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Genome-wide identification and expression analysis of the Small Ubiquitin-like Modifier (SUMO) gene family in *Triticum aestivum* L.

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Abstract

Background Post-translational modification of proteins by SUMO is critical for a wide range of cellular and developmental processes. Although SUMO proteins have been extensively studied in animals and, to some extent, in *Arabidopsis*, their precise functions in other crop plants are still largely unknown.

Results In this research, we identified 31 *TaSUMO* genes in genome of wheat. Phylogenetic tree unveiled that genes clustered into thirteen subfamilies. Chromosomal mapping unveiled the dispersal of 31 *TaSUMO* genes across 11 wheat chromosomes. The eleven pairs of duplicated gene were identified in the SUMO family. Ka/Ks ratio revealed that 8 duplicated *TaSUMO* genes went through purifying purification. Furthermore, it was noted that *TaSUMO* genes displayed significant conversation in their gene structure. In addition, analysis of promoters uncovered the presence of numerous cis-regulatory elements in the promoter region of the *TaSUMO* genes. The differential expression patterns were observed among *TaSUMO* family members across various tissues and in response to multifaceted stress conditions. Moreover, this investigation explored the miRNAs targeted to *TaSUMO* genes and expression profile in various tissues.

Conclusion Thus, the results of this study establish a strong basis for further investigation of the functions of *TaSUMO* genes across different tissues, developmental stages, phytohormone responses, and diverse stress in wheat.

Keywords Gene expression, TaSUMOs, MiRNA, Phylogenetic tress, Post-translational modifications and wheat

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Introduction

To cope with continuous environmental fluctuations and developmental signals, plants have developed a variety of molecular mechanisms that enable them to swiftly sense and respond at the transcriptional, translational, and post-translational levels [1, 2]. Post-translational modifications (PTMs) play a crucial role in regulating protein stability, subcellular localization, enzymatic activity, and interactions with other biomolecules [3–5]. PTMs encompass a variety of modifications, including phosphorylation, glycosylation, persulfidation, ubiquitination, acetylation, S-nitrosylation, carbonylation, SUMOylation, and lipidation [6–9]. PTMs are increasingly recognized as essential components of cell signaling, acting as fine-tuning mechanisms that modulate cellular responses to environmental changes and developmental progress [10, 11]. Small Ubiquitin-like Modifier (SUMO) is a ubiquitin-like protein that regulates diverse cellular functions, including cell cycle regulation, DNA repair, enzymatic stability and activity, and sub-nuclear localization by modifying target proteins through post-translational processes [12–16]. In addition, SUMO also regulates various processes, including receptor function, channel activity, G-protein signaling, exocytosis, cytoskeletal organization, mitochondrial dynamics, and autophagy [15, 17–20]. The sumoylation is highly similar to the ubiquitination. SUMOylation occurs through a various enzymatic reactions involving E1 SUMO activation enzymes, E2 SUMO conjugation enzymes, and E3 SUMO ligation enzymes [14]. Sumoylation typically occurs at lysine (K) residues within the SUMO consensus sequence CKXD/E, where C represents a hydrophobic amino acid, K is lysine, X can be any amino acid, and D/E denotes glutamic or aspartic acids [21, 22].

SUMO is classified as a low molecular weight protein, typically comprising between 100 and 130 amino acids [15, 23]. SUMO protein was first identified in tomato that interacts with the ethylene-inducing xylanase produced by *Trichoderma viride* [24]. They have been found to control numerous plant biological processes, including root development, plant reproduction, overall plant development, and tolerance to both biotic and abiotic stresses [25–30]. SUMOylation mediated by *AtSUMO1*, *AtSUMO2*, *OsSUMO1*, *OsSUMO2*, *ZmSUMO1*, *GmSUMO1*, and *GmSUMO2* is rapidly induced in response to stress [29, 31–33]. SUMO system influences plant development by promoting the accumulation of SUMO conjugates under abiotic stress [34]. The SUMO E3 ligase named *SIZ1* has been identified as a regulator of growth and heat stress in *Arabidopsis* [35], and enhanced drought tolerance in tobacco [36]. Ecd4 is a SUMO-specific protease that regulates flowering time and processes precursor SUMO proteins in *Arabidopsis* [37]. It has also been reported that the SUMO protease

OTS1 acts as a positive regulator of rice seed germination and root development under saline conditions [38]. Ectopic expression of *ZmSCE1e* gene increased SUMO conjugation levels and enhanced drought and salt tolerance in tobacco [39]. Further, the SUMO system modulates ABA signaling and mediates resistance to bacterial and viral disease [40, 41]. Hence, SUMO genes are regarded as key regulators of various environmental responses. Although the molecular mechanisms of the SUMO system are well characterized in *Arabidopsis*, its functions in many economically significant agricultural crops are still not fully understood. However, there are currently only a few reports on the role of SUMO in crops, particularly in wheat [42]. Wheat has become a crucial global food source, but it is highly susceptible to both biotic and abiotic stresses [43, 44]. Therefore, this study conducted an in silico analysis of the SUMO gene family in wheat, encompassing biochemical characteristics, phylogenetic and syntenic relationships, gene organization, motif, promoter analysis, expression profiles, and potential miRNA targeting of the *TaSUMO* genes. Thus, further research is needed to explore the functional characterization of SUMO genes in wheat, focusing on plant developmental processes and responses to various stresses.

Materials and methods

Identifying the TaSUMO genes

To identify the SUMO genes in the genome of wheat, genomic data were sourced from <http://plants.ensembl.org/index.html>. We used SUMO sequences from *Arabidopsis* as query for our search. AtSUMO protein sequences were acquired from TAIR database. To identify TaSUMO proteins within the wheat genome, a BLASTp was carried out with proteome of wheat, a bit score more than 100 and an e value threshold was 10^{-5} . Subsequently, the SUMO motifs were authenticated using various tools including HMMscan (<https://www.ebi.ac.uk/Tools/hmmer/search/hmmscan>), InterPro (<https://www.ebi.ac.uk/interpro/>), and PROSITE (<https://prosite.expasy.org/scanprosite/>). To mitigate redundancy, duplicate sequences were eliminated, yielding a distinctive collection of potential SUMO transporter proteins. Following this, protein sequences harboring the SUMO motif were chosen and assigned names according to their chromosomal locations.

Biophysical characterization, chromosomal mapping and phylogenetic analysis of TaSUMO genes

The biochemical properties of TaSUMO proteins such as pI, protein length, and MW, were analyzed utilizing the ExPASy webserver [45] and the isoelectric point calculator [46]. For predicting the subcellular location of TaSUMO proteins, we utilized the following tools: PSORT (<https://www.psорт.org/psортb/>), Bacello (<https://www.bacello.org/>)

[://busca.biocomp.unibo.it/bacello/](http://busca.biocomp.unibo.it/bacello/)), and BUSCA (<https://busca.biocomp.unibo.it/>). To ascertain the distribution of *TaSUMO* genes across chromosomes of wheat based on their genomic positions. Specifically, we employed the Ensembl Plants genome browser alongside genome annotation data from the International Wheat Genome Sequencing Consortium (IWGSC) to obtain detailed chromosomal localization of wheat genes (<http://plants.ensembl.org/index.html>). Subsequently, PhenoGram was employed to visualize the position of SUMO genes on the chromosomes of wheat (<https://visualization.ritchielab.org/phenograms/plot>). Further, protein sequences of AtSUMOs, OsSUMOs, TaSUMOs, BraSUMOs, ZmSUMOs, GmSUMOs, PpSUMOs and SmSUMOs were retrieved from Ensembl Plants. Phylogenetic tree was created using MEGA X [47]. Protein sequences were aligned using the ClustalW, and the NJ method was used for the production of tree with 1000 replicates.

Gene duplication, synteny, gene structure analysis of TaSUMOs

MCSan were utilized to identify gene duplication events and conduct synteny analysis [48]. Subsequently, the Ka/Ks, also known as the ratio of nonsynonymous to synonymous substitutions, was computed via TBtools [49]. The Gene Structure Display Server 2.0 was used to visualize the positions of introns, exons, and UTR (<http://gsds.guo-lab.org/>).

Conserved motif analysis, 3-D structure and cis-regulatory elements, analysis of TaSUMO

Additionally, for each TaSUMO protein sequence, conserved protein motifs were recognized via MEME (<http://meme-suite.org/tools/meme>). Phyre2 was employed to predict the three-dimensional structures of all 31 TaSUMO protein sequences [50]. Further, promoter element analysis was conducted using the upstream sequence of the *TaSUMOs* (2000 bp), employing PlantCARE server [51].

