Journal of Experimental Biology and Agricultural Sciences, October - 2024; Volume – 12(5) page 730 – 741

Journal of Experimental Biology and Agricultural Sciences

http://www.jebas.org

ISSN No. 2320 – 8694

Real-time and *in silico*-based characterization of the heat stress-responsive gene *TaGASR1* from Indian bread wheat

Satish Kumar[1,2], Jasdeep C. Padaria[2], Hardeep Singh Tuli[1], Pawan Kumar[3], Ritu Chauhan[4], Damandeep Kaur[5], Sachin Kumar Mandotra[1], Diwakar Aggarwal[1*]

[1]Department of Bio-Sciences and Technology, Maharishi Markandeshwar Engineering College, Maharishi Markandeshwar (Deemed to be University), Mullana, Ambala 133207, Haryana India
[2]National Institute for Plant Biotechnology, Pusa Campus, New Delhi-110012, India
[3]Institute of Plant Sciences, Agricultural Research Organization (ARO), The Volcani Center, Rishon LeZion, 7505101, Israel
[4]Department of Biotechnology, Graphic Era Deemed to be University, Dehradun 248002, Uttarakhand, India
[5]University Center for Research & Development (UCRD), Chandigarh University, Gharuan, Mohali, Punjab, India

Received – August 14, 2024; Revision – November 05, 2024; Accepted – November 17, 2024
Available Online – November 29, 2024

DOI: http://dx.doi.org/10.18006/2024.12(5).730.741

KEYWORDS

High-temperature

Real-time PCR

in silico studies

Gibberellin-stimulated transcript

Genetic resource

ABSTRACT

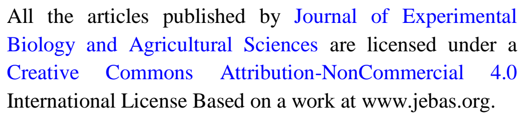
Wheat is a staple food for 80% of the global population, offering essential protein, calories, and nutrients. Earlier wheat heat interaction studies revealed that increasing temperatures can severely hinder plant growth and development, increasing overall productivity and sensitivity to extreme temperatures during seed emergence and anthesis. In this study, *TaGASR1* (*gibberellic acid-stimulated regulator 1*), a potential candidate for heat stress resistance, was isolated, and its expression was found to be significantly greater in HD3086 wheat than in HD2894 wheat at both the seedling and anthesis stages after exposure to 42 °C heat stress (HS). Furthermore, *in silico* studies validated the molecular findings, revealing a CDS region of 297 nucleotides with 2 ORFs, with ~93% sequence similarity to the *TaGASR1* gene from the TAM107 wheat variety. A 3D model of the target protein was designed using the C8C4P9.1 template, showing 95.92% sequence similarity and 100% query coverage with the gibberellin-stimulated transcript. Furthermore, studies of the conserved motifs and protein-protein interactions of the *TaGASR1* protein have identified three major functional partners: cold acclimation proteins, ABA-inducible proteins, and protein phosphatase 2C, emphasizing its role in abiotic stress responses. Hence, the *TaGASR1* gene is a promising candidate for further studies, as it positively responds under HS conditions. Therefore, future research should focus on its role across different species to cultivate heat-tolerant varieties, supporting sustainable development amid climate change. This would

* Corresponding author

E-mail: diwakaraggarwal@yahoo.co.in (Diwakar Aggarwal)

Peer review under responsibility of Journal of Experimental Biology and Agricultural Sciences.

Production and Hosting by Horizon Publisher India [HPI] (http://www.horizonpublisherindia.in/).
All rights reserved.



All the articles published by Journal of Experimental Biology and Agricultural Sciences are licensed under a Creative Commons Attribution-NonCommercial 4.0 International License Based on a work at www.jebas.org.

encourage breeders and researchers to use this gene to advance wheat crop development, considering current and anticipated environmental conditions.

1 Introduction

The increase in urbanization and population has resulted in a shortage of cultivated land, which ultimately has been a great issue for agriculturists. Environmental change has also worsened the current scenario, making it difficult for plants to survive; ultimately, food security is a great warning for plant biologists. A comprehensive understanding of how environmental stress enhances yield under challenging conditions is essential to navigate this scenario. Expeditious climate modifications and global warming lead to a decrease in the productivity of crops and hinder their growth, which is a major challenge in the cultivation period. The deprivation of crop production is caused by various environmental conditions, including abiotic factors such as salinity, drought and heat stress (HS). The maximum temperature is often expected in major wheat growing zones, sometimes above 5°C above the standard temperature (Hatfield and John 2015; Grosse-Heilmann et al. 2024).

Wheat (*Triticum aestivum* L.) is adapted for cultivation in a wide range of climatic conditions, making it the most important crop in the world, with an approximate production of 784.91 million metric tons in 2023-2024 (https://www.statista.com/statistics/267268/production-of-wheat-worldwide-since-1990/). It is estimated that by 2050, food production will need to increase by 60-70% to meet rising demands. However, continuously increasing the temperature may also induce osmotic stress, resulting in high salt concentrations and a decrease in yield per unit to approximately 2.8 tonnes ha^{-1} (Masarmi et al. 2023). Therefore, combined stresses often impact wheat plants, highlighting the need for research on the adverse effects of heat stress. Future efforts should focus on developing genotypes that can withstand varying environmental conditions. Therefore, quality and production rates are affected worldwide by these types of environmental stresses, including heat and drought, during the growing season.

For food safety, the advancement of heat-tolerant varieties and enhanced pre-breeding materials is vital for any breeding program because of the increase in climatic temperature, which ultimately affects productivity (Ortiz et al. 2008; Tripathi et al. 2016; Sarkar et al. 2021). Proteins and genes related to the environment can be determined via transcriptomic and proteomic data, but further research is needed to develop various methods for adjusting to high temperature and climate variation (Altenbach 2012; Jiang et al. 2020). In wheat, embryonic cells are affected at 45 °C, which subsequently harms seed germination (Essemine et al. 2010; Khaeim et al. 2022). Heat stress promotes abscission, and leaf senescence causes a reduction in growth and photosynthesis (Kosova et al. 2011; Farhad et al. 2023). However, if a genotype develops with agronomic rehearses, such effects can be achieved (Asseng et al. 2011; Chapman et al. 2012; Gawdiya et al. 2023).

Genetic modification is the only way to increase the stress resistance level of complex genome crops, such as wheat crop systems, in response to various environmental stresses (Chapman et al. 2012; Villalobos-López et al. 2022). Therefore, it is necessary to study the complexity of each crop genome and characterize heat stress-responsive TFs (transcription factors) from this large Hsfs family of crops involved in genetic improvement to increase heat tolerance, which could provide insight into wheat-heat interactions (Clavijo et al. 2017). Genetic engineering techniques such as transgenic approaches would more effectively alter the plant response to different stresses, which require the desired gene of interest (Zheng et al. 2012; Parmar et al. 2017). In recent studies, the complete genomes of many crop species have been sequenced, which proves their complexity level and the presence of genes, TFs and Hsps, as reported for genetic improvement to overcome high-temperature conditions (Wang et al. 2016; Clavijo et al. 2017). Therefore, several genes reported from different crop systems, including 21 from tomato, 97 from Rice and 82 from wheat, are involved in heat stress tolerance (Gua et al. 2015; Duan et al. 2019; Liu et al. 2023).

