extract *Solanum tuberosum*-specific *hsf* gene sequences. The

gene and protein sequences of the *hsfs* were downloaded and used for phylogenetic analysis and *in-silico* characterization. Phylogenetic analysis was performed to elucidate the relationships among *hsf* genes of potato genome. Phylogenetic analysis of the protein sequences was performed using <https://itol.embl.de/> (Letunic *et al.*, 2024).

Gene structure analysis and chromosomal location

To further explore the structural details of *hsf* genes, advanced bioinformatics tools and resources were used. Deciphering the gene structures Dicots PLAZA 4.0 server (<https://www.vandepoelelab.be/plaza/>) and tools were used to obtain the exon-intron organization, splice variants, and putative regulatory elements of the genes. The software, MapChart, (Voorrips, 2002) was used for physical mapping of locations of *hsf* genes on the chromosomes of *S. tuberosum*.

In-silico characterization of *hsf* proteins

To get more information about the nature of the *hsf* protein, the grand average of hydropathy (GRAVY), Isoelectric point (pI), and molecular weight of the potato *hsf* proteins were estimated. For this purpose, various tools including ProtParam tool available on Expert Protein Analysis System (ExPASy) proteomics server (<https://web.expasy.org/cgi-bin/protparam/protparam>; Gasteiger *et al.*, 2005) were used.

Plant material and growing conditions

Previously reported drought tolerant cultivar (Kufri Jyoti) and drought susceptible cultivar (Kufri Pukhraj) (henceforth referred to as K. Jyoti and K. Pukhraj, respectively) were used in the present study. For studies pertaining to temperature, high temperature tolerant cultivar, Kufri Surya, and high temperature susceptible cultivar Kufri

Chandramukhi (henceforth referred to as K. Surya and K. Chandramukhi, respectively) were used. Freshly harvested good quality breeder seed potatoes were procured from ICAR- Central Potato Research Institute, Regional Station Modipuram, Uttar Pradesh, India. The tubers were kept at ambient temperature under dark conditions for chitting. Well sprouted tubers of each of these cultivars were planted in plastic pots and grown in poly house and /or controlled environment plant growth chambers (M/s Conviron, Model E-15, Canada) for imposing drought and high temperature stress, respectively.

Imposition of drought condition

Drought experiment was conducted in the controlled environment poly house. Well sprouted tubers of K. Jyoti and K. Pukhraj were planted in plastic pots and grown in poly house. Till 30 days of Planting, the plants were watered to the full field capacity. The filed capacity of the pots was previously calculated as per the standard method. After 30 days of planting, one set of plants were watered and maintained at 50% of the field capacity (and named as drought treated plants). While the other set was maintained at full field capacity (control plants). On 60th day of planting leaf samples from the fixed leaf position (4th leaf position from the top) were picked from both the control plants as well as those under drought. Approximately 100 mg leaf samples were weighed and frozen in liquid nitrogen and subsequently, stored at -80°C till further use.

Imposition of high temperature condition

For high temperature stress experimentations, previously reported high temperature resistant and susceptible cultivars K. Surya and K. Chandramukhi,

respectively were used for the study. The experiment was conducted in controlled environment chambers (M/s Conviron, Model E-15, Canada). Initially, in both the chambers, the plants were grown at 16 h photoperiod with day and night temperature of 22°C, and 18°C, respectively. For imposing high temperature stress on one set of plants; after 30 days of planting the temperature of one of the two growth chambers were changed. The constant temperature of 26°C ± 2°C with the same photoperiod of 16 h was set and maintained. At 60th day of planting roots, and leaf samples (from the 4th leaf position from the top) were picked from both the control plants as well as those under high temperature. Approximately 100 mg leaf as well as root samples were weighed and frozen in liquid nitrogen and subsequently, stored at -80°C till further use.

RNA isolation and cloning of the *hsf* genes

Leaf and root tissue samples collected from the plants and stored at -80°C were used for RNA isolation. Total RNA was isolated using NucleoSpin® RNA kit (M/s Macherey-Nagel, USA) following the user's instruction. Complementary DNA (cDNA) was synthesized from 2 µg of DNase-treated total RNA as a template in 20 µl reaction volume by using cDNA synthesis kit (Invitrogen, USA) (Dutt *et al*, 2023). Degenerate primers for various *hsf* genes were designed from the conserved regions for all the 15 *hsf* genes. The partial gene sequences were amplified by PCR. The amplicons were cloned in pGEM-T Easy Vector (Promega, USA), plasmids were isolated using Fermentas GeneJET™ Plasmid Miniprep Kit (Fermentas Inc., USA), and sequencing was performed using Big Dye terminator cycle sequencing mix (Version 3.1; Applied Biosystems, USA) using an

automated DNA sequencer (ABI 3130 xl Genetic Analyzer, Applied Biosystems, USA). Protocols were followed essentially as described by the respective manufacturer.