GO enrichment analysis, expression profiling, identification of potential miRNA targets, and analysis of protein-protein interactions of TaSUMOs

GO enrichment analysis of TaSUMO proteins were performed using the AgriGO tool [52]. TPM values for various tissues and stress were sourced from the Wheat Expression Database (<http://www.wheat-expression.com/>). Heatmaps and PCA plots were generated using ClustVis [53] and TBtools [49]. To recognize potential miRNAs in wheat, sequences of miRNA was retrieved from PmiREN (pmiren.com/download). Plant Small RNA Target Analysis Server was used to identify potential miRNA targets for the *TaSUMO* genes, applying the server default parameters (<https://www.zhaolab.org/psRNATar>

[get/analysis](https://www.zhaolab.org/psRNATar)). In addition, the TaSUMOs protein network was analyzed using the STRING (<https://string-db.org/cgi>).

Plant material, stress treatments, and qRT-PCR analysis

Seeds from five wheat cultivars such as KSU-L, KSU-K, KSU-Y, KSU-105, and KSU-115 were obtained from the King Saud University collection. The seeds were initially germinated in a soil mix within small plastic pots. After 10 days, the seedlings were transferred to larger plastic pots and acclimatized for two days in a growth room. Subsequently, the seedlings were subjected to different stress treatments as follows: cold stress was applied at 4 °C for 1 day, heat stress at 37 °C for 6 h, and drought stress at 22–25 °C with no watering for 4 days. Control plants were maintained at 22–25 °C under standard conditions. After treatment, tissues (roots, leaves, and stems) from both control and stressed seedlings were collected for RNA extraction and stored at – 80 °C. To synthesize cDNA, 1 µg of total RNA was reverse transcribed using the High-Capacity cDNA Reverse Transcription Kit (Applied Biosystems), following the instruction of manufacturers protocol. Quantification of SUMO mRNA expression was performed using qRT-PCR. The qPCR reactions were conducted on a QuantStudio™ 7 Flex System (Applied Biosystems) with the SolisFAST® Solis-Green® qPCR Mix (Solis BioDyne), following the manufacturers protocol. Specific primers for the SUMO genes and the housekeeping gene Actin are detailed in Table S6. Relative expression levels were calculated following the method described by [54, 55], and the resulting values were used to create the graph. Details of all primers used in this study are provided in Table S6.

Results

Identification and biophysical characterization of TaSUMO proteins

In order to pinpoint TaSUMO proteins in the wheat genome, a BLASTp search was executed, utilizing sequences sourced from diverse plant species. We collected 36 full-size SUMO protein sequences from various species including *Arabidopsis thaliana* (7), *Oryza sativa* (5), *Brassica rapa* (10), *Zea mays* (3), *Glycine max* (6), *Physcomitrella patens* (2), and *Selaginella moellendorffii* (3). Subsequently, we compiled these sequences to create a local sequence database. This search yielded 31 putative *TaSUMO* genes (Table S1; Table S2), having the existence of conserved SUMO motif. Further, we confirmed the presence of the conserved SUMO motif through validation using bioinformatic tools such as Pfam, CDD, and SMART. This number indicates a significantly higher count of SUMO genes in wheat compared to the SUMOs documented in other plant species, such as *Arabidopsis thaliana*, *Oryza sativa*, *Zea mays*, and *Glycine max*.

This outcome could be credited to the elevated ploidy level and expansive genome characteristic of wheat. Wheat originates from the fusion of A, B, and D genome. The amino acid range spans from 86 (TaSUMO13) to 367 (TaSUMO30), as illustrated in Table S1. Molecular weights (MW) vary between 9.79 KDa (TaSUMO13) and 41.28 KDa (TaSUMO30). Isoelectric points (pI) range from 4.59 (TaSUMO31) to 9 (TaSUMO16). We observed GRAVY values ranging from -0.063 (TaSUMO13) to -0.772 (TaSUMO28). These results demonstrated that most of TaSUMO proteins possess a highly hydrophilic nature. Further, to explore the dispersal pattern of TaSUMO proteins, we established a correlation between their Mw and pI, as depicted in Fig. S1. Our findings indicate that TaSUMO proteins exhibit a similar distribution pattern based on both pI and MW. Moreover, all 31 TaSUMOs were identified as situated at nucleus (Table S1).

Phylogeny analysis of SUMO proteins

A phylogenetic tree was created utilizing the NJ method with MEGA X. SUMO protein sequences sourced from diverse species, for instance *A. thaliana* (7), *O. sativa* (5), *Triticum aestivum* (31), *Brassica rapa* (10), *Zea mays* (3), *Glycine max* (6), *Physcomitrella patens* (2), and *Selaginella moellendorffii* (3) (Table S3). Based on the observed branching patterns in the phylogenetic tree, SUMO proteins from various plant species were classified into thirteen distinct clusters (Fig. 1).

The cluster VII notably distinguished itself by containing the largest number of members, totaling 16 genes, representing 23.88% of the overall count. In contrast, the cluster IX displayed a smaller membership, consisting of only a single gene. An intriguing finding was that cluster I, VIII, IX, X, XI, XII and XIII were monocot specific while the cluster II, and III were dicot specific suggesting gene amplification occurred before the split between monocot and dicot (Fig. 1; Fig. S2).

Chromosomal mapping, gene duplication occurrences, and syntenic connections of TaSUMO genes

To explore chromosomal dispersal of the *TaSUMO* genes, we mapped 31 *TaSUMO* genes on eleven chromosomes of wheat (Fig. 2A, Fig. S3 and Table S1).

Notably, B sub-genome exhibited the maximum number of *TaSUMOs* (13), followed by sub-genome D (12), while sub-genome A consist least *TaSUMOs* genes (6) (Fig. 2B). Overall, all the genes in the SUMO family were unevenly distributed among the three subgenomes of wheat. Chromosome 1B hosts the highest number of *TaSUMOs*, with ten genes identified (Fig. S3). On the other hand, chromosome 1 A, 2B, and 4 A each harbor a single *TaSUMO* gene, representing the minimum count within our analysis. Interestingly, no *TaSUMO*

genes were detected on 4B, 4D, 5 A, 5B, 5D, 6 A, 6B, 6D, 7 A and 7B chromosomes. Therefore, the results indicate an unequal dispersion of *TaSUMO* gene family members across wheat chromosomes. Within our study, we focused on exploring gene duplication among the *TaSUMO* family members. We pinpointed eight duplicated pair of *TaSUMO* genes (Fig. S4 and Table S4).

A phylogenetic tree depicting the *TaSUMO* genes illustrates numerous events of gene duplication (Fig. S5). Throughout its evolutionary history, the *TaSUMO* gene family has experienced duplications, as shown in Fig. S4 and Table S4. This process has led to the emergence of multiple copies of gene, which may possess a range of functions. Thus, our results revealed that the growth of the SUMO gene family in wheat is primarily attributable to whole-genome duplications. To explore the pressure of selection influencing the duplicated *TaSUMO* genes, we performed Ka/Ks analysis for eight *TaSUMO* genes (Table S4). Ka/Ks value was consistently below one for 8 *TaSUMO* genes, suggesting that duplicated *TaSUMO* genes experienced purifying selection. Additionally, we investigated the syntenic analysis of *TaSUMO* genes with other plants, for instance, *A. thaliana*, *O. sativa*, (*A*) *tauschii*, and (*B*) *distachyon*. To search orthologous genes in these plant species genomes, we utilized MCSScan (Fig. 3).

These findings collectively underscored a conserved evolutionary process experienced by the *TaSUMO* family genes.

Gene organization, motif and 3D structure analysis of the TaSUMOs

Gene structure, which refers to the organization of specific sequence in a gene, offers crucial insights into the conserved characteristics and evolutionary variations among genes. The analysis reveals evident conservation in introns and exons number among distinct subgroups (Fig. S6; Fig. S7).

Further, it was noted that *TaSUMO* genes display extensive conservation in their gene structure. Most of the *TaSUMO* genes were the intron less. However, *TaSUMO24*, *TaSUMO25*, *TaSUMO26*, *TaSUMO27*, *TaSUMO28*, and *TaSUMO30* displayed 1 to 2 introns (Fig. S7). In addition, for a deeper comprehension of biological roles of *TaSUMO* genes, we performed conserved motifs analysis in TaSUMO proteins utilizing webserver (Fig. S8A and B).