Similarly, the *gibberellic acid-stimulated regulator gene* (*GASR*) is a well-known heat stress-responsive gene involved in several biological processes belonging to the *GA-stimulated transcript* (*GAST*) family of genes, whose expression can be altered according to environmental conditions (Cheng et al. 2019). Earlier studies identified 15 *AtGASR*, 11 *OsGASR* and 37 *TaGASR* genes in various plant systems, such as *Arabidopsis thaliana*, Rice and wheat, respectively. Two homologues of *GASR*, namely, *OsGASR1* and *OsGASR2,* were identified in Rice and play a role in the division and differentiation of panicles. Furthermore, both were highly expressed in the florets and branches, as confirmed through transient expression experiments (Furukawa et al. 2006). The wheat *TaGASR1* gene has been reported in the heat-tolerant wheat variety TAM107, which shares 51.52% similarity with the rice *OsGASR1* gene (Zhang et al. 2017). The results revealed the existence of HS elements and several cis-elements involved in various HS-related pathways. Furthermore, their over-expression in *A. thaliana* and wheat heat-susceptible varieties enhances their tolerance to heat stress and decreases ROS accumulation (Zhang et al. 2017). Furthermore, 37 *TaGASR* genes were identified in common wheat (*Triticum aestivum* L.), designated TaGASR1-37.

In the present study, the *TaGASR1* gene was isolated from the heat-tolerant Indian bread wheat variety HD3086 known as 'Pusa Gautami'. The expression pattern of the *TaGASR1* gene was

analyzed through real-time PCR and subsequently cloned and inserted into the pJET1.2 blunt-end sequencing vector for further confirmation via Sanger sequencing. The obtained sequence data for the *TaGASR1* gene were submitted to the online NCBI database. *In-silico* analysis was carried out to validate the molecular work by performing multiple sequence alignments with orthologous species, phylogenetic tree analysis, secondary and 3D protein structure prediction, location in plant cells and conserved domain analysis, which demonstrated its importance in providing heat stress (HS) resistance in host plant species. Characterizing this gene through expression analysis and *in silico*-based studies could improve understanding of its structure and functions under HS conditions.

2 Materials and methods

2.1 Plant material and stress treatment

Seeds of high-temperature resistant and susceptible varieties of Indian bread wheat cv. HD3086, commonly called 'Pusa Gautami' and cv. HD2894 were obtained from National Seed Corporation, IARI, New Delhi, India, and grown in 6-inch pots at MMDU (Mullana, India) in growth chambers with 60 to 70% relative humidity. The photoperiod was 16/8 h light and dark at 25 ± 2 °C, with the optimum light intensity applied to the plants (100 $\mu mol/m^2/s$). Plants of both cultivars were exposed to extreme temperature treatments of 37 °C and 42 °C in a BOD incubator at the seedling stage (15 days old) and anthesis phase for 4 h by continuously increasing the temperature (1 °C per 10 min) (Vishwakarma et al. 2018). For subsequent experimentation, control plants were also maintained under normal growth conditions (25 ± 2° C). The leaf tissues were harvested from stressed and unstressed plants, immediately frozen in liquid N_2 and placed at -80 °C (ultradeep freezer) for further RNA extraction.

2.2 RNA isolation, cDNA synthesis, and semiq and real-time PCR analysis

Using a Plant Total RNA Kit (Sigma, USA), total RNA was isolated from heat-stressed and unstressed frozen leaves of HD3086 and HD2894 and passed through an on-column DNase I Kit (Sigma, USA) to remove unwanted gDNA contamination. To check purity and integrity, all the eluted RNA products were analyzed via a nanodrop (Thermo Scientific, USA), and their concentration was checked by the naked eye on a 1.2% TBE gel. Approximately 1µg of RNA template from both heat-stressed and normal-grown plants was used for cDNA synthesis via a SMART PCR cDNA synthesis kit (Clontech Laboratories, USA) according to the instructions provided by the manufacturers.

Semiquantitative PCR was performed to analyze the expression of the *TaGASR1* gene at the seedling and grain-filling stages via gene-specific primers with ~100 ng of template (cDNA). To compare the intensity of the TaGASR1 bands, *TaActin* was detected and analyzed on 1.2% agarose after PCR. qRT–PCR was performed for expression analysis of the *TaGASR1* gene (Bio-Rad, USA), and the housekeeping gene (*TaActin*) was used as an endogenous control to normalize the target gene transcripts via the following reaction program: 3 min at 94 °C (initial denaturation), 35 cycles of target amplification followed by 94 °C for 15 sec, 60 °C for 15 sec, and 72 °C for 15 sec. The generated melting curves describe the interaction of the templates with the gene-specific primers, and each reaction was performed in three biological replicates. Furthermore, the Ct value was recorded for all samples separately, and the fold change in the *TaGASR1* gene was calculated according to the $2^{-\Delta\Delta Ct}$ equation (Livak and Schmittgen 2001; Rao et al. 2013).

2.3 Full-length gene isolation

The coding DNA sequence of the *TaGASR1* gene was amplified from the HD3086 (heat stress-tolerant) wheat variety via gene-specific primers. The forward (F) and reverse (R) primers already contained *Bam*HI and *Sac*I restriction sites (Table 1). PCR was carried out to amplify specific sequences of the gene via the proofreading polymerase enzyme and *TaGASR1* primers via the following program: 3 min at 94 °C (initial denaturation), followed by 29 cycles of target amplification (94 °C - 30 sec, 60 °C - 30 sec, 72 °C - 30 sec) with extension for 10 min at 72 °C.

Table 1 List of primers used in the study

Primer name	Sequence (5′–3′)
TaGASR1	F- GGATCCTGCTCCTCGTCTTGCTCGT
	R- GAGCTCAGAAGCCGTTGGTGCGTT
β-actin	F- GAAGCTGCAGGTATCCATGAGACC
	R- AGGCAGTGATCTCCTTGCTCATC

The band intensity of the PCR target product was observed (1.5% agarose) and eluted via a gel extraction kit (Qiagen, USA). The extracted product was cloned and inserted into the pJET1.2 blunt-end easy vector (Thermo Scientific, USA) *via* transformation into *E.coli* cells. Vector backbone-specific flanking primers were used for screening colonies; positive clones were inoculated in 10 mL of Luria broth supplemented with ampicillin (100 µg/mL) and incubated on a shaker at 200 rpm and 37 °C overnight. To check the presence of the insert, the recombinant plasmid was isolated via a miniprep kit (Qiagen, USA) and digested with the *Eco*RI enzyme. The accession number was received after the CDS was submitted to the NCBI database.

2.4 *In silico*-based studies

Computer-assisted protein sequence studies are needed to confirm the results and reference several biological observations.

ProtParam software tools were used to calculate the amino acids present in the TaGASR1 protein (http://web.expasy.org/protparam/) (Gasteiger et al. 2005; Roy et al. 2011). The location of the *GASR1* gene in plant cells was studied via the CELLO v.2.5 online tool (http://cello.life.nctu.edu.tw/cgi/main.cgi) (Yu et al. 2006; Yu et al. 2014). Total reading frames were predicted through the NCBI ORF tool (http://www.ncbi.nlm.nih.gov/orffinder/). The UK/Phyre2tool (Phyre2 (http://www.sbg.bio.ic.ac) was used to analyze the secondary structure of the protein, whereas Swiss model software (https://swissmodel.expasy.org) was applied to the 3D structure of the TaGASR1 protein. Furthermore, the generated models were checked through Ramachandran plot analysis via the Vadar 1.8 version tool (http://vadar.wishartlab.com/) (Schwede et al. 2003; Park et al. 2023). To predict the interaction of the TaGASR1 protein with the homologous protein, STRING (https://string-db.org/) software tools were utilized (Szklarczyk et al. 2019). The MEME software tool was applied to predict the conserved domain motifs in TaGASR1(http://meme-suite.org/) (Bailey et al. 2009; Bailey et al. 2015). MEGA11 software performed sequence alignment and constructed a phylogenetic tree for the TaGASR1 protein and its orthologous species (www.megasoftware.net/) (Tamura et al. 2021).