Semi-quantitative expression analysis by RT-PCR

Semi-quantitative RT-PCR based expression analysis was performed to study the expression level of cloned *hsf* genes in response to drought and high temperature stress *vis-a-vis* respective controls. The RNA isolated from the plant tissue (leaf and root in case of drought, and leaf in case of high temperature experiment) as mentioned above. To analyze the expression level of the cloned genes, total RNA was isolated from leaf tissue (100 mg) using the NucleoSpin® RNA kit (M/s Macherey-Nagel, USA) following the user's instruction. cDNA was synthesized from DNA-free RNA using SuperScript® III Reverse Transcriptase (Invitrogen, USA). The cDNA was used as a template for Reverse transcription-PCR reaction using gene specific primers and cycling conditions were optimized to obtain amplification under the exponential phase (Table 1). 26S rRNA based primer pair was used as internal control for expression studies (Singh *et al.*,

2004). The amplicons were analysed and quantified using the Alpha Digi Doc Gel Documentation and Image analysis system (Bio-Rad Molecular Imager ChemiDoc XRS Imaging system, USA). Each experiment was repeated at least twice with three biological replicates each time and the representative figure of one experiment is shown in the manuscript. Integrated density values (IDV) of the amplicons were calculated and compared after normalization for the control gene (26S rRNA).

RESULTS AND DISCUSSION

hsf genes, their distribution in potato genome and their structures

Fifteen *hsf* genes were identified in the potato genome from Dicots PLAZA 4.0. The chromosomal locations of these genes revealed that three *hsf* genes found to be scattering on chromosomes 2, 4, 6,7, 8, 9,10,11,12 (Fig. 1). Of the 15 *hsfs*, three each were found to be located on chromosome 2 (PGSC0003DMG400028416, P G S C 0 0 0 3 D M G 4 0 0 0 4 1 3 6 1 , P G S C 0 0 0 3 D M G 4 0 1 0 0 4 0 2 3) and chromosome 8 (PGSC0003DMG400017846, P G S C 0 0 0 3 D M G 4 0 0 0 0 8 2 2 3 , PGSC0003DMG400014811). Two *hsf* genes were located each on chromosomes 4 (P G S C 0 0 0 3 D M G 4 0 0 0 2 7 2 8 3 , P G S C 0 0 0 3 D M G 4 0 0 0 0 7 9 6 2) and chromosome 10 (PGSC0003DMG401008167, PGSC0003DMG402008167). Remaining 5 *hsf* genes were found to be present on chromosomes 6 (PGSC0003DMG400027036), 7 (PGSC0003DMG400032793), chromosome 9 (PGSC0003DMG400006447), chromosome 11 (P G S C 0 0 0 3 D M G 4 0 0 0 4 3 2 3 4), chromosome 12(PGSC0003DMG400000380) (Fig. 1, Table 2)

The structural analysis of predicted potato *hsf* genes (Fig. 2) indicated the presence of only one intron in potato *hsf*

Table 1. Primer and PCR conditions used for expression analysis of *hsf* genes of potato

S. No.	Name of primer	Sequence of primers (5'-3')	PCR condition
1	HP1-F	CACATGGTCCAGATGGAGTG	Initial denaturation at 94 °C for 3 min, followed by 35 cycles of 94 °C, 30 s; 55–60 °C, 40 s; 72 °C, 50 s. Final extension at 72 °C for 7 min
2	HP1-R	TTTCTCCTTCCCCATCTCCT	
3	HP2-F	CGGTAGAGAATGGGACCAAA	
4	HP2-R	CACTCGGATGGGTTTGAGAT	
5	HP4-F	AGAGAAGAGGCCACAGCAAA	
6	HP4-R	GACGAAGGCGAGGATTGATA	
7	HP9-F	GCAGCAACAGCAGAGTACGA	
8	HP9-R	GACCTGACATTGCTGCTTGA	
9	HP10-F	TCGTTGTTTGAATCCACCT	
10	HP10-R	CCGCAGATGACTGAAGCATA	

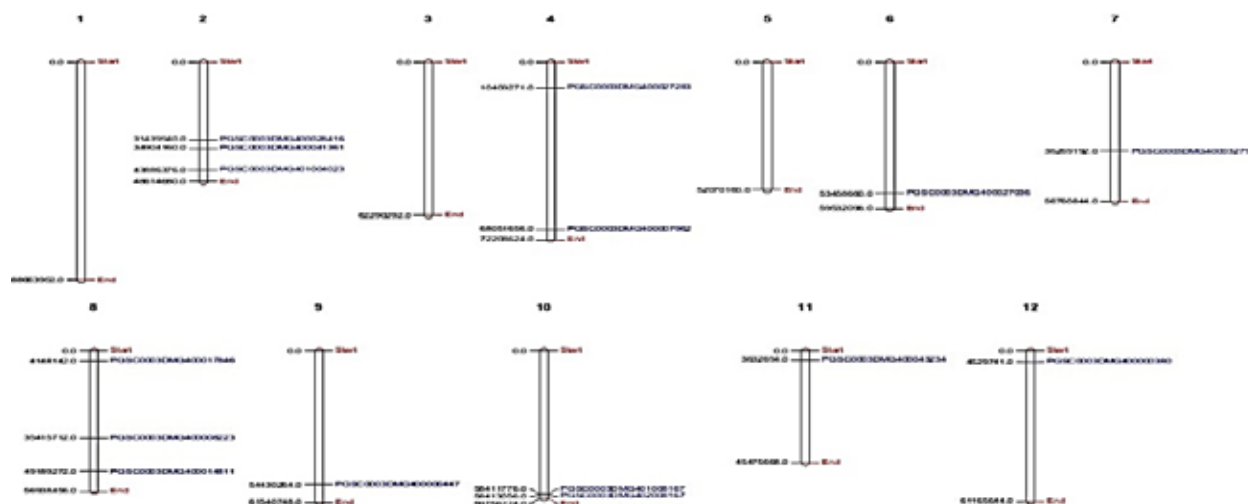


Fig. 1. Locations of *hsf* genes on different chromosomes of potato.