It was observed that all members of SUMO family possessed conserved SUMO motif. Furthermore, the majority of TaSUMO proteins exhibited three to six motifs, highlighting the remarkably conserved characteristics of SUMO proteins across evolutionary stages and implying comparable functionalities among them. A collective count of 4 motifs was recognized in each SUMO protein. The size and optimal matching sequences of motifs were

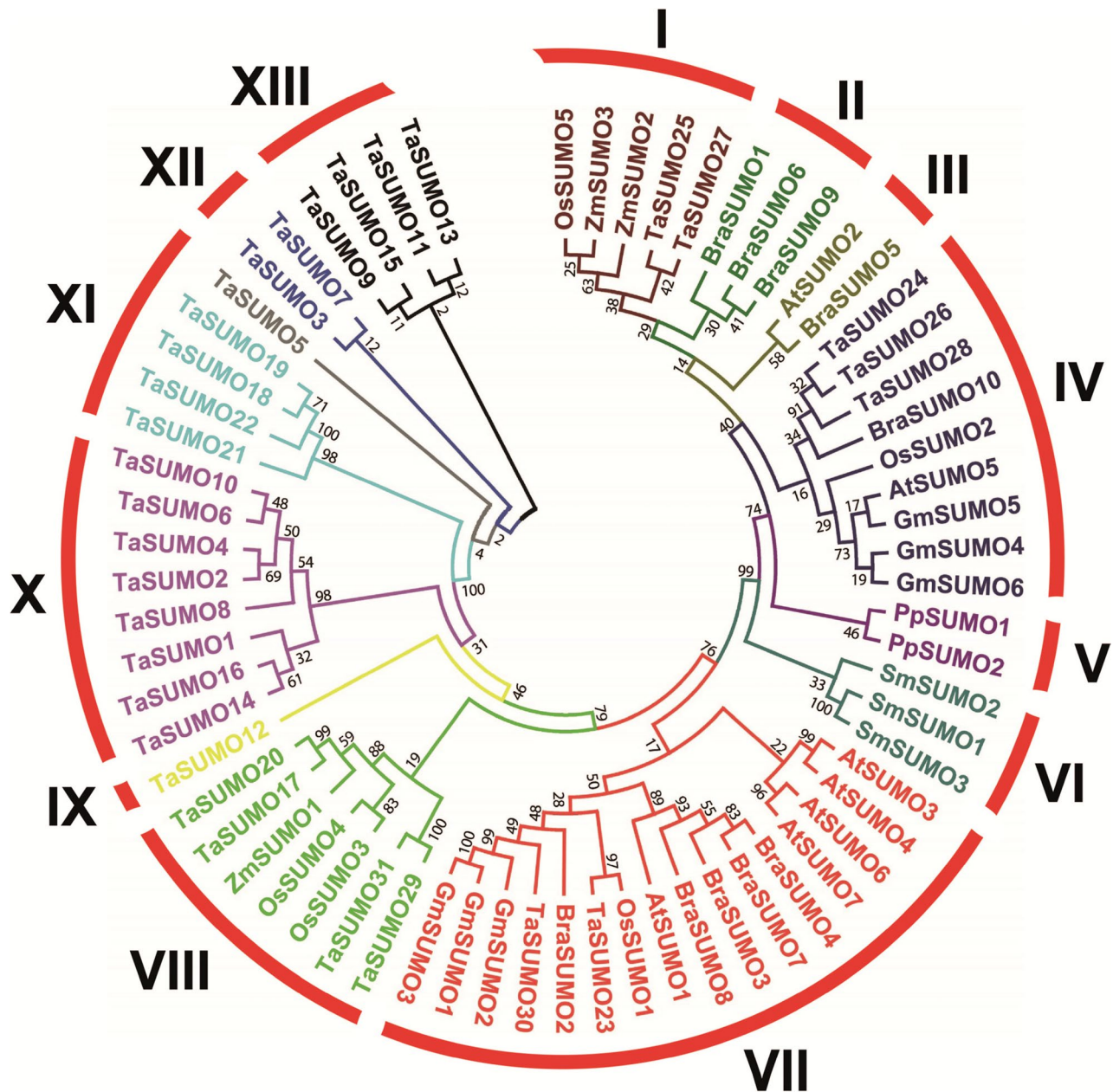


Fig. 1 The phylogenetic relationships of SUMO proteins among various crop species, including *Arabidopsis thaliana* (7), *Oryza sativa* (5), *Triticum aestivum* (31), *Brassica rapa* (10), *Zea mays* (3), *Glycine max* (6), *Physcomitrella patens* (2), and *Selaginella moellendorffii* (3), were analyzed. The phylogenetic tree was generated using MEGA X with the NJ method with bootstrap 1000 replicates

illustrated in Fig. S8A, accompanied by the corresponding motif logos in Fig. S8B. The arrangement, type, and abundance of motifs within SUMO sequences typically remained uniform in the same subfamily, yet diverged across different subfamilies. This observation suggests that certain SUMO members might have lost of specific motifs over evolutionary progression, consequently leading to the emergence of novel functionalities. Furthermore, alignment of TaSUMO protein sequence, it was noticed that all 31 TaSUMO proteins harbored the conserved SUMO motif (Fig. 4; Fig. S9A).

Moreover, this study involved predicting 3-D structure of TaSUMO proteins (Fig. S9B). Hence, these discoveries are anticipated to contribute significantly to understand the precise role of TaSUMO proteins in controlling various biological processes in wheat.

Examination of Cis-regulatory elements of TaSUMO

In order to elucidate the potential role of *TaSUMO* genes, we conducted an analysis of the upstream sequence of *TaSUMO* genes using the PlantCARE. Our examination revealed existence of several cis-regulatory elements

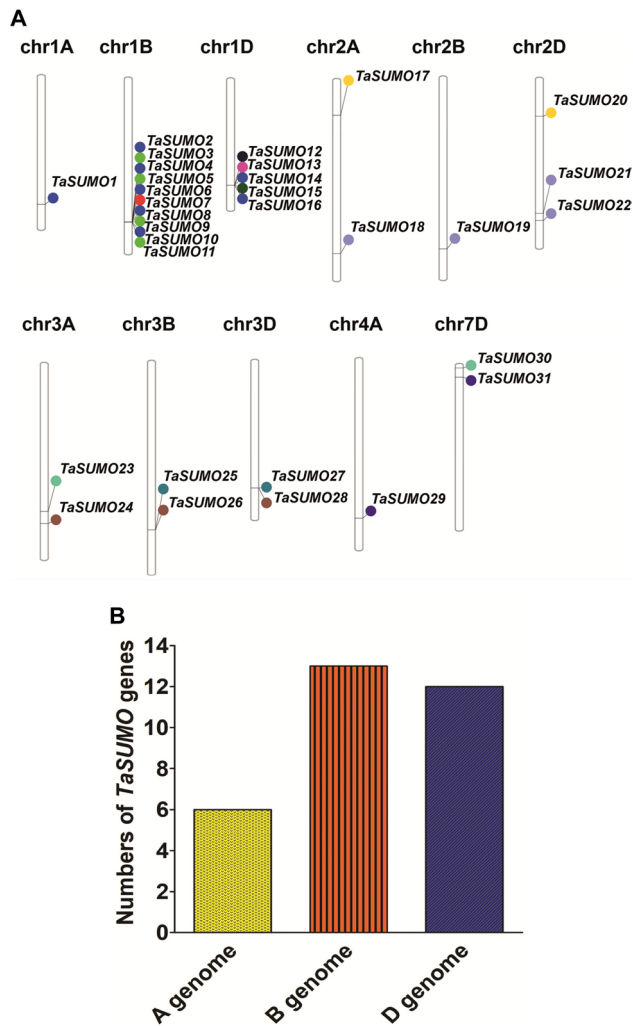


Fig. 2 The chromosomal positions of *TaSUMO*s and their spreading among different sub-genomes. **A** A diagrams depicting the arrangement of *TaSUMO*s on chromosomes of wheat. **B** *TaSUMO*s are scattered throughout sub-genomes of wheat

(CREs) within this region of the *TaSUMO* family genes. The identified elements span a range of functional categories, including responsiveness to light, phytohormones, and various stressors (Fig. 5A and B, and Table S5).

Within the *TaSUMO* genes, we identified five CREs responsive to phytohormones. These encompass auxin response element, the gibberellin response element, the MeJA response element (MeJARE), the abscisic acid response element (ABRE), and the salicylic acid response element. CREs linked to light response, ABRE, MeJARE, defense and stress responsiveness were primarily observed in promoters of *TaSUMO* genes (Fig. 5B). Consequently, these outcomes advocate that *TaSUMO* genes may exert a crucial influence on plant growth, development, and the response to diverse stressors. Moreover, *TaSUMO*s promoter harbor the CREs associated with a

multitude of functions, such as seed specific regulation, cell cycle regulation, endosperm expression and circadian regulation. The presence multiple CREs in *TaSUMO*s promoter indicates their potential involvement in various cellular processes. Together, these results offer essential insights into the regulatory roles of these genes, thereby enhancing our understanding of the intricate regulatory functions of *TaSUMO* genes and their importance in shaping the broader physiological landscape of wheat.

Analyzing the enrichment of *TaSUMO* genes using gene ontology (GO)

In order to enhance our comprehension about functions of *TaSUMO* genes, we performed GO analysis. *TaSUMO* family members were accurately annotated using AgriGO (Fig. S10A, S10B and Table S7). These results were further validated through eggNOG-Mapper (Table S8). This thorough annotation contributes to a deeper understanding the role of *TaSUMO* genes across different cellular processes. *TaSUMO* genes indicated noteworthy enriched in biological category, including regulation of molecular function (GO:0065009), RNA processing (GO:0006396), and cellular protein catabolic process (GO:0044257) (Fig. S10A). Further, in molecular category, *TaSUMO* genes exhibited enrich in protein tag (GO:0031386) and protein binding (GO:0005515) (Fig. S10B). This enrichment underscores the contribution of *TaSUMO* genes in diverse cellular protein catabolic process and ubiquitin mediated degradation of functions.