2.5 Statistical analysis

Student's t-test was performed for data analysis, and the significant results are marked with an asterisk (*) if the p-value was ≤ 0.05.

3 Results and Discussion

3.1 RNA isolation, semiq PCR and real-time PCR

The RNA isolated from the HD3086 and HD2894 wheat genotypes was sown with intact 28S and 18S ribosomal RNA bands at their respective locations. A good-quality isolated RNA-synthesized smear of cDNA was obtained. Under thermal stress conditions, *TaGASR1 transcripts* were more abundant in the HD3086 wheat variety than in the HD2894 variety, according to the semi-Q–PCR results. Furthermore, this transcript was highly upregulated in the HD3086 variety at both the seedling and heading stages compared with the HD2894 variety, indicating that the transcripts provide additional thermal stress tolerance to the HD3086 variety (Figure 1).

qRT–PCR of the *TaGASR1* gene revealed various expression patterns with increased heat stress (HS) exposure. The *TaGASR1*gene was upregulated 2.4 and 3.8 fold during the seedling and anthesis stages in the HD3086 wheat variety under high-temperature stress conditions (42 °C) (Figure 1). In the case of the HD2894 variety, the expression of this gene increased from 1.2 - 1.44 fold after exposure to 42 °C HS (heat stress). Similarly, semiquantitative studies revealed the same pattern by analyzing the band intensity of the *TaGASR1* gene compared with that of the internal control *TaActin*. However, based on the semiq- and qRT–PCR results, this gene can be isolated from the HD3086 wheat variety. The qRT-PCR study validated the gene expression differences, with specificity confirmed through melting curve analysis of the amplified products, which revealed single peaks across varying temperatures, as reported by Padaria et al. (2013; 2014).

3.2 Full-length gene isolation and cloning

An intact CDS band (encoding the DNA sequence) of the *TaGASR1* gene appeared at ~300 bp and was cloned and inserted into the empty pJET1.2 vector, as confirmed through colony PCR and restriction endonuclease examination. Furthermore, sequencing analysis of the *TaGASR1* gene revealed its exact size of 297 bp, and the obtained sequence was submitted to the NCBI

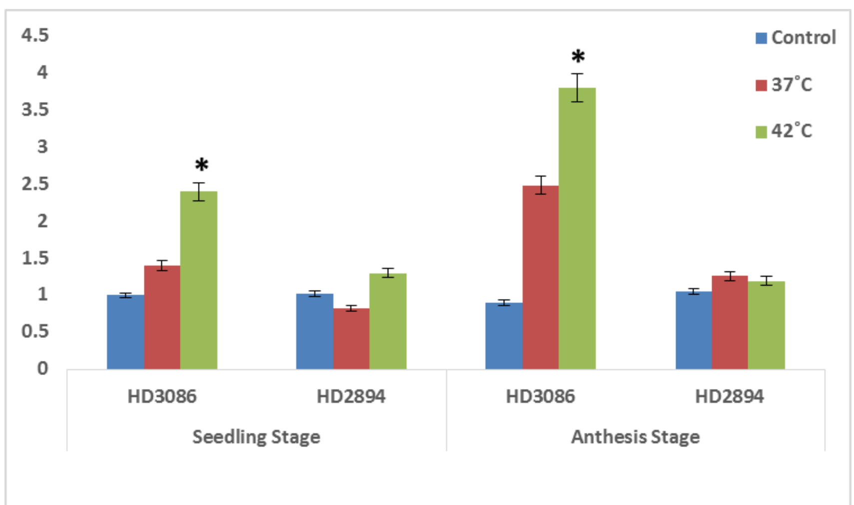


Figure 1 Expression analysis of wheat genotypes, cv. HD3086 and HD2894, before and after the exposure to 37 °C and 42 °C heat stress.

database (accession number: PQ582396). Linearized vector backbone and insert (transgene) bands were observed at ~3 kb and ~297 bp when successfully digested with the *Bam*HI and *Sac*I enzymes. However, these results confirmed that the gene *TaGASR1* (CDS) isolated from the wheat variety HD3086 was successfully cloned and inserted into a pJET sequencing vector. The pipeline used from gene isolation to cloning and sequence confirmation was similar to that used in earlier studies (Vishwakarma et al. 2018; Panzade et al. 2021; Kumar et al. 2023).

3.3 *In silico*-based studies of *the TaGASR1* gene

An *in silico*-based study was carried out using available online software tools to validate the results obtained from the molecular analysis. The physicochemical properties of the target protein, such as the theoretical pI of TaGASR1, molecular formula, chemical formula, and aliphatic index, were computed, and the instability index, amino acid composition, hydropathicity index and total number of positive and negative residues were also described (Table 2). The protein GASR1 has a molecular weight of 10360.36 and a theoretical pI value of 9.30, revealing its basic nature. This analysis also revealed the presence of 17 positively and 6 negatively charged amino acid residues in the GASR1 protein complex, revealing its stability in alkaline environments because these amino acid residues can form ionic bonds in the protein exterior with oppositely charged amino acid residues. The predicted protein was characterized through the ExPASy tool ProtParam, developed by Gasteiger et al. (2005), and the present study showed uniformity with earlier analyses using the same tool (Vishwakarma et al. 2018; Panzade et al. 2021; Kumar et al. 2023).

Table 2 Physicochemical properties of the TaGASR1 protein

Parameters	TaGASR1
Theoretical pI	9.30
Molecular weight	10360.36
Positivechargedamino acids (Arg + Lys)	17
Negative charged amino acids (Asp + Glu)	6
Total no. of Atoms	1442
Molecular formula	$C_{431}H_{732}N_{138}O_{125}S_{16}$
Ext. coefficient	2980
Absorbance at 0.1% (=1 g/L)	0.288
Instability index (unstable)	76.16
Aliphatic index	70.92
Hydropathicity	0.042
localization	Extracellular matrix

Table 3 Amino acid composition of the TaGASR1 protein

Amino acid composition:					
Ala (A)	15	15.3%	Lys (K)	6	6.1%
Arg (R)	11	11.2%	Met (M)	3	3.1%
Asn (N)	0	0.0%	Phe (F)	1	1.0%
Asp (D)	3	3.1%	Pro (P)	7	7.1%
Cys (C)	13	13.3%	Ser (S)	8	8.2%
Gln (Q)	1	1.0%	Thr (T)	3	3.1%
Glu (E)	3	3.1%	Trp (W)	0	0.0%
Gly (G)	7	7.1%	Tyr (Y)	2	2.0%
His (H)	0	0.0%	Val (V)	4	4.1%
Ile (I)	0	0.0%	Pyl (O)	0	0.0%
Leu (L)	11	11.2%	Sec (U)	0	0.0%

Table 4 Atomic composition of the TaGASR1 protein

Atomic composition:		
Carbon	C	431
Hydrogen	H	732
Nitrogen	N	138
Oxygen	O	125
Sulphur	S	16

Further details of the amino acids and atomic composition of the TaGASR1 protein sequence are given in Tables 3 and 4. A total of 16 amino acids were formed in the complete structure of the protein, of which Ala, Arg, Cys, and Leu presented relatively high Mole percentages (Table 3). The most prevalent atoms involved in the consensus protein atomic composition, such as 431 carbon atoms, 732 hydrogen atoms, 125 oxygen atoms, 138 nitrogen atoms and only 16 sulphur atoms, are given in Table 4. CELLO v.2.0 revealed the subcellular location of the *TaGASR1* gene in the extracellular matrix with 3.432% reliability, whereas the nuclear region was less reliable at 0.546 (Figure 2A).