genes (Fig. 2) The *hsf* gene accession no. PGSC0003DMG402008167 was found to be intron-less (Table 2 and Fig. 2). The length of cDNAs in present studies ranged from 0.826 kbp to 6.315 kbp. The variation in gene length and exon-intron structure could indicate different regulatory roles or adaptability mechanisms among the *hsf* genes in potato.

Our findings offer a detailed examination of the *hsf* gene family in potato, including its

distribution, structure, evolutionary history and functional expression under stress conditions. The number of *hsf* gene family members varies among different species. For example, *Triticum urartu* has 17 members (Li *et al.* 2021), *Arabidopsis thaliana* has 21 *hsf* members (Li *et al.* 2003), *Aegilops tauschii* has 19 members (Yang *et al.* 2014) and maize (*Zea mays*) has 25 members (Lin *et al.* 2011). The uneven distribution of genes on various chromosomes suggests specific evolutionary pressures or functional diversifications associated with particular chromosomal locations. The structural analysis revealed that most *hsf* genes possess two exons. The variation in gene length and exon-intron structure could indicate different regulatory roles or adaptability mechanisms among the *hsf* genes.

In silico characterization of deduced *hsf* proteins of potato

After moving from genome exploration to protein-level analyses, the work investigated *hsf* proteins using *in silico* analysis. During this phase, the Expert Protein Analysis System (ExPASy) (<https://www.expasy.org/>) server became a crucial tool for

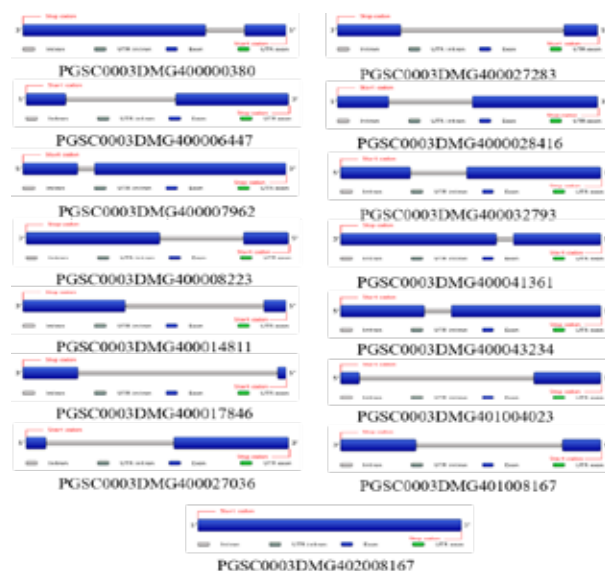


Fig. 2. Gene structure of potato *hsf* genes of potato genome.

Table 2. Details of *hsf* genes structure in potato.

Sr. no.	Gene ID	Chr. number	mRNA coordinates		Exon	Intron	Size (Kbp)
			Start	End			
1.	PGSC0003DMG400028416	2	31439941	31441450	2	1	1.510
2.	PGSC0003DMG400041361	2	34904161	34905484	2	1	1.324
3.	PGSC0003DMG401004023	2	43886377	43889920	2	1	3.544
4.	PGSC0003DMG400027283	4	10409271	10411469	2	1	2.199
5.	PGSC0003DMG400007962	4	68051653	68053098	2	1	1.446
6.	PGSC0003DMG400027036	6	5348661	53463194	2	1	4.543
7.	PGSC0003DMG400032793	7	36269190	36271391	2	1	2.202
8.	PGSC0003DMG400017846	8	4148142	4154456	2	1	6.315
9.	PGSC0003DMG400008223	8	35415712	35418551	2	1	2.840
10.	PGSC0003DMG400014811	8	49189274	49194971	2	1	5.698
11.	PGSC0003DMG400006447	9	54430264	54432804	2	1	2.541
12.	PGSC0003DMG401008167	10	5841178	5841061	2	1	2.284
13.	PGSC0003DMG402008167	10	58413057	58414061	1	0	1.005
14.	PGSC0003DMG400043234	11	3632694	3633519	2	1	0.826
15.	PGSC0003DMG400000380	12	4529741	4531488	2	1	1.748

predicting important protein parameters as the molecular weight, instability index, isoelectric point (*pI*), and grand average of hydropathy (GRAVY). These parameters play a pivotal role in understanding the physicochemical characteristics of proteins, shedding light on their stability, structure, and potential functional roles within cellular processes. *In silico* analysis was performed on the 43 *hsf* deduced protein sequences obtained from NCBI and 15 *hsf* deduced protein sequences obtained from Dicots PLAZA 4.0. The lengths of *hsf* proteins were found ranging from 55 amino acids (PGSC0003DMG402008167) to 536 amino acids (XP_015160911.1 and XP_015160909.1) having molecular weights ranging from 12.20 kDa to 60.25 kDa, respectively (Table 3). Theoretical *pI* values ranged from 4.71 to 9.58. All the 15 *hsf* proteins of potato were found to have negative GRAVY value which ranged from -0.931 to -0.387. The negative GRAVY values of *hsf* suggested hydrophilic nature of these proteins.