Transcriptomic profile of *TaSUMO* genes

An expression patterns of *TaSUMO* genes were thoroughly investigated across a range of tissues and stress conditions. The primary objective of this thorough analysis was to enhance comprehension of the functional roles played by *TaSUMO* genes. TPM value was used to create heatmap and conduct PCA (Fig. 6A, B, and Fig. S11A and S11B).

It was noted that 31 *TaSUMO* genes exhibited unique expression profiles in various tissues, and response to stress conditions (Fig. 6A and B). Notably, *TaSUMO* genes demonstrated a varying expression pattern across diverse tissues, for example, in spike_z32, *TaSUMO*26, *TaSUMO*27, *TaSUMO*28 and *TaSUMO*29 exhibited significant elevation, whereas spike_z32 *TaSUMO*20, and *TaSUMO*23 were observed to be raised. The expression of *TaSUMO*20 was highly induced in spike_z65. Further, the expression levels of *TaSUMO*19 and *TaSUMO*31 were notably increased in stem_z65. In grain_z85, *TaSUMO*26, *TaSUMO*27, *TaSUMO*28, and *TaSUMO*29 were observed to be up-regulated. Furthermore, *TaSUMO*22 expression displayed raised in stem_z32. Furthermore, distinctive transcript level was also evident for *TaSUMO* genes under diverse environmental stress (Fig. 6B). The

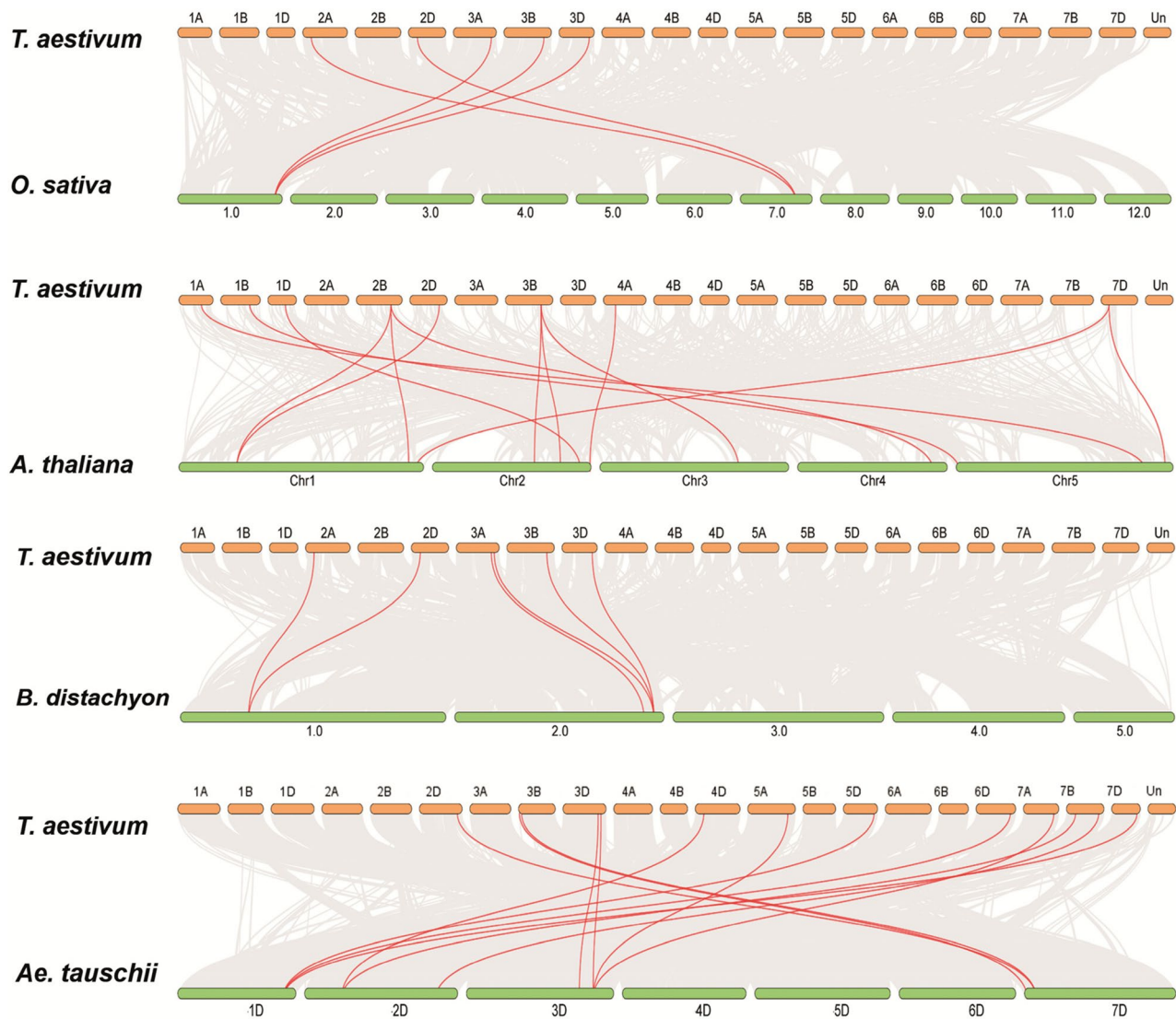


Fig. 3 A syntentic analysis was conducted on *TaSUMO* genes across various crop and model species including *Oryza sativa*, *Arabidopsis thaliana*, *Brachypodium distachyon*, and *Aegilops tauschii*, is shown. The gray color in background represents collinear blocks in the genome of wheat and different plant species, while red lines indicate the identified orthologous genes among wheat and in different species plant genomes

expression of *TaSUMO24*, *TaSUMO25*, *TaSUMO27*, *TaSUMO28* and *TaSUMO29* exhibited notable elevation in DS+HS_6h, while *TaSUMO24*, *TaSUMO25*, *TaSUMO26*, *TaSUMO27* and *TaSUMO28* HS_6h were significantly induced in HS_6h. In addition, *TaSUMO31* showed increased expression levels in DS_6h. In DS_1h, *TaSUMO29* and *TaSUMO31* exhibited up-regulation. Furthermore, the RNA-seq data were further validated using qRT-PCR (Fig. S12). These results were consistent with and exhibited similar expression trends to those observed in RNA-seq data. Collectively, these findings suggest that *TaSUMO* genes play significant roles in various developmental processes and exhibit responsiveness to environmental stresses in wheat.

Identifying MiRNAs and targeted TaSUMOs

The psRNATarget was used to pinpoint putative miRNAs that targeted the *TaSUMO* genes. Out of 31 *TaSUMO* genes, nine were found that targeted by 9 miRNAs, including *TaemiR3711*, *TaemiR5384*, *TaemiR9674*, *TaemiR9676*, *TaemiRN4284*, *TaemiRN4305*, *TaemiRN4320*, *TaemiRN4331* and *TaemiRN4389*. These miRNAs target *TaSUMO* genes such as *TaSUMO1*, *TaSUMO23*, *TaSUMO24*, *TaSUMO25*, *TaSUMO26*, *TaSUMO27*, *TaSUMO28*, *TaSUMO29* and *TaSUMO30* (Table S9). Further, we conducted an examination of the miRNA network, elucidating its specific targets within the wheat SUMO genes (Fig. 7A).

Furthermore, we scrutinized the transcript level of miRNA in diverse tissue (Table S10). These putative