Only two open reading frames were detected through the NCBI ORF finder tool in the TaGASR1 protein sequence, one on the positive strand and the second on the negative strand (Figure 2B). A total of 41% alpha helices, zero beta sheets and 16% TM helices were detected during the secondary structure prediction of the consensus protein sequence of TaGASR1 with 20% disorder (Figure 3A). C8C4P9.1 was the template selected to predict the 3D structure of the TaGASR1 protein sequence. This C8C4P9.1 gene encodes a gibberellin-stimulated transcript, the alpha-fold DB model of the wheat (*Triticum aestivum*) *GAST1* gene, and no ligand binding site is detected. The consensus protein sequence showed 95.92% sequence similarity and 100% query coverage with the predicted model C8C4P9.1 sequence (Figure 3D). All the predicted models were further confirmed by plotting a Ramachandran plot through the Vadar tool, which revealed the presence of glycine, proline and preproline amino acid residues, including 78 in the core region, which was the most favoured region; 6 in the allowed region, 3 in the generous region and 1 in the disallowed region (Figure 3B).

Our consensus protein sequence showed the most favourable protein-protein interactions with the GAST1 protein sequence of *Triticum aestivum,* which encodes gibberellin-stimulated transcript fragments. The consensus protein sequence showed 95.9% identity, a 191.4-bit score value and a lower e value (1e-48) with the GAST1 sequence. Furthermore, out of the 10 predicted functional partners, Wcor615, Wrab18 and PP2C also interacted with the cold acclimation protein WCOR615, ABA inducible protein and protein phosphatase 2C (Figure 3C).

Only 12 orthologous species of the *TAGASR1* gene were processed for the analysis of motif domains, and only four conserved motifs were identified in all the sequences, such as PTGRSGSRDECPCYRDMLTAGPRKRPKCP, GDAASGFCAG KCAVRCGRSRARGA, CMKYCGLCCEECACV, and AALLLVLLAAASLLQ (Figure 4). Similarly, 20 motifs were observed in TaGASR1 to 37 in an earlier study. Of these, 5 represent the GASA domain, 2 represent the variable region, and 3 represent the peptide signal putative region (Cheng et al. 2019). All the sequences were aligned for pair-wise multiple sequence alignment to interpret SNPs, which are highly similar and less similar in amino acid residues (Figure 5).

All the aligned protein sequences were further processed via the MEGA v.11 software tool. Using the neighbour-joining method, the phylogenetic tree was predicted with a bootstrap value of 1000. Our consensus protein sequence was highly similar to that of only the grass family species, meaning it is highly conserved in Poaceae family crop systems. This study was consistent with earlier studies related to wheat *GASR1* genes and other heat stress (HS)-related genes, and the identified TaGASR1 protein sequence also showed 93% identity with TaGASR1 isolated from the TAM107wheat variety (Cheng et al. 2019; Kumar et al. 2023).

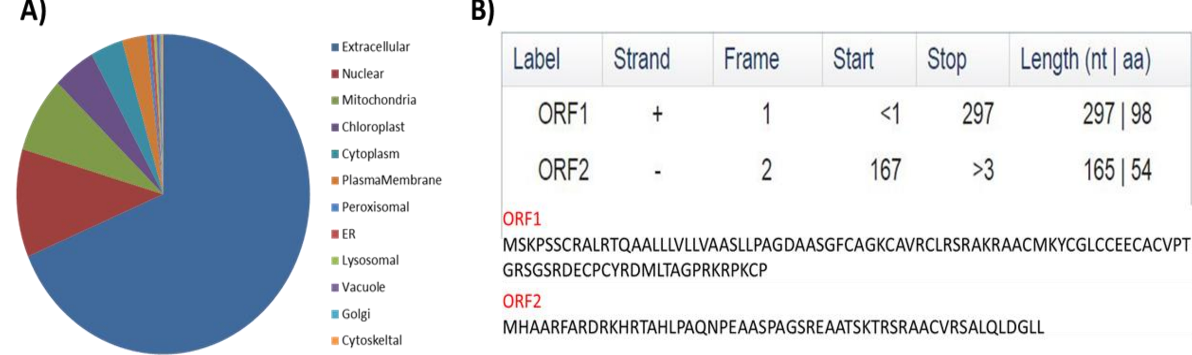


Figure 2 Insilico analysis of protein sequences. A) Subcellular location of the TaGASR1 gene; B) presence of open reading frames

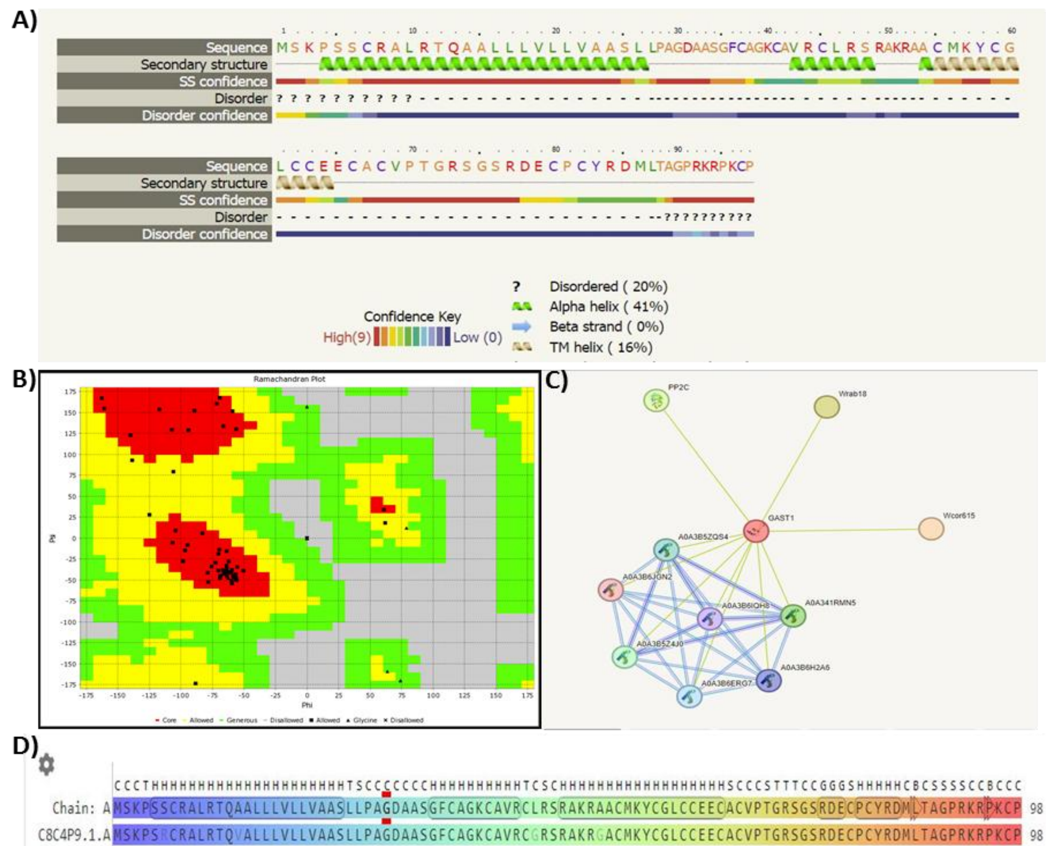


Figure 3 Characterization of the TaGASR1 protein sequence through online tools: A) secondary structure; B) Ramachandran plot; C) protein-protein interaction study via STRING software; D) predicted model template alignment with the consensus protein sequence