The maximum GRAVY value of -0.387 was for protein PGSC0003DMG402008167 and minimum for XP_006363482.1 and PGSC0003DMG400006447 *hsf* gene proteins (-0.931) (Table 3).

In silico analysis of *hsf* proteins demonstrated considerable variability in protein length and molecular weight. This diversity in protein size and characteristics may reflect functional specialization within the *hsf* gene family. The theoretical *pI* values and GRAVY scores suggested a range of hydrophilic properties, potentially influencing the protein's stability and interaction with other cellular components. Although all the *hsf* proteins were found to be hydrophilic nature, yet their degree of hydrophilicity difference from each other as evidenced by different GRAVY value of these *hsf* proteins of potato. The GRAVY is an important physicochemical parameter that reflects the overall hydrophobic or hydrophilic nature of a protein. Proteins with

Table 3. Molecular weight, theoretical *pI* and GRAVY value of predicted potato HSF gene proteins.

S. no.	Protein ID	Numbers of nucleotide in CDS/ ORF	Numbers of amino acids in deduced protein	Mol. Wt. of deduced protein kDa	Theoretical <i>pI</i>	GRAVY
1.	PGSC0003DMG400000380	1107	368	40.97	5.86	-0.614
2.	PGSC0003DMG400006447	1089	362	42.26	5.46	-0.931
3.	PGSC0003DMG400007962	1119	372	42.38	7.76	-0.714
4.	PGSC0003DMG400008223	1062	353	40.71	4.92	-0.659
5.	PGSC0003DMG400014811	1497	498	55.72	5.01	-0.671
6.	PGSC0003DMG400017846	1527	508	56.25	5.15	-0.658
7.	PGSC0003DMG400027036	1446	481	54.42	5.17	-0.578
8.	PGSC0003DMG400027283	744	247	28.70	8.68	-0.754
9.	PGSC0003DMG400028416	1023	340	38.45	6.59	-0.909
10.	PGSC0003DMG400032793	1086	361	41.76	5.28	-0.825
11.	PGSC0003DMG400041361	1236	411	46.95	4.92	-0.809
12.	PGSC0003DMG400043234	741	246	28.71	9.30	-0.651
13.	PGSC0003DMG401004023	909	302	33.29	5.75	-0.754
14.	PGSC0003DMG401008167	735	244	28.38	6.13	-0.857
15.	PGSC0003DMG402008167	168	55	6.02	8.12	-0.387

positive GRAVY values are generally more hydrophobic and are often associated with membranes or lipid environments, whereas proteins with negative GRAVY values are more hydrophilic, making them more likely to be soluble in aqueous environments. GRAVY analysis provides valuable insights into protein solubility, folding, stability, membrane association, and subcellular localization. Proteins with lower GRAVY values are generally more hydrophilic, which might be advantageous in stress conditions where solubility and interactions with water-soluble molecules are critical.

Phylogenetic analysis of potato *hsf* genes

This phylogenetic framework (Fig. 3) provided a comprehensive overview of the evolutionary dynamics, gene duplication events, and divergence patterns within the potato *hsf* gene family, shedding light on their evolutionary history and potential functional

specialization. This analysis involved 58 *hsf* protein sequences. All ambiguous positions were removed for each sequence pair (pairwise deletion option). Total of three major groups were deduced from the phylogenetic

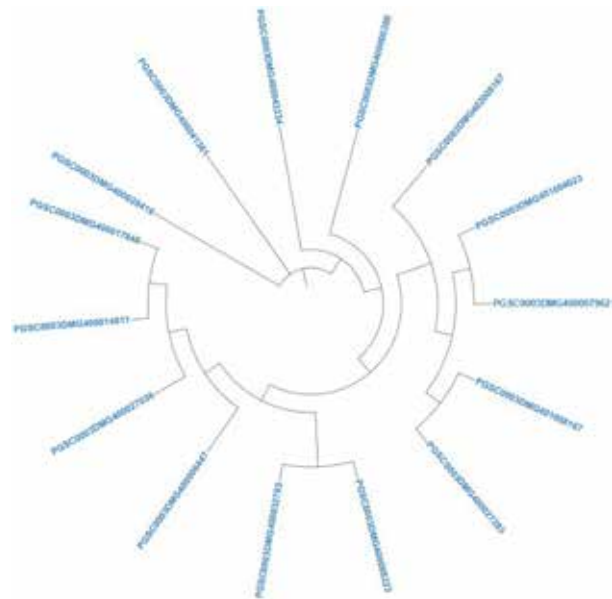


Fig. 3. Phylogenetic tree of potato *hsf* genes.