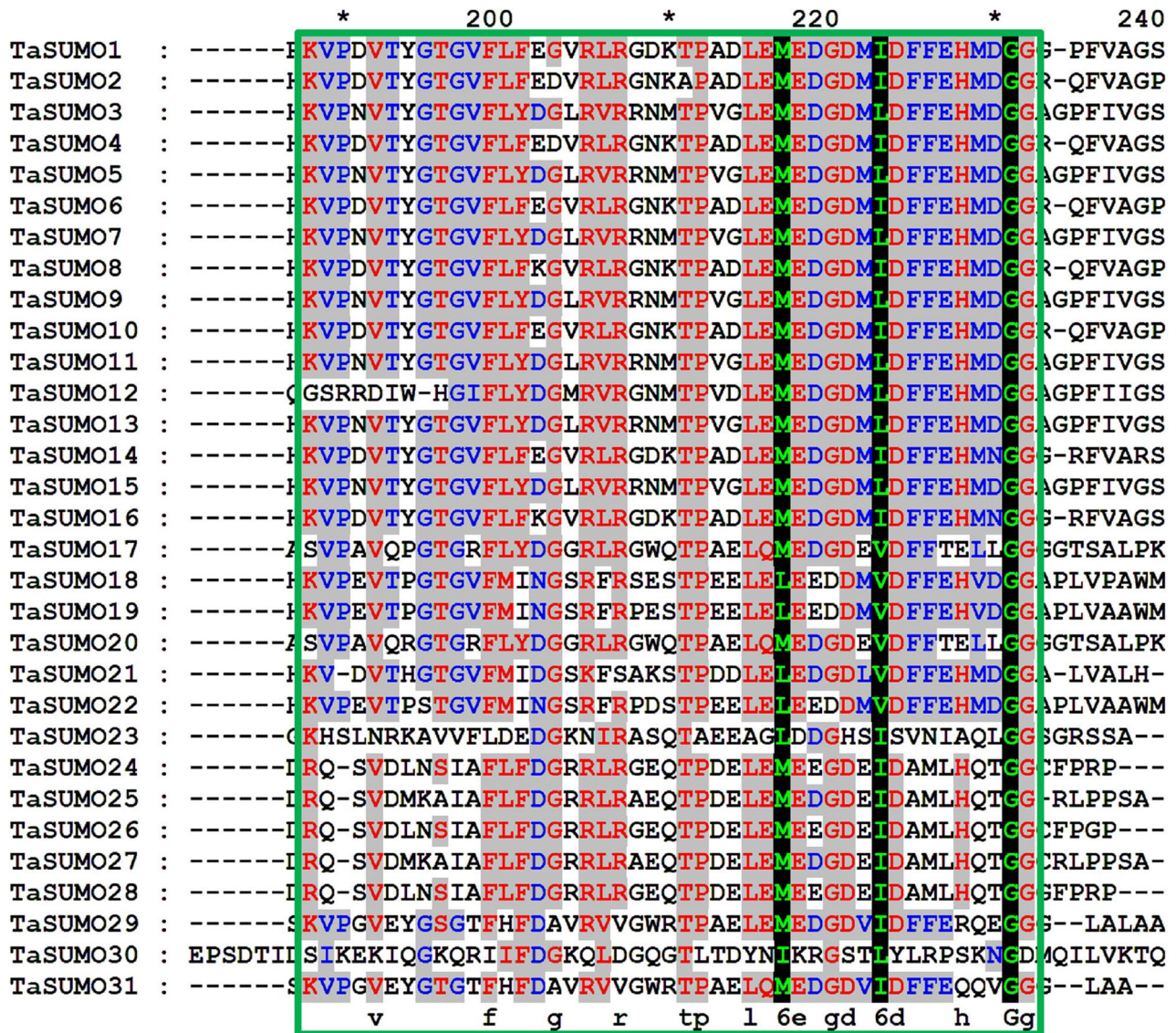


Fig. 4 The amino acid sequence alignment of TaSUMO proteins reveals a conserved small ubiquitin-related modifier (SUMO) motif, characterized by a diglycine (GG) sequence. This conserved motif is highlighted within a green box

miRNA exhibit distinct expression patterns in various tissues in wheat (Fig. 7B). These miRNAs could ultimately interfere with and affect the developmental processes in wheat. As a result, these results offer invaluable insights to comprehending the particular roles of these miRNA in various fundamental process in the wheat.

Examining the protein-protein interactions of TaSUMOs

We constructed a protein-protein interaction (PPI) network between TaSUMO proteins and wheat proteins (Fig. 8 and Table S11).

A total of 31 TaSUMO proteins were identified to interact within the TaSUM family. Interestingly, TaSUMO30 was found to interact with 24 proteins, including TaSUMO1, TaSUMO2, TaSUMO4, TaSUMO5,

TaSUMO7, TaSUMO8, TaSUMO10, TaSUMO11, TaSUMO13, TaSUMO14, TaSUMO15, TaSUMO16, TaSUMO17, TaSUMO19, TaSUMO20, TaSUMO22, TaSUMO23, TaSUMO24, TaSUMO25, TaSUMO26, TaSUMO27, TaSUMO28, TaSUMO29, and TaSUMO31. These findings provide valuable insights that could stimulate further functional analysis of TaSUMO proteins, advancing our understanding of their roles in various biological processes in wheat.

Discussion

SUMO is a protein similar to ubiquitin that influences various cellular functions. It modulates processes such as cell cycle regulation, DNA repair, enzyme stability and activity, and sub-nuclear localization by modifying

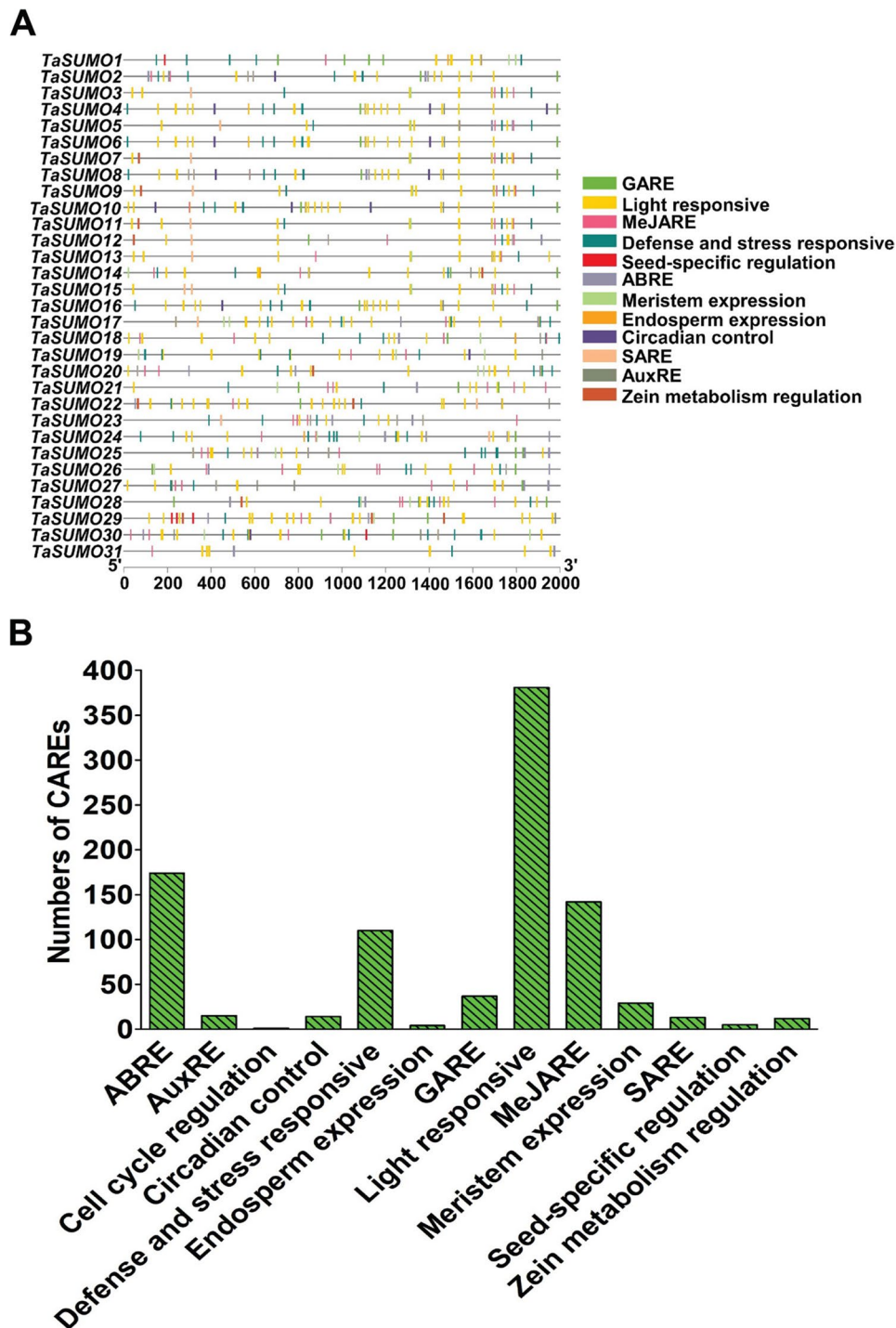


Fig. 5 Identified CREs in promoter regions of *TaSUMO* genes. **A** Several CREs were identified in *TaSUMO* promoter, each illustrated in distinct colors. **B** *TaSUMO* promoters harbor numerous CREs

target proteins through post-translational mechanisms [12–16]. In addition, SUMO also plays a role in regulating a wide range of cellular processes, including receptor function, channel activity, G-protein signaling, exocytosis, cytoskeletal organization, mitochondrial dynamics, and autophagy [15, 17–20]. Therefore, a genome-wide

investigation is essential to uncover the functions of the *TaSUMOs* in plant development and their response to diverse stresses. The complete wheat genome enables comprehensive characterization of SUMO genes. This study marks the identification of 31 SUMO genes in the wheat genome (Table S1). The 31 *TaSUMO* genes

