Pak. J. Bot., 58(13): November 2026.

ARTICLE DOI: http://dx.doi.org/10.30848/PJB2026-11(13)

GENOME-WIDE IDENTIFICATION AND TEMPERATURE STRESS RESPONSE ANALYSIS OF THE SOT GENE FAMILY IN PEPPER (*CAPSICUM ANNUUM*)

YAN MIAO AND FENG LIU*

College of Horticulture, Hunan Agricultural University, Changsha 410000, China

*Corresponding author's email: jwszjx@hunau.edu.cn

Abstract

Sulfotransferases (SOTs) play a crucial role in regulating secondary metabolism and responding to abiotic stress in plants; however, systematic studies on SOTs in peppers (*Capsicum annuum*) remain limited. To investigate the compositional characteristics of the pepper SOT gene family and its response mechanisms to high- and low-temperature stresses, this study utilized whole-genome data from the "Zhangshugang" pepper variety to identify and analyze the structure of SOT gene family members. Additionally, transcriptomic data from the PepperHub database were integrated to examine their expression patterns across different tissues and under temperature stress conditions. The results indicated that 25 *CaSOT* family members were identified in the Capsicum genome, distributed across 6 chromosomes. Phylogenetic analysis classified the *CaSOT* genes into four subfamilies, with high consistency in gene structure and conserved motif composition among different subfamilies. The colinearity of *CaSOT* genes with potato was stronger than that of *Arabidopsis*, reflecting the evolutionary affinity among Solanaceae crops. Analysis of cis-acting elements in the promoter region revealed that the *CaSOT* gene promoter was rich in elements associated with light response, hormone response, and abiotic stress conditions such as high and low temperatures. Transcriptomic and qRT-PCR analyses revealed significant expression differences in *CaSOT* genes under temperature stresses conditions at different time points. Some *CaSOT* genes were associated with high- or low-temperature stress, e.g. *CaSOT6*, *CaSOT7*, *CaSOT2*, *CaSOT12*, *CaSOT18*, *CaSOT22* and *CaSOT14*. This study provides important theoretical foundations and candidate gene resources for further elucidating the molecular mechanisms of temperature stress responses in pepper and for molecular breeding aimed at enhancing stress tolerance.

Key words: Pepper; SOT gene family; Gene identification; Temperature stress; Bioinformatic analysis

Introduction

Pepper (*Capsicum annuum* L.) is an important vegetable and cash crop, holding significant value in the food, seasoning, health, and processing industries. However, in recent years, temperature stress has become one of the key abiotic stress factors. Under low-temperature stress, chili peppers exhibit reduced flower bud differentiation, pollen viability, and germination capacity, which affects growth and development and limits yield and quality (Guo *et al.*, 2013; Zhang *et al.*, 2022). High temperatures lead to increased cell membrane permeability, inhibit photosynthetic efficiency, and promote the massive accumulation of reactive oxygen species (ROS) (Zhang *et al.*, 2024). Thus, temperature stress, both higher and low temperature stress, severely limiting pepper growth and development, yield formation, and quality improvement (Lee *et al.*, 2018). Therefore, elucidating the molecular response mechanisms of pepper under high- or low-temperature stress is of great significance for identifying heat- and cold-tolerant genes and for breeding pepper varieties with improved tolerance to temperature stress.

The adaptation of plants to temperature stress is a complex process regulated by integrated physiological and molecular networks, involving multiple layers such as signal perception, transcriptional regulation, metabolite biosynthesis, and the maintenance of cellular homeostasis. Studies demonstrated that secondary metabolites, including flavonoids, phenolic acids, and their derivatives, played a crucial role in plant antioxidant defense and stress adaptation (Rao & Zheng, 2025). The bioactivity, stability, and subcellular localization of these metabolites often require precise regulation through specific modification reactions. Sulfotransferases (SOTs) are involved in regulating a wide range of important endogenous compounds in plants. Known substrates include flavonoids, glucosinolates, brassinosteroids, jasmonic acid, and salicylic acid (Mistry *et al.*, 2024). SOTs mediate the transfer of sulfate moieties from the universal donor 3'-phosphoadenosine 5'-phosphosulfate (PAPS) to the hydroxyl groups of diverse substrates (Hirschmann *et al.*, 2014), concurrently generating 3'-phosphoadenosine 5'-phosphate (PAP). These structural elements are essential for their catalytic activity (Negishi *et al.*, 2001). These functional characteristics suggest that SOTs may indirectly influence plant adaptation to environmental stresses by modulating the activity of endogenous protective compounds and signaling molecules (Wawrzynska & Sirko, 2024; Han *et al.*, 2025).

The SOT gene family has been systematically identified in model plants such as *Arabidopsis thaliana*, rice (*Oryza sativa*), and potato (*Solanum tuberosum*) (Faraji *et al.*, 2021). For instance, in *Arabidopsis thaliana*, sulfation has been shown to regulate flavonoid metabolism, thereby influencing plant tolerance to ultraviolet radiation. Glucosinolates, which are important secondary metabolites in *Brassicaceae* species, require sulfation catalyzed by

Received: 30-03-2026 Revised: 18-05-2026 Accepted: 02-06-2026 Online: 02-07-2026

SOTs as a key step in their biosynthesis; among them, *AtSOT18* exhibits the highest affinity