tree (Fig. 3). Yu *et al.* 2022 comprehensively identified 2950 *Hsf* family genes from the whole genome of 111 representative plants and conducted systematic comparative analysis to reveal their basic characteristics, conserved motif, duplications and losses, evolutionary, expression patterns, and phylogenetic relationships. The phylogenetic analysis of potato *hsf* genes revealed three major groups, each possibly representing different evolutionary lineages or functional specializations. This classification provides insights into the evolutionary dynamics and gene duplication events within the potato *hsf* gene family. The phylogenetic relationships and groupings observed in our study align with known patterns of gene evolution and functional diversification in plants, highlighting the complexity of stress response mechanisms. The genome evolutionary mechanisms and expansions of gene families are mainly depended on gene duplication events. The major duplication patterns for gene families are tandem duplication and segmental duplication (Vision *et al.* 2000; Kong *et al.* 2007).

Amplification and cloning and sequencing of *hsf* genes

Of the primers designed for all the fifteen *hsf* genes, only five *hsf* genes viz., *HP1*, *HP2*, *HP4*, *HP9*, and *HP10*, from various potato tissue samples, were successfully amplified. The amplicons were then cloned and sequenced. In order to establish the identity of the cloned genes the obtained nucleotide sequences were subjected to BLAST analysis. The Blast analysis confirmed the identity of the amplified and sequenced amplicons to be *hsf* genes. Sequences of partially amplified genes viz: *HP1*, *HP2*, *HP4*, *HP9*, and *HP10* were redesignated as *St_hsf1*, *St_hsf2*, *St_hsf4*, *St_hsf9* and *St_hsf10* high temperature shock transcription factor gene, respectively. These

partial gene sequences were submitted to NCBI and were allocated accessions nos. MW001209, MW001210, MW001211, MW001212, and MW001213, respectively. The deduced amino sequences of these cloned genes are presented in Fig. 4.

Expression analysis of amplified genes

In order to gain insights into the role of HSPs in potato under abiotic stresses, the expression dynamics of the cloned *hsf* genes (*HP1*, *HP2*, *HP4*, *HP9* and *HP10*) in response to drought and high temperature stress was studied. As described under Materials

NCBI Acc no. MW001209, Gene: *HP1*
 ccaat ggt cca gat gga gta ggt tgg ttt tta atg gaa gga gag ata gta agt ttt agt ttt
 H C P D G V G S E S M E U Q I V K F E S
 gac ttt caa gac att gca gga tgg ttt gac agc cca gat ttc aat aca gtt gtt gat cca
 D F Q S L A G C F D S P D F H T V V U Q
 caa ctc cca gaa act ggt tta gga ggt gaa gac atg cct ttt gaa ggt ggt act gtt gaa
 C L F E T S L S A E A M P F E U S T V A
 tcc gaa gga tgg aca gta gaa gat gaa att ttt aat tgt tca gat agt gaa ata gga gga
 S E C L T V A K E L F N C S D R E I G C
 gtt taa ttt ttt aac ccc gaa ggt ttt aat ttc aac aag aag aat gta gtt agt att caa
 A S F F H D E G S H F K G K H V A S L Q
 tta gaa gtt atg cct gag taa ttt gaa ttt ttt aca gag gag atg ggg aag gag aac
 L E V M P E Y F A S F F E E M G K E N
 NCBI Acc no. MW001210, Gene: *HP2*
 catta gaa aat gga aac aag aag aag atg ttt att gtt gaa aat cca gag gag taa aca
 V E N C T K E K A R L Y S U E H N S Y T
 aag att aag tgg gag ata cag aca att ttt ttt ttt gat gaa tgg aat aat att cca gtt
 T I N S E I H T L F S C D E S S H S P V
 gag gaa caa aag ggt aag gga aat aat aat agt agc cca gag atg gta ttt gag aac tat
 E Q N G R G H H N S S D E M V S E H Y
 ata ttt tgg gag aag atg atg gaa gat gaa atg att ttt gat aat gga gaa gaa aca gat
 I L M F R L M E D D M I C E S A E T G
 aat tat cca agt gag ata gtt att gaa att gag gat ttt att aca aac cca tcc gag ttt
 N Y Q S E E I V L E L S D L I S N P S E
 NCBI Acc no. MW001211, Gene: *HP4*
 aga gaa ggt ggt cca gaa aat ggt aca aac cca tca gat ttt ccc cag aat ttt aat gga
 R E E A T A H G T T H S D F A Q N F H G
 gaa aca gaa atg atg cct aag ttt aac aac tgg tta gaa aac aag gag act tat aat ttc
 E R E I T A H F H K S L E R R E T T H F
 gaa ttt aat gag ttt ttt atg gta tat ata agt aat ttt gtt ata aac taa aca gaa
 E F H S L F F R L T L S E F G L H S R E
 aac gta agt aat ttt gaa gta atc taa aac taa aca taa atc ttt gtt gaa gaa taa gaa
 E V S H F R V I S R S I H L A E S A
 gaa gaa ttt gaa aca tcc gat aat ttt att aat taa taa ttt cca taa aat ccc gaa
 A D C A T S D T F L H T S V I L S T L A
 ttt gaa
 F V
 NCBI Acc no. MW001212, Gene: *HP9*
 togtt gtt tgg aat cca cct gag ttc gct aga gac ttt att cgt aac taa ttt aag cat aac
 V V M N P V E F A R D L L V E I F E H N
 aat ttc tcc agt ttt atc aca cca cct aat att ttt tgg ttt gaa aat gtt gtt gtt gaa
 N F S E F I H Q S N T Y C A K V U D D E
 caa tgg gag ttt tgg aac gag gag ttt tta aca ggt gag aat ttt ttt gag aat att
 Q H E F A H E E F L R G Q R H L L X H I
 taa aca gaa aag cgt atc att agt cca ttt gaa aca ggt gag taa gtt gtt taa ttt aag
 T S R E P I H S H S A T S Q E V A S L T
 gat tgg gag aca gag tat gaa gat gaa att gag agt ttt aag aca gaa aac agt atg
 D R E R Q S Y E D E I E R L E R E N S M
 ctt gag taa ttt gtt g
 L Q S S A
 NCBI Acc no. MW001213, Gene: *HP10*
 togtt gtt tgg aat cca cct gag ttc gct aga gac ttt att cgt aac taa ttt aag cat aac
 V V M N P V E F A R D L L V E I F E H N
 aat ttc tcc agt ttt atc aca cca cct aat att ttt tgg ttt gaa aat gtt gtt gtt gaa
 N F S E F I H Q S N T Y C A K V U D D E
 caa tgg gag ttt tgg aac gag gag ttt tta aca ggt gag aat ttt ttt gag aat att
 Q H E F A H E E F L R G Q R H L L X H I
 taa aca gaa aag cgt atc att agt cca ttt gaa aca ggt gag taa gtt gtt taa ttt aag
 Y H R E P I H S H S A T S Q E V A S L T
 gat tgg gag aca gag tat gaa gat gaa att gag agt ttt aag aca gaa aac agt atg
 D R E R Q S Y E D E I E R L E R E N S M
 ctt gag taa ttt gtt g
 L Q S S A