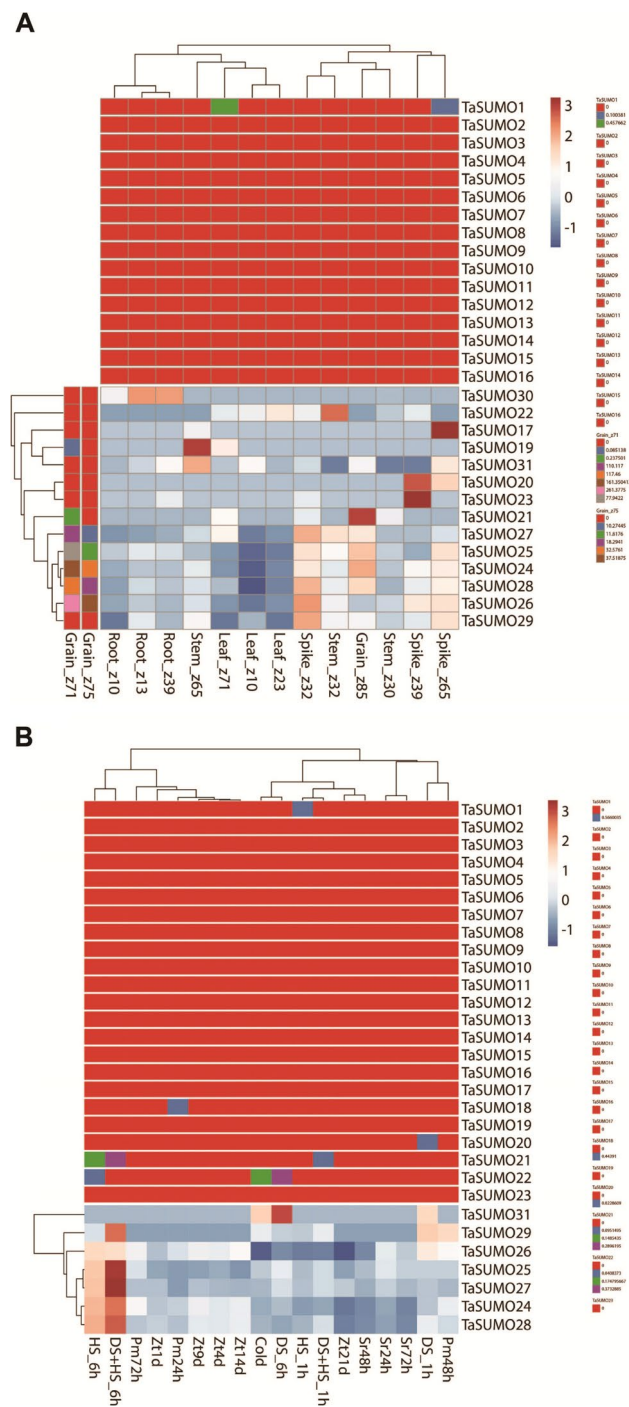


Fig. 6 Expression pattern of *TaSUMO* genes were examined under diverse tissues and stress conditions. **A** The expression patterns of *TaSUMO*s across various wheat tissues. **B** An expression profile of *TaSUMO* genes under different stress conditions

demonstrated a range of physicochemical properties. Our analysis of the GRAVY values revealed that *TaSUMO* proteins are hydrophilic, aligning with their roles in cellular functions such as post-translational modification. Hydrophilic proteins, including *SUMO*, are

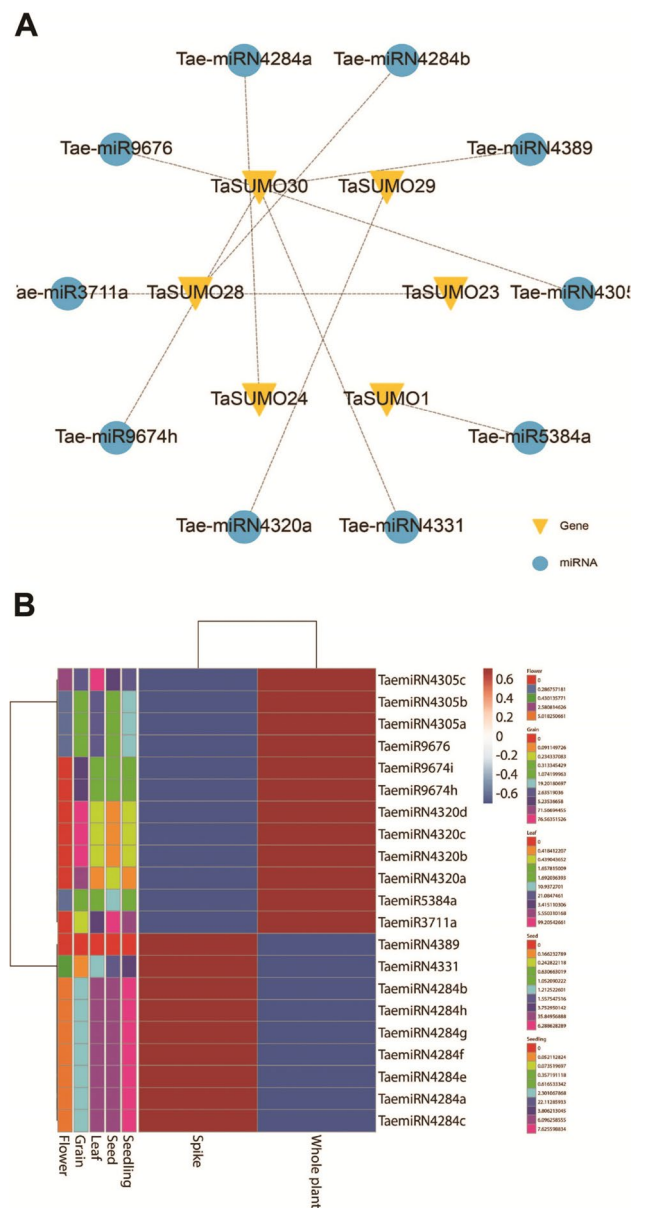


Fig. 7 The miRNA network, along with its specific targets within Wheat *SUMO* genes, and the expression profiles of potential miRNAs were analyzed. **A** The network comprises miRNAs and targeted *TaSUMO* genes, with miRNAs represented by circles and potential target *TaSUMO* genes by triangles. **B** The heatmap displays the transcript levels of miRNAs across different tissues

generally active in dynamic cellular environments and are commonly found in the nucleus. Here, they interact with DNA and regulatory proteins, playing a crucial role in modulating stress responses and development. The corresponding *TaSUMO* proteins exhibited a uniform distribution pattern in both pI and MW (Fig. S1; Table S1). For instance, proteins with different pI values may interact with various cellular partners depending on the pH of the cellular compartment. This interaction could influence their localization and function during