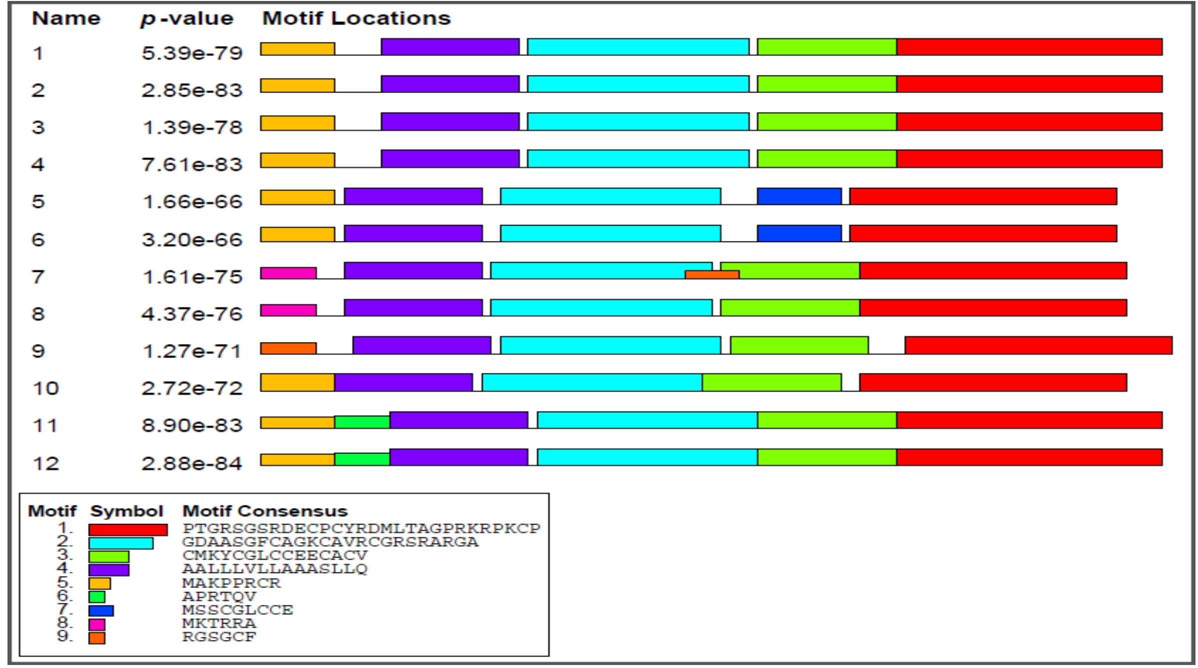


Figure 4 Conserved motif identification in the TaGASR1 protein and its orthologous species

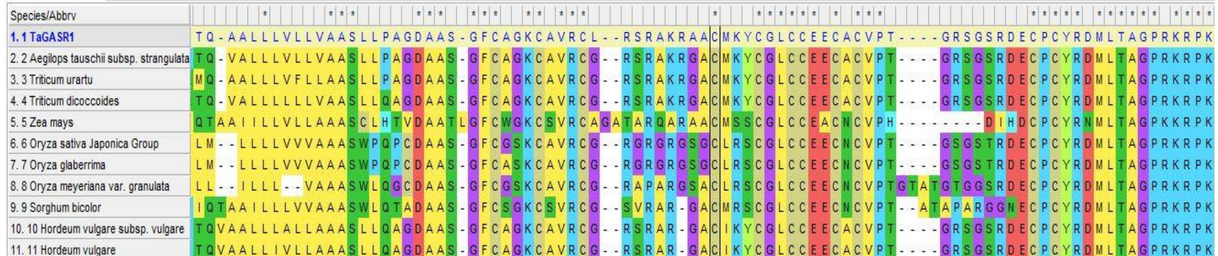


Figure 5 MSA (multiple sequence alignment) of the *TaGASR1* gene with its orthologue species

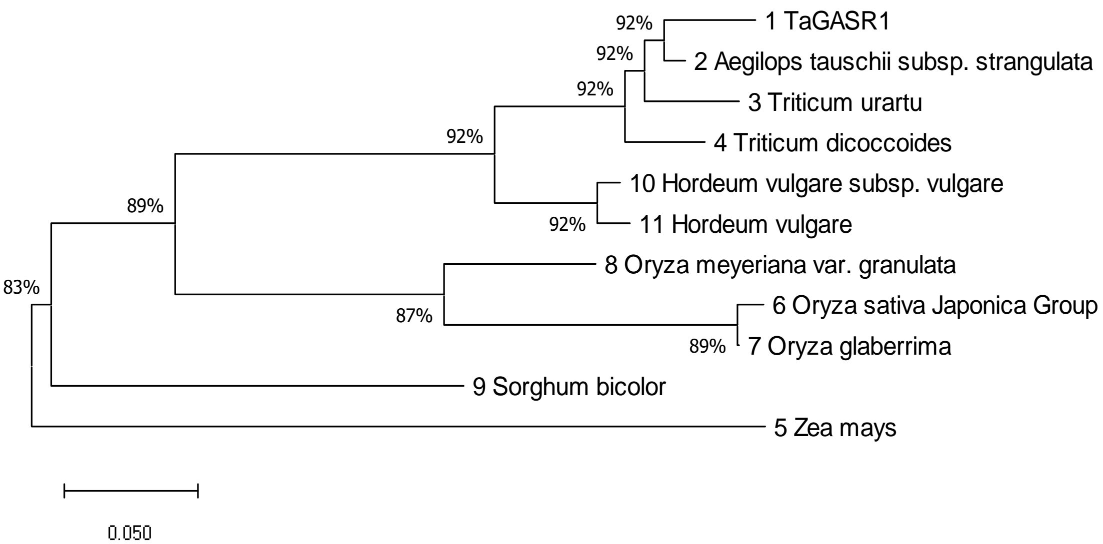


Figure 6 Phylogenetic tree prediction of the TaGASR1 protein with orthologous species via the neighbor-joining method

The protein sequence is closely associated with *Aegilops tauschii* subsp. *Strangulata,* which means that they have higher sequence similarity and query coverage than others. Other species, such as *Triticum urartu*, *Triticum dicoccoides*, *Hordeum vulgare* and *Hordeum vulgare* subsp. *vulgare* were clustered in the same group and presented 92% query coverage. The coverage of the remaining species ranged from 83-89%, indicating a distant evolutionary relationship with the consensus sequence (Figure 6). Similarly, the identified TaGASR1 protein sequence shares 87-89% identity with the Rice and *Arabidopsis thaliana* GASR proteins, revealing its involvement in abiotic stress tolerance (Cheng et al. 2019; Yang et al. 2023).

Wheat is considered a primary staple food crop compared with other cereal crops because of its high nutritional value and good source of protein, which is 15.1% per 100 g, 364 kcal of calories, 71g of carbohydrates, 20% of vitamins B-6 and minerals (19% of iron, 36% of magnesium and 3% of calcium), etc., per 100 g, which completes the daily diet of the total population of the world (Shewry and Hey 2015; Khalid et al. 2023). All these valuable parameters can be reduced due to extreme temperature exposure, especially during the flowering and grain-filling phases, which ultimately causes considerable damage to the wheat reproductive stage and leads to a reduction in grain number, quality and weight (Farooq et al. 2011; Jamil et al. 2019; Matsunaga et al. 2021; Ullah et al. 2022). For the identification and isolation of genes responsible for HS, RNA sequencing and *in silico*-based studies are key methods in plant systems (Wang et al. 2009).