Fig. 4. Accession numbers, nucleotide sequences and the deduced amino acid sequences of partially cloned *hsf* genes from potato

and methods section, K. Jyoti, K. Pukhraj cultivars of potato were used for drought. And for high temperature stress K. Surya and K. Chandramukhi potato cultivar were used. Integrated density value (IDV) based expression analysis of selected amplified *hsf* genes in K. Jyoti, K. Pukhraj for drought stress while K. Surya and KCM (KCM) for high temperature stress conditions. The expression analysis of amplified genes (*HP1*, *HP2*, *HP4*, *HP9* and *HP10*) was performed under drought and high temperature stress conditions in potato.

In case of drought tolerant cultivar i.e. K. Jyoti expression of all the *hsf* genes i.e. *HP1*, *HP2*, *HP4*, *HP9* and *HP10* were increased both in leaf as well as root tissues (Fig. 5). In comparison to control, increase in expression in leaf tissue of plants under drought, was found to be 51%, 1263 %, 232 %, 66 % and 1042 % of *HP1*, *HP2*, *HP4*, *HP9* and *HP10*, respectively. In roots, 126%, 448%, 13911%, 501%, 94% higher expression of *HP1*, *HP2*, *HP4*, *HP9* and *HP10* was observed in roots of plants exposed to drought (Fig. 5).

On the other hand, expression of all these 5 *hsf* genes was found to decrease in leaf tissue of plants under drought as compared to that in respective control (Fig. 5). The decrease

in expression was 35 %, 23%, 96%, 24% and 62%, of *HP1*, *HP2*, *HP4*, *HP9* and *HP10*, respectively. In roots, 86 %, 95%, 94% and 97% decrease in expression was observed of *HP2*, *HP4*, *HP9* and *HP10*, respectively (Fig. 6). However, 28% increased expression of *HP1* was observed in roots of plants under drought, as compared to the respective control (Fig. 6)

In case of high temperature stress, the expression analysis showed that expression of most of the cloned *hsf* genes decreased in both high temperature tolerant (K. Surya) as well as susceptible cultivars (K. Chandramukhi) (Fig. 7). However, the decrease was comparatively more in case of K. Chandramukhi (high temperature susceptible cultivar) as compared to that in K. Surya (high temperature tolerant cultivar). Seventy-seven, and 32% decrease in expression of *HP2* and *HP10* genes was observed in leaves of K. Surya exposed to high temperature as compared to respective control. Not much change in expression was observed of *HP1*, *HP4* and *HP9* genes. In case of K. Chandramukhi, the decrease in expression was observed to be 44%, 62%, 87%, 98% and 83% of *HP1*, *HP2*, *HP4*, *HP9* and *HP10*, respectively (Fig. 7).