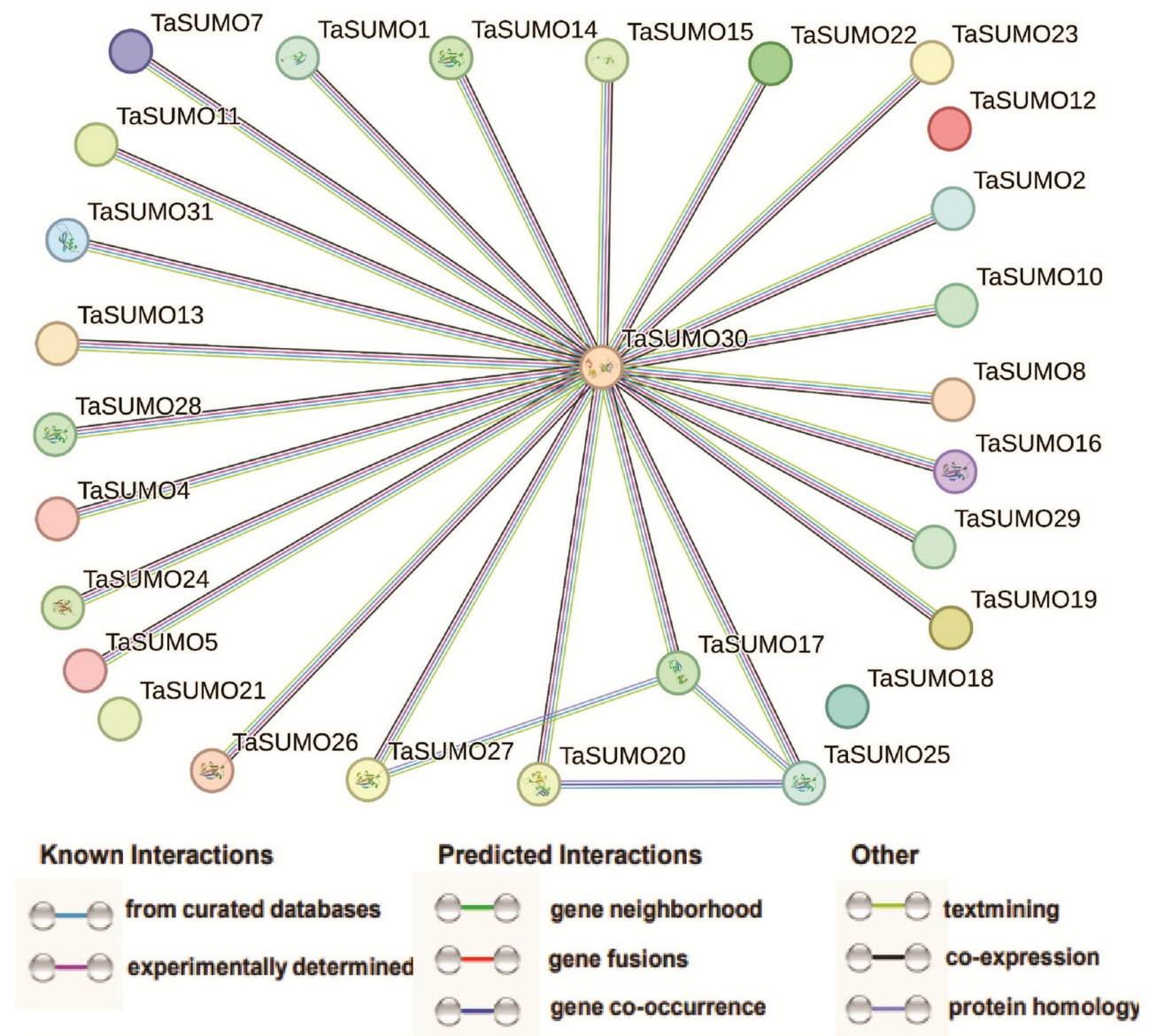


Fig. 8 The interactions between TaSUMO proteins were investigated by constructing a network of PPI using STRING v12.0

stress responses or developmental stages. The variation in molecular weight, when considered alongside pI, offers valuable insights into potential post-translational modifications and functional diversity. Phylogenetic analysis unveiled that SUMO proteins from diverse plant species were grouped into thirteen distinct clusters, as illustrated in Fig. 1. Cluster VII stood out by having the largest number of members, comprising 16 genes and accounting for 23.88% of the total. In contrast, cluster IX contained only a single gene. Interestingly, clusters I, VIII, IX, X, XI, XII, and XIII were specific to monocots, while clusters II and III were exclusive to dicots, indicating that clusters that are specific to monocots or dicots may reflect lineage-specific diversification, which could relate to the distinct functional needs of monocots and dicots. For example,

SUMO proteins in dicots might be more involved in specific stress responses or developmental processes that are less critical in monocots, and vice versa. Upon further analysis, we found that clusters I, VIII, IX, X, XI, XII, and XIII also show signs of gene amplification in monocots, though the extent of amplification and divergence varies compared to cluster II and III. This suggests that while gene amplification occurred in multiple clusters after the monocot-dicot divergence, the evolutionary pressures and functional differentiation of SUMO genes may vary across clusters (Fig. 1). These duplications play a vital role in enhancing and diversifying gene families, facilitating the adaptation and evolutionary processes of organisms over time [56–58]. Chromosome mapping revealed the dispersion of *TaSUMO* genes across eleven chromosomes

of wheat (Fig. 2A, Fig. S3 and Table S1). Similar patterns have been observed in several other plant gene families, indicating the presence of a potentially conserved regulatory mechanism [57–61]. Gene clusters are notably found at Chr1B and Chr1D (Fig. 2A). The uneven distribution of *TaSUMO* genes across the eleven wheat chromosomes suggests that gene gain and loss, driven by segmental duplication and whole-genome duplication, have occurred. These evolutionary processes often result in variations in gene numbers across different chromosomes. In this study, we pinpointed eight duplicated pair of *TaSUMO* genes (Fig. S4 and Table S4). To explore the pressure of selection influencing duplicated *TaSUMO* genes, we performed Ka/Ks computation for eight *TaSUMO* genes (Table S4). Consistently, Ka/Ks ratio for eight *TaSUMO* genes remained below one, suggesting that these duplicated *TaSUMO* genes went through negative selection. However, the negative selection indicated that the *TaSUMO* genes have been conserved throughout evolution, likely due to their essential roles in critical biological processes such as stress response, development, and cellular regulation. This conservation suggests that mutations causing significant changes to protein function are likely harmful and therefore eliminated by selective pressure. In certain instances, duplicated genes under negative selection may diverge to develop specialized functions, allowing different *TaSUMO*s paralogs to contribute to distinct biological pathways such as specialization enhances an organisms ability to adapt to various environmental stresses and developmental cues. Conversely, if negative selection preserving duplicated genes with overlapping functions may lead to functional redundancy, which acts as a safeguard for essential biological processes. This redundancy ensures that even if mutations occur in some gene copies, the organism can retain stability and functionality through the compensatory roles of the remaining gene copies. Tandem repeats are instrumental in the creation of gene clusters, while fragment repeats are involved in the expansion of homologous genes [62]. In addition, our investigation encompassed an examination of the syntenic analysis between *TaSUMO* genes and other crucial plant species, including *A. thaliana*, *O. sativa*, (*A. tauschii*, and (*B. distachyon* (Fig. 3). These findings underscore a conserved evolutionary pattern within the *TaSUMO* gene family.

The organization of specific sequences within a gene, known as gene structure, reveals important insights into the conserved traits and evolutionary variations across different genes. The analysis shows a clear conservation in the number of introns and exons among different subgroups (Fig. S6; Fig. S7). Additionally, *TaSUMO* genes exhibit significant conservation in their gene structure, with most *TaSUMO* genes lacking introns (Fig. S7). However, *TaSUMO24*, *TaSUMO25*, *TaSUMO26*, *TaSUMO27*,

TaSUMO28, and *TaSUMO30* displayed 1 to 2 introns. Numerous researchers shown an importance of introns in evolutionary history of diverse plant gene [63–65]. Gene family show considerable erraticism in intron number, fluctuating from genes with little or intronless or larger number of intron in plants [64, 65]. The disparity in the number of exons and introns is thought to reflect evolutionary processes and can be used as a record of these changes [66]. Further, conserved motifs analysis of *TaSUMO* revealed that SUMO family members possessed a conserved SUMO motif (Fig. 4 and Fig. S9A). Additionally, 3D structure of *TaSUMO* proteins were predicted (Fig. S9B). The majority of *TaSUMO* proteins exhibited three to six motifs, highlighting the remarkably conserved characteristics of SUMO proteins across evolutionary stages and implying comparable functionalities among them. A collective count of 4 motifs was recognized in each SUMO protein. The size and optimal matching sequences of motifs were illustrated in Fig. 8A, accompanied by the corresponding motif logos in Fig. 8B. Therefore, these findings would be crucial for understanding and elucidating the precise roles of *TaSUMO* proteins in governing various cellular processes related to plant development and stress response in wheat.

The cis-elements located in the promoter region are crucial for regulating gene expression [67–69]. Our study uncovered multiple CREs within promoter region of *TaSUMO*s. These CREs encompass different functional categories, including responses to light, phytohormones, and different stress conditions (Fig. 5A and B, and Table S5). CAREs associated with light response, ABRE, MeJARE, defense and stress responsiveness were predominantly found within the promoters of *TaSUMO* genes (Fig. 5B). *AtSUMO1*, *AtSUMO2*, *OsSUMO1*, *OsSUMO2*, *ZmSUMO1*, *GmsSUMO1*, and *GmsSUMO2* is rapidly induced in response to stress [29, 31–33]. The SUMO E3 ligase named *SIZ1* has been identified as a regulator of growth and heat stress in *Arabidopsis* [35], and enhanced drought tolerance in tobacco [36]. SUMO system modulates ABA signaling and mediates resistance to bacterial and viral disease [40, 41]. NPR1 SUMOylation by SUMO3 following SA treatment highlights the complex role of SUMO in SA-mediated defense responses. This SUMOylation process functions as a tightly regulated switch that triggers the activation of PR gene expression [70]. BR signaling is negatively controlled by the SUMO E3 ligase *SIZ1*, which modulates the stability and activity of BES1 through SUMOylation [71]. Furthermore, *TaSUMO*s promoter harbor the CREs related with a multitude of functions including seed specific regulation, cell cycle regulation, endosperm expression and circadian regulation. The presence of these CREs in *TaSUMO*s promoters indicates their potential involvement in a range of cellular processes. Therefore, these

findings suggest that *TaSUMO* genes might play a crucial function in governing plant growth, development, and response to various plant hormones.