In the present study, the TaGASR1 gene was isolated from the Indian bread wheat variety, known as a heat-tolerant variety; similarly, the same variety, HD3086, was used in an earlier study (Kumar et al. 2023). Gene expression was analyzed through semiquantitative and real-time PCR, and molecular work was subsequently validated via *in-silico* analysis, similar to the pipeline used in earlier studies (Vishwakarma and Sharma 2018; Panzade et al. 2021; Kumar et al. 2023). To the best of our knowledge, this is the first report of *TaGASR1* gene identification from the Indian bread wheat variety HD3086. The expression patterns of the *TaGASR1* gene during the seedling and grain-filling stages under heat stress conditions were investigated for the first time, and the results were consistent with previous studies (Vishwakarma et al. 2018; Kumar et al. 2023). The single peaks formed during the melting curve analysis of the *TaGASR1* gene amplification

products through qRT–PCR confirmed the specificity of the qRT–PCR amplification compared with the use of β-actin as an endogenous control (Padaria et al. 2013; 2014). Several studies have suggested that the genes belonging to the *GAST1* family are always involved in plant development and response to various environmental stresses, including the transition to flowering (Zhang et al. 2009), flower and fruit development (Moyano-Canete et al. 2013), hormonal signal transduction pathways (Rubinovich et al. 2014), and biotic and abiotic stress (Sun et al. 2013; Mao et al. 2011). The consensus protein sequence showed 95.92% sequence similarity and 100% query coverage with the generated template for 3D model prediction through the SWISS-MODEL tool, and the generated template C8C4P9.1 encoded a gibberellin-stimulated transcript, which belongs to the *GAST1* family of genes. Furthermore, our consensus protein sequence also showed higher sequence identity (95.9%) with GAST1 family proteins analyzed through STRING software, where out of ten functional partners, three showed protein-protein interactions with cold acclimation protein, ABA inducible protein and protein phosphatase 2C, which indicated their involvement in abiotic stress tolerance (Bhaskara et al. 2012; Sah et al. 2016).

Zhang et al. (2017) reported that the overexpression of the *TaGASR1* gene in an *A. thaliana* model plant system provides heat stress tolerance and reduces the accumulation of ROS after heat stress induction. However, the consensus CDS of *TaGASR1* has 93% sequence similarity with the TaGASR1 previously reported by Zhang et al. (2017). Only hypothetical pathways of *TaGASR1* are known to date, so more studies related to molecular analysis and *in silico* work, which may provide information about its role in the response to thermotolerance or tolerance to other abiotic stresses, are needed. Furthermore, the *TaGASR1* gene can generate transgenic plants with enhanced thermal stability. *In silico* studies related to *TaGASR1* could help researchers identify specific sites to change to improve their functionality at the gene level. BLAST of the consensus protein sequence revealed hits with mostly Poaceae family member species, indicating that this gene was highly conserved in the grass family, and the phylogenetic tree revealed its close evolutionary relationship, with 92% query coverage clustered in the same group. Therefore, *in silico* and real-time PCR-based studies of the *TaGASR1* gene could enhance the understanding of wheat-heat interactions. By introducing this type of GA-stimulated transcript into major cereal staple food crops, climate-smart traits can be developed through transgenic approaches.

Conclusion

In conclusion, the *TaGASR1* gene from the Indian bread wheat variety HD3086 was characterized for heat stress tolerance via real-time PCR and *in silico*-based studies. Multiple sequence alignment and phylogenetic clustering might be helpful to researchers for further studies because of their conserved nature, especially in the Poaceae family species. Furthermore, the *TaGASR1* gene can generate various climate-adapted crops via transgenic technologies to overcome the adverse impacts of HS.

Acknowledgements

The authors would like to thank the National Institute for Plant Biotechnology, Pusa Campus, New Delhi and Maharishi Markandeshwar (Deemed to be University), and Mullana-Ambala for providing the necessary facilities and encouragement to carry out the work.

Funding declarations

Not applicable.

Conflict of interest

The authors declare no conflicts of interest.

Ethical approval

Not applicable

References

Altenbach, S. B. (2012). New insights into the effects of high temperature, drought and postanthesis fertilizer on wheat grain development. *Journal of Cereal Science*, *56*, 39–50. https://doi.org/10.1016/j.jcs.2011.12.012

Asseng, S., Foster, I., & Turner, N. C. (2011). The impact of temperature variability on wheat yields. *Global Change Biology*, *17*, 997–1012. https://doi.org/10.1111/j.1365-2486.2010.02262.x

Bailey, T. L., Boden, M., Buske, F. A., Frith, M., Grant, C. E., Clementi, L., & Noble, W. S. (2009). MEME SUITE: tools for motif discovery and searching. *Nucleic acids research*, *37*, W202-W208.

Bailey, T. L., Johnson, J., Grant, C. E., & Noble, W. S. (2015). The MEME suite. *Nucleic acids research*, *43*(W1), W39-W49. https://doi.org/10.1093/nar/gkv416

Bhaskara, G. B., Nguyen, T. T., & Verslues, P. E. (2012). Unique drought resistance functions of the highly ABA-induced clade A protein phosphatase 2Cs. *Plant Physiology*, *160*, 379–395. https://doi.org/10.1104/pp.112.202408

Chapman, S. C., Chakraborty, S., Dreccer, M. F., & Howden, S. C. (2012). Plant adaptation to climate change-opportunities and priorities in breeding. *Crop and Pasture Science*, *63*, 251–268. http://dx.doi.org/10.1071/CP11303

Cheng, X., Wang, S., Xu, D., Liu, X., Li, X., et al. (2019). Identification and Analysis of the *GASR* Gene Family in Common

Wheat (*Triticum aestivum* L.) and Characterization of *TaGASR34*, a Gene Associated With Seed Dormancy and Germination. *Frontiers in Genetics*, *10*, 980. https://doi.org/10.3389/fgene.2019.00980

Clavijo, B. J., Venturini, L., Schudoma, C., Accinelli, G. G., Kaithakottil, G., et al. (2017). An improved assembly and annotation of the allohexaploid wheat genome identifies complete families of agronomic genes and provides genomic evidence for chromosomal translocations. *Genome Research*, *27*(5), 885–896. https://doi.org/10.1101%2Fgr.217117.116

Duan, S., Liu, B., Zhang, Y., Li, G., & Guo, X. (2019). Genome-wide identification and abiotic stress-responsive pattern of heat shock transcription factor family in *Triticum aestivum* L. *BMC in Genomics*, *20*, 257. https://doi.org/10.1186/s12864-019-5617-1

Essemine, J., Ammar, S., & Bouzid, S. (2010). Impact of heat stress on germination and growth in higher plants: physiological, biochemical and molecular repercussions and mechanisms of defence. *Journal of Biological Sciences*, *10*, 565–572. http://dx.doi.org/10.3923/jbs.2010.565.572

Farhad, M., Kumar, U., Tomar, V., Bhati, P. K., Krishnan J, N., Barek, V., Brestic, M., & Hossain, A. (2023). Heat stress in wheat: a global challenge to feed billions in the current era of the changing climate. *Frontiers in Sustainable Food Systems*, *7*, 1203721. https://doi.org/10.3389/fsufs.2023.1203721

Farooq, M., Bramley, H., Palta, J. A., & Siddique, K. H. M. (2011). Heat stress in wheat during reproductive and grain filling phases. *Critical Reviews in Plant Science*, *30*, 491–507. https://doi.org/10.1080/07352689.2011.615687