Differential expression dynamics of these five *hsfs* suggests that these *hsf* genes

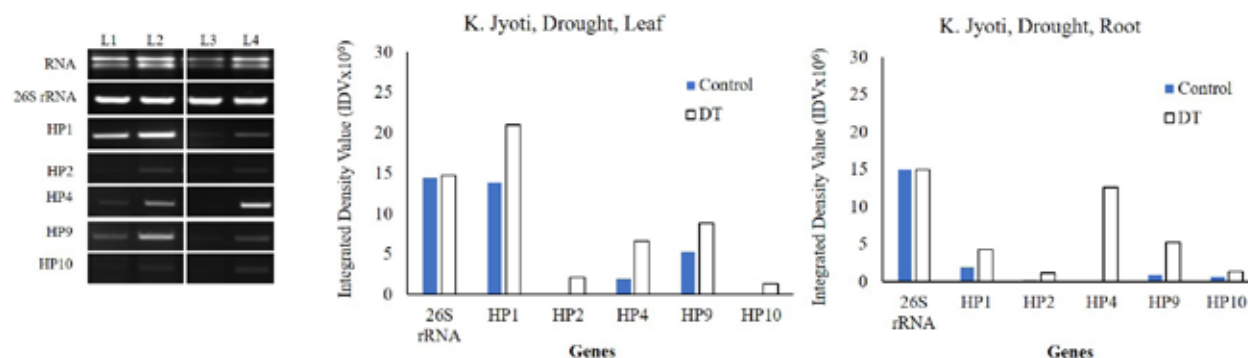


Fig. 5. Expression pattern of *hsf* genes in response to drought in potato leaf. Total RNA was extracted from leaves of potato plants subjected to different levels of water stress vis-a vis control; and subjected to semi-quantitative RT-PCR based expression analysis. A constitutive 26S rRNA gene was used as an internal control for equal loading. Lane L1: K. Jyoti, Leaf, Control; L2: K. Jyoti, Leaf, Drought Treatment; L3: K. Jyoti, Root, Control; L4: K. Jyoti, Root, Drought Treatment.

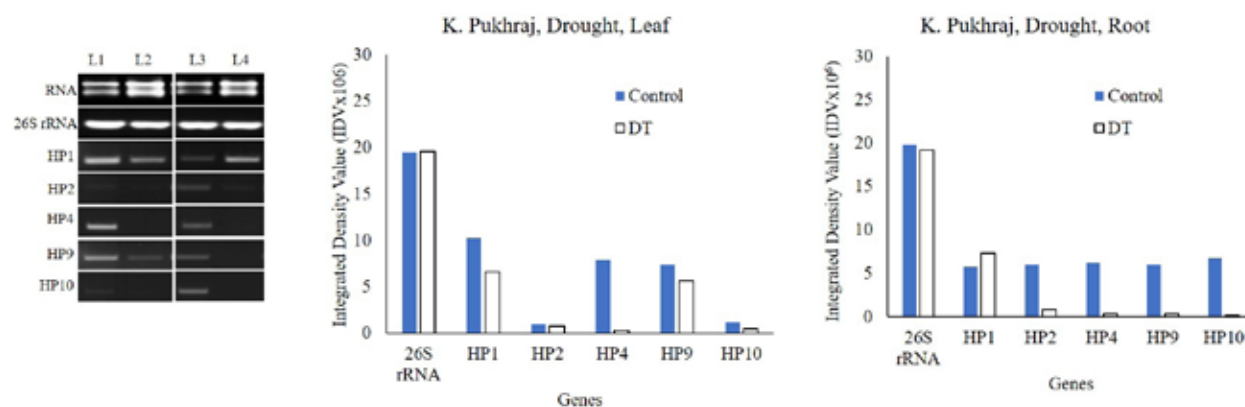


Fig. 6. Expression pattern of *hsf* genes in response to drought in potato root. Total RNA was extracted from root of potato plants subjected to different levels of water stress vis-a vis control; and subjected to semi-quantitative RT-PCR based expression analysis. A constitutive 26S rRNA gene was used as an internal control for equal loading. Lane L1: K. Pukhraj, Leaf, Control; L2: K. Pukhraj, Leaf, Drought Treatment; L3: K. Pukhraj, Root, Control; L4: K. Pukhraj, Root, Drought Treatment.