SUMO proteins have been found to regulate a variety of plant biological processes, including root development, plant reproduction, overall growth, and tolerance to both biotic and abiotic stresses [25–30]. Our investigation revealed that the 31 *TaSUMO* genes exhibited unique expression profiles in various tissues and stress conditions (Fig. 6A and B). In spike_z32, *TaSUMO26*, *TaSUMO27*, *TaSUMO28* and *TaSUMO29* exhibited significant elevation, whereas spike_z32 *TaSUMO20*, and *TaSUMO23* were observed to be raised. The expression of *TaSUMO20* was highly induced in spike_z65. Further, the expression levels of *TaSUMO19* and *TaSUMO31* were notably increased in stem_z65. In grain_z85, *TaSUMO26*, *TaSUMO27*, *TaSUMO28*, and *TaSUMO29* were observed to be up-regulated. Furthermore, *TaSUMO22* expression displayed raised in stem_z32. These findings indicate that the identified SUMOs have distinct expression profiles specific to various tissues. Ecd4 is a SUMO-specific protease that regulates flowering time and processes precursor SUMO proteins in *Arabidopsis* [37]. It has also been reported that the SUMO protease OTS1 acts as a positive regulator of rice seed germination and root development under saline conditions [38]. In *Arabidopsis*, *AtSUMO1* is expressed constitutively across most plant tissues, except for the root apex, lateral root primordia and vasculature [26]. The double homozygous *sum1 sum2* mutants are embryo-lethal, indicating that SUMO1 and SUMO2-mediated SUMO conjugation are essential for plant development [72]. Additionally, recent studies have demonstrated that SIZ1 acts as a repressor of in vitro shoot regeneration by regulating the expression of key regeneration factors in *Arabidopsis* [73]. Hu et al. underscored the pivotal role of pangenomics in revealing genome-wide diversity across cultivated varieties and their wild relatives. They further emphasized the need to integrate pangenomic analyses with functional annotation to more accurately link structural variations to phenotypic traits [74]. These results showed that *TaSUMO* genes may participate in the development of different tissue of wheat. Furthermore, distinctive transcript level was also evident for *TaSUMO* genes under different environmental stress. The expression of *TaSUMO24*, *TaSUMO25*, *TaSUMO27*, *TaSUMO28* and *TaSUMO29* exhibited notable elevation in DS + HS_6h, while *TaSUMO24*, *TaSUMO25*, *TaSUMO26*, *TaSUMO27* and *TaSUMO28* HS_6h were significantly induced in HS_6h. In addition, *TaSUMO31* showed increased expression levels in DS_6h. In DS_1h, *TaSUMO29* and *TaSUMO31* exhibited up-regulation. Ectopic expression of *ZmSCE1e* gene increased SUMO conjugation levels and enhanced drought and salt tolerance in tobacco [39]. Ectopic expression of *AtSUMO1*

and *AtSUMO2* do not affect overall plant growth but does alleviate ABA-mediated growth inhibition by triggering the expression of ABA-responsive genes, including RD29A [41]. SUMOylation regulates ABA signaling at various levels. An elevated in conjugation of SUMO was observed in drought stress, with this effect being partially regulated by ABA and SIZ1 [75]. These genes are potential targets for gene editing to improve abiotic tolerance in wheat under changing climatic conditions. The GO enrichment analysis revealed enrichment in several biological process categories. Collectively, these findings indicate that *TaSUMO* genes are essential in different developmental process and both biotic and environmental stress in wheat. Consequently, this study provides a foundation for gaining valuable insights into the functions of *TaSUMO* genes in future research.

In addition, nine miRNAs were found to target nine out of the 31 *TaSUMO* genes (Fig. 7A, Table S9). The miRNAs identified exhibit unique expression patterns in different tissues in wheat (Fig. 7B). These miRNAs could potentially interfere with various developmental processes and stress tolerance in wheat. Recent advances in computational biology have significantly advanced our understanding of gene regulation and genome variability in plants. Kuang et al. highlighted the potential of machine learning (ML) approaches for predicting plant miRNAs, while also identifying key challenges, including limited validated datasets and biological noise. They proposed future directions involving integrative ML frameworks tailored to the complexity of plant genomes [76]. This perspective aligns with our approach, where computational predictions serve as a critical foundation for subsequent experimental validation. Several heat stress-responsive miRNAs have been identified in various plant species, highlighting their crucial regulatory roles under high temperature stress (HS) [77]. These findings emphasize the crucial role of plant miRNAs in coordinating the response to HS. The SUMOylation-mediated stress response (SSR) emerges as one of the earliest and most rapid responses to HS [37, 78]. Under HS, plants activate the SSR, causing many SUMO molecules to shift from the cytoplasm to the nucleus, which enhances the SUMOylation of several target proteins. This conserved mechanism, found in diverse organisms such as plants, yeast, and humans, serves as a protective umbrella that shields essential proteins involved in stress management [79, 80]. Recent research has demonstrated that chromatin SUMOylation is involved in regulating global HS response genes, with the SUMO E3 ligase SAP and MIZ1 (SIZ1) playing a central role in this process. In SIZ1 deletion mutants, there is a marked decrease in the heat-induced SSR and a significant reduction in chromosomal distribution [81]. Chromatin-associated SUMOylation play a key role in regulating miRNAs in *Arabidopsis* [82].

The Xia group identified SUMO peaks in the promoter regions of several miRNAs under HS. Through small RNA sequencing (sRNA-seq), they detected significant alterations in the expression of these miRNAs during HS in both wild-type plants and *siz1* mutants. Further, yeast one-hybrid assays were performed to identify potential transcription factors (TFs) involved in this process. Notably, these TFs were shown to undergo SUMOylation *in vitro*. Collectively, these findings highlight the critical role of SUMOylation in modulating miRNA expression during the HS response in plants. Hence, this work uncovers a novel function for SUMOylation in regulating miRNAs under high-temperature stress [82]. Furthermore, differential expression of miR824 and miR398 were detected before and after high-temperature stress in wild-type Col-0 and *SIZ1*-deleted mutants [82]. The thermosensitive SUMOylation of TaHsfA1, a class A heat shock TF, acts as a dynamic molecular switch, regulating the activation and deactivation of the HS response in wheat [42]. Consequently, these findings provide valuable insights into understanding the specific roles of these miRNAs in a range of biological processes in wheat. Moreover, our PPI findings reveal that a total of 31 TaSUMO proteins were identified to interact within the TaSUMO family. Interestingly, TaSUMO30 was found to interact with 24 proteins (Fig. 8 and Table S11). These insights open new avenues for future research into the functional characterization of TaSUMO genes, providing a deeper understanding of their roles in various biological processes in wheat. In this study, we identified thirty-one SUMO genes in the wheat genome through the application of advanced bioinformatics tools. This comprehensive analysis provides a foundational framework for investigating the molecular mechanisms by which SUMO genes contribute to stress responses in cereal crops. Our findings offer valuable insights that pave the way for functional characterization of TaSUMO genes across various tissues, in response to phytohormones, and under diverse and complex stress conditions in wheat.

Conclusion

This study identified 31 SUMO genes in the wheat genome. Chromosomal mapping showed that these TaSUMO genes are distributed across 11 wheat chromosomes. Additionally, eight duplicated gene pairs were identified, with Ka/Ks ratio analysis indicating that these duplicated TaSUMO genes have undergone purifying selection. The promoter region of TaSUMOs contain multiple CREs associated with light response, phytohormones, and stress tolerance. Furthermore, differential expression patterns among TaSUMO family members were observed across various tissues and in response to diverse stress conditions. In addition, the investigation of miRNAs targeting TaSUMO genes, alongside

their varying transcript levels in different tissues, adds a regulatory dimension to our understanding of post-transcriptional control within the SUMO family. The insights from this study provide a strong foundation for elucidating the functions of TaSUMO genes in tissue-specific responses, phytohormonal signaling, and stress adaptation. Given the changing climate, manipulating these TaSUMO genes and their associated miRNAs through genome editing tools could significantly improve wheat yield and enhance stress tolerance. Thus, this work paves the way for developing more resilient wheat cultivars with improved productivity, making a vital contribution to future food security.

Abbreviations

SUMO	Small Ubiquitin-like Modifier
PTMs	Post-translational modifications
TPM	Transcripts per million
MW	Molecular weight
pl	Isoelectric point
CREs	Cis-regulatory elements
BLASTp	Basic local alignment search tool for protein
NCBI-CDD	National Center for Biotechnology Information-Conserved Domain Database
qRT-PCR	Quantitative reverse transcription polymerase chain reaction
cDNA	Complementary DNA
ABRE	Abscisic acid response element
AuxRE	Auxin response element
MeJARE	MeJA response element
SARE	Salicylic acid response element
GARE	Gibberellin response element
miRNAs	microRNA

Supplementary Information

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Supplementary Material 1

Supplementary Material 2

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Authors' contributions

M.S.K. conceptualized and designed the research, M.S.K. and F.B.M. drafted the original manuscript. M.S.K. and F.B.M. supervised the study. M.S.K. led the investigation and conducted the **in silico** analysis. S.F.A. and T.K.A. carried out the qRT-PCR experiment. B.S.K., S.K.L. and S.F.A., C.B.H. writing, review and editing, M.S.K. and K.K.R., methodology, M.L.R., M.B., M.K., S.T., A.K. and S.K. visualization and help in editing and revising the manuscript, S.F.A. and T.K.A. for the valuable input and formal analysis. All authors have reviewed and approved the final published version of the manuscript.

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Data availability

We utilized publicly available databases for this study. All publicly sourced resources have been clearly cited in the Materials and Methods section, along with their respective online links.

Declarations**Ethics approval and consent to participate**

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Consent for publication

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Competing interests

The authors declare no competing interests.

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