Furukawa, T., Sakaguchi, N., & Shimada, H. (2006). Two *OsGASR* genes, rice GAST homologue genes that are abundant in proliferating tissues, show different expression patterns in developing panicles. *Genes and Genetic System*, *81*, 171−180. https://doi.org/10.1266/ggs.81.171

Gasteiger, E., Hoogland, C., Gattiker, A., Duvaud, S., Wilkins, M. R., Appel, R. D., & Bairoch, A. (2005). Protein identification and analysis tools on the ExPASy server. In: J.M Walker (Eds). *The Proteomics Protocols Handbook* (*112*, pp. 571–607). Springer Protocols Handbooks, Humana Press, New Jersey, USA. https://doi.org/10.1385/1-59259-890-0:571

Gawdiya, S., Kumar, D., Shivay, Y. S., Radheshyam, Nayak, S., Ahmed, B., &Mattar, M. A. (2023). Nitrogen-Driven Genotypic Diversity of Wheat (*Triticum aestivum* L.) Genotypes. *Agronomy*, *13*(10), 2447. https://doi.org/10.3390/agronomy13102447

Grosse-Heilmann, M., Cristiano, E., Deidda, R., & Viola, F. (2024). Durum wheat productivity today and tomorrow: A review of influencing factors and climate change effects. *Resources, Environment and Sustainability*, *17*, 100170. https://doi.org/10.1016/j.resenv.2024.100170

Guo, M., Lu, J. P., Zhai, Y. F., Chai, W. G., Gong, Z. H., & Lu, M. H. (2015). Genome-wide analysis, expression profile of heat shock factor gene family (*CaHsfs*) and characterization of *CaHsfA2* in pepper (*Capsicum annuum* L.). *BMC Plant Biology*, *15*, 1-20. https://doi.org/10.1186/s12870-015-0512-7

Hatfield, J. L., & John, H. P. (2015). Temperature extremes: Effect on plant growth and development. *Weather and Climate Extremes*, *10*, 4-10. https://doi.org/10.1016/j.wace.2015.08.001

Jamil, M., Ali, A., Gul, A., Ghafoor, A., Napar, A.A., Ibrahim, A. M., Naveed, N. H., Yasin, N. A., &Mujeeb-Kazi, A. (2019). Genome-wide association studies of seven agronomic traits under two sowing conditions in bread wheat. *BMC in Plant Biology*, *19*, 1–18. https://doi.org/10.1186/s12870-019-1754-6

Jiang, C., Bi, Y., Mo, J., Zhang, R., Qu, M., Feng, S., & Essemine, J. (2020). Proteome and transcriptome reveal the involvement of heat shock proteins and antioxidant system in thermotolerance of Clematis florida. *Scientific reports*, *10*(1), 8883. https://doi.org/10.1038/s41598-020-65699-2

Khaeim, H., Kende, Z., Balla, I., Gyuricza, C., Eser, A., &Tarnawa, Á. (2022). The effect of temperature and water stresses on seed germination and seedling growth of wheat (*Triticum aestivum* L.). *Sustainability*, *14*(7), 3887. https://doi.org/10.3390/su14073887

Khalid, A., Hameed, A., &Tahir, M. F. (2023). Wheat quality: A review on chemical composition, nutritional attributes, grain anatomy, types, classification, and function of seed storage proteins in bread making quality. *Frontiers in Nutrition*, *10*, 1053196. https://doi.org/10.3389/fnut.2023.1053196

Kosova, K., Vitamvas, P., Prasil, I. T., & Renaut, J. (2011). Plant proteome changes under abiotic stress-contribution of proteomics studies to understanding plant stress response. *Journal of Proteomics*, *74*, 1301–1322. https://doi.org/10.1016/j.jprot.2011.02.006

Kumar, S., Vishwakarma, H., Loitongbam, A., &Aggarwal, D. (2023). Multiprotein- bridging factor 1c from *Triticum aestivum* L. confers tolerance to high-temperature stress in transgenic *Nicotiana tabacum*. *Plant Cell Tissue and Organ Culture*, *154*, 443–456. https://doi.org/10.1007/s11240-023-02548-w

Liu, H., Zeng, B., Zhao, J., Yan, S., Wan, J., & Cao, Z. (2023). Genetic Research Progress: Heat tolerance in Rice. *International Journal of Molecular Science*, *24*(8), 7140. https://doi.org/10.3390/ijms24087140

Livak, K. J., & Schmittgen, T. D. (2001). Analysis of relative gene expression data using real-time quantitative PCR and the 2− ΔΔCT method. *Methods*, *25*(4), 402-408. https://doi.org/10.1006/meth.2001.1262

Mao, Z. C., Zheng, J. Y., Wang, Y. S., Chen, G. H., Yang, Y. H., Feng, D. X., & Xie, B. Y. (2011). The new CaSn gene belonging to the snakin family induces resistance against root-knot nematode infection in pepper. *Phytoparasitica*, *39*, 151−164. http://dx.doi.org/10.1007/s12600-011-0149-5

Masarmi, A. G., Solouki, M., Fakheri, B., Kalaji, H. M., Mahgdingad, N., Golkari, S., &Yousef, A. F. (2023). Comparing the salinity tolerance of twenty different wheat genotypes on the basis of their physiological and biochemical parameters under NaCl stress. *Plos One*, *18*(3), e0282606. https://doi.org/10.1371/journal.pone.0282606

Matsunaga, S., Yamasaki, Y., Toda, Y., Mega, R., Akashi, K., & Hjijoms, T. (2021). Stage-specific characterization of physiological response to heat stress in the wheat cultivar Norin 61. *International Journal of Molecular Science*, *22*, 6942. https://doi.org/10.3390/ijms22136942

Moyano-Canete, E., Bellido, M. L., Garcia-Caparros, N., Medina-Puche, L., Amil-Ruiz, F., Gonzalez-Reyes, J. A., Caballero, J. L., Munoz-Blanco, J., & Blanco-Portales, R. (2013). *FaGAST2*, a strawberry ripening-related gene, acts together with *FaGAST1* to determine cell size of the fruit receptacle. *Plant and Cell Physiology*, *54*, 218−236. https://doi.org/10.1093/pcp/pcs167

Ortiz, R., Sayre, K. D., Govaerts, B., Gupta, R., Subbarao, G. V., Ban, T., Hodson, D., Dixon, J. M., Ortiz-Monasterio, J. I., & Reynolds, M. (2008). Climate change: can wheat beat the heat? *Agriculture, Ecosystems and Environment*, *126*, 46– 58. https://doi.org/10.1016/j.agee.2008.01.019

Padaria, J. C., Bhatt, D., Biswas, K., Singh, G., & Raipuria, R. (2013). In-silico prediction of an uncharacterized protein generated from heat stress responsive SSH library in wheat (*Triticum aestivum* L.). *Plant Omics Journal*, *6*, 150–156.