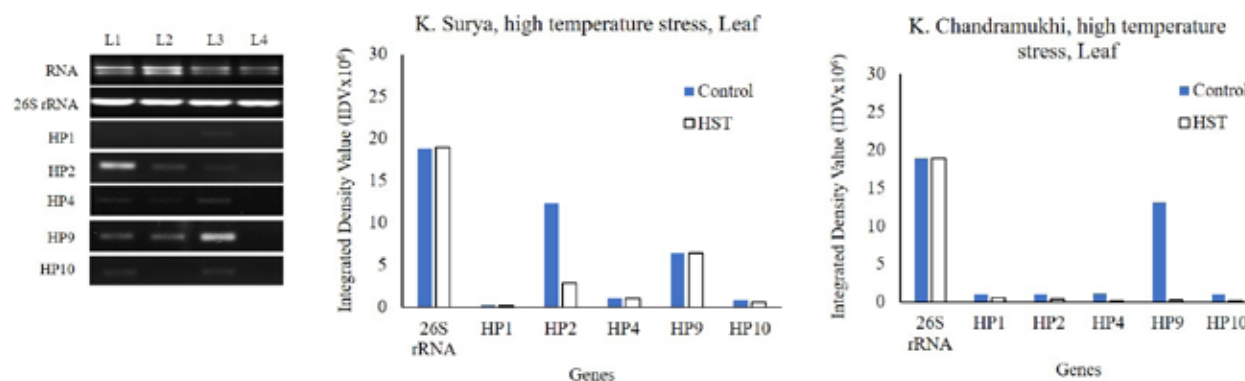


Fig. 7. Expression pattern of *hsf* genes in response to high temperature stress in potato. Total RNA was extracted from leaves of potato plants subjected to high temperature (vis-a vis control); and subjected to semi-quantitative RT-PCR based expression analysis. A constitutive 26S rRNA gene was used as an internal control for equal loading. Lane L1: K. Surya, Leaf, Control (18°C); L2: K. Surya, Leaf, high temperature stress (26°C); L3: K. Chandramukhi, Leaf, Control (18°C); L4: K. Chandramukhi, Leaf, high temperature stress (26°C).

may be playing diverse roles in potato in response to various abiotic stresses like drought and high temperature stresses. Mishra *et al.* 2002 generated transgenic tomato plants with altered expression of high temperature stress transcription factor *HsfA1*. *HsfA1a* has been reported as an exceptional master transcription factor of induced high temperature tolerance in tomato but no similar *hsfs* were found in *Arabidopsis* (Lohmann *et al.*, 2004; Von Koskull-Döring *et al.*, 2007). Previous researchers have also contributed valuable insights into the *hsf* gene family

and their roles in stress responses. Tang *et al.* (2016) identified twenty-seven *hsf* members by bioinformatics and phylogenetic analyses, classifying them into A, B, and C groups according to their structural and phylogenetic features. They found that the *hsfs* were located on 10 of the 12 chromosomes, except chromosomes 1 and 5, and that 18 of these genes formed 9 paralogous pairs. Expression profiles of *hsfs* in 12 different organs and tissues revealed distinct spatial expression patterns and potential roles in growth and development. Promoter and quantitative

real-time PCR analysis demonstrated that these genes responded to various stresses, indicating their diverse regulatory roles under adverse conditions. Additionally, Zhao *et al.* (2018) identified 48 putative potato *Hsp20* genes and found that most possessed no, or only one, intron. Their phylogenetic analysis grouped these genes into 12 subfamilies and identified stress response cis-elements in nearly all of them. Real-time quantitative PCR showed that the *Hsp20* genes responded to multiple abiotic stresses, with significant upregulation under high temperature stress. Furthermore, Zhu *et al.* (2021) analysed the *hsf* gene *SpHsfA4c* isolated from a wild potato (*Solanum pinnatisectum* Dun), showing that *SpHsfA4c* encodes a protein with conserved *HsfA* characteristics. Overexpression of *SpHsfA4c* in potato lines resulted in enhanced high temperature tolerance, improved cell membrane stability, increased antioxidant capacity and osmotic adjustment ability; demonstrating its positive regulation of high temperature tolerance and potential as a candidate gene for genetic engineering in potato breeding.

The expression patterns of *hsf* genes in different tissues have been described in many species, such as *Arabidopsis*, pepper, rice and tomato in several different researches (Siddique *et al.*, 2008; Guo *et al.*, 2015; Ouyang *et al.*, 2009; Yu *et al.*, 2016). There is no uniform gene expression pattern for *hsf* genes in different plants. According to the RNA-seq data of potato different *hsf* proteins might have diverse functions and different *hsf* genes such as *StHsp20-22* and *StHsp20-41* exhibited incompatible expression patterns in various tissues (Zhao *et al.*, 2018).

CONCLUSION

The present study reveals that all genome-wide landscape of *hsf* genes in potato

genome and difference and similarities in their structure and biochemical properties. Higher expression of the cloned *hsf* genes in drought and high temperature tolerant as compared to that of in susceptible potato cultivars suggests their involvement in imparting stress tolerance to potato plants. These results highlight the importance of *hsf* genes in regulating plant responses to high temperature and drought stresses, thus providing a model which could be used for enhancing plant resilience. The integration of molecular, bioinformatics and functional analyses in this study provides a robust framework for further research. In conclusion, our study confirms the critical roles played by *hsf* in a stress response system and sets the stage for future studies that aim at building more climate resilient potato cultivars. Future studies should focus on functional characterization of these genes to elucidate their specific roles in stress tolerance.

DECLARATION OF COMPETING INTEREST

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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CONFLICT OF INTEREST

“Som Dutt and Hemant B. Kardile are the Editor-in-Chief and Senior Editor of Potato Journal, respectively and were not involved in the editorial review or decision-making

process for this manuscript. The peer review and editorial handling were managed entirely independently by an alternative editor.” Other authors declare that they have no conflict of interest

ETHICAL STATEMENT

This article does not contain any studies with human participants or animals performed by any of the authors

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