Padaria, J. C., Vishwakarma, H., Biswas, K., Jasrotia, R. S., & Singh, G. P. (2014). Molecular cloning and in-silico characterization of high temperature stress responsive *pAPX* gene isolated from heat tolerant Indian wheat cv. Raj 3765. *BMC in Research Notes*, *7*, 713. https://doi.org/10.1186/1756-0500-7-713

Panzade, K. P., Vishwakarma, H., Awasthi, O. P., & Padaria, J. C. (2021). Molecular cloning and *in silico* analysis of heat stress responsive gene *ClpB1* from *Ziziphus nummularia* genotypes. *Indian Journal of Experimental Biology*, *59*, 316–327. http://nopr.niscpr.res.in/handle/123456789/57223

Park, S. W., Lee, B. H., Song, S. H., & Kim, M. K. (2023). Revisiting the Ramachandran plot based on a statistical analysis of static and dynamic characteristics of protein structures. *Journal of Structural Biology*, *215*, 107939. https://doi.org/10.1016/j.jsb.2023.107939

Parmar, N., Singh, K. H., Sharma, D., Singh, L., Kumar, P., Nanjundan, J., & Thakur, A. K. (2017). Genetic engineering strategies for biotic and abiotic stress tolerance and quality enhancement in horticultural crops: a comprehensive review. *3 Biotech*, *7*, 1-35. https://doi.org/10.1007/s13205-017-0870-y

Rao, X., Huang, X., Zhou, Z., & Lin, X. (2013). An improvement of the 2ˆ (–delta delta CT) method for quantitative real-time polymerase chain reaction data analysis. *Biostatistics, bioinformatics and biomathematics*, *3*(3), 71.

Roy, S., Maheshwari, N., Chauhan, R., Sen, N. K., & Sharma, A. (2011). Structure prediction and functional characterization of secondary metabolite proteins of Ocimum. *Bioinformation*, *6*(8), 315. https://doi.org/10.6026/97320630006315

Rubinovich, L., Ruthstein, S., & Weiss, D. (2014). The Arabidopsis Cysteine-Rich GASA5 Is a Redox-Active Metalloprotein that Suppresses Gibberellin Responses. *Molecular Plant*, *7*, 244−247. https://doi.org/10.1093/mp/sst141

Sah, S. K., Reddy, K. R., & Li, J. (2016). Abscisic Acid and Abiotic Stress Tolerance in Crop Plants. *Frontiers in Plant Science*, *7*, 571. https://doi.org/10.3389/fpls.2016.00571

Schwede, T., Kopp, J., Guex, N., & Peitsch, M. C. (2003). SWISS-MODEL: an automated protein homology -modelling server. *Nucleic Acids Research*, *31*(13), 3381-3385.

Sarkar, S., Islam, A. A., Barma, N. C. D., & Ahmed, J. U. (2021). Tolerance mechanisms for breeding wheat against heat stress: A review. *South African Journal of Botany*, *138*, 262-277. https://doi.org/10.1016/j.sajb.2021.01.003

Shewry, P. R., & Hey, S. J. (2015). The contribution of wheat to human diet and health. *Food and energy security*, *4*(3), 178-202. https://doi.org/10.1002/fes3.64

Sun, S., Wang, H., Yu, H., Zhong, C., Zhang, X., Peng, J., & Wang, X. (2013). GASA14 regulates leaf expansion and abiotic stress resistance by modulating reactive oxygen species accumulation. *Journal of Experimental Botany*, *64*, 1637−1647. https://doi.org/10.1093/jxb/ert021

Szklarczyk, D., Gable, A. L., Lyon, D., Junge, A., Wyder, S., & Huerta-Cepas, J. (2019). STRING v11: protein–protein association networks with increased coverage, supporting functional discovery

in genome-wide experimental datasets. *Nucleic Acids Research*, *47*(D1), D607-D613.https://doi.org/10.1093/nar/gky1131

Tamura, K., Stecher, G., & Kumar, S. (2021). MEGA11: Molecular Evolutionary Genetics Analysis Version 11. *Molecular Biology and Evolution*, *38*(7) 3022–3027. https://doi.org/10.1093/molbev/msab120

Tripathi, A., Tripathi, D. K., Chauhan, D. K., Kumar, N., & Singh, G. S. (2016). Paradigms of climate change impacts on some major food sources of the world: a review on current knowledge and future prospect. *Agriculture Ecosystems and Environment*, *216*, 356–373. https://doi.org/10.1016/j.agee.2015.09.034

Ullah, A., Nadeem, F., Nawaz, A., Siddique, K. H. M., & Farooq, M. (2022). Heat Stress effects on the reproductive physiology and yield of wheat. *Journal of Agronomy and Crop Science*, *208*, 1–17. https://doi.org/10.1111/jac.12572

Villalobos-López, M. A., Arroyo-Becerra, A., Quintero-Jiménez, A., & Iturriaga, G. (2022). Biotechnological advances to improve abiotic stress tolerance in crops. *International Journal of Molecular Sciences*, *23*(19), 12053. https://doi.org/10.3390/ijms231912053

Vishwakarma, H., & Sharma, J. (2018). Real time and in-silico based analysis of heat stress responsive transcription factor *MBF1c* from wheat. *International Journal of Advanced Scientific Research and Management*, *3*(11), 2455–6378.

Vishwakarma, H., Junaid, A., Manjhi, J., Singh, G. P., Gaikwad, K., & Padaria, J. C. (2018). Heat stress transcripts, differential expression, and profiling of heat stress tolerant gene *TaHsp90* in Indian wheat (*Triticum aestivum* L.) cv. C306. *PLoS One*, *13*(6):e0198293. https://doi.org/10.1371%2Fjournal.pone.0198293

Wang, X., Yan, B., Shi, M., Zhou, W., Zekria, D., Wang, H., & Kai, G. (2016). Over expression of a *Brassica campestris* HSP70 in tobacco confers enhanced tolerance to heat stress. *Protoplasma*, *253*(3), 637– 645.https://doi.org/10.1007/s00709-015-0867-5

Wang, Z., Gerstein, M., & Snyder, M. (2009). RNA-Seq: a revolutionary tool for transcriptomics. *Nature reviews genetics*, *10*(1), 57-63. https://doi.org/10.1038/nrg2484

Yang, M., Liu, C., Zhang, W., Wu, J., Zhong, Z., Yi, W., & He, Y. (2023). Genome-Wide Identification and Characterization of Gibberellic Acid-Stimulated Arabidopsis Gene Family in Pineapple (Ananascomosus). *International Journal of Molecular Sciences*, *24*(23), 17063. https://doi.org/10.3390/ijms242317063

Yu, C. S., Chen, Y. C., Lu, C. H., & Hwang, J. K. (2006). Prediction of protein subcellular localization. Proteins: Structure, Function and Bioinformatics, *64*, 643-651.https://doi.org/10.1002/prot.21018

Yu, C. S., Cheng, C. W., Su, W. C., Chang, K. C., Huang, S. W., Hwang, J. K., & Lu, C. H. (2014). CELLO2GO: a web server for protein subCELlularLOcalization prediction with functional gene ontology annotation. *PloS one*, *9*(6), e99368. https://doi.org/10.1371/journal.pone.0099368

Zhang, L., Geng, X., Zhang, H., Zhou, C., Zhao, A., Wang, F., &Peng, H. (2017). Isolation and characterization of heat-responsive gene *TaGASR1* from wheat (*Triticum aestivum* L.). *Journal of Plant Biology*, *60*, 57–65. https://doi.org/10.1007/s12374-016-0484-7

Zhang, S., Yang, C., Peng, J., Sun, S., & Wang, X. (2009). *GASA5*, a regulator of flowering time and stem growth in *Arabidopsis thaliana*. *Plant Molecular Biology*, *69*, 745−759. https://doi.org/10.1007/s11103-009-9452-7

Zheng, B., Chenu, K., Dreccer, M. F., & Chapman, S. C. (2012). Breeding for the future: what are the potential impacts of future frost and heat events on sowing and flowering time requirements for Australian bread wheat (*Triticum aestivum*) varieties? *Global Change Biology*, *18*, 2899–2914. https://doi.org/10.1111/j.1365-2486.2012.02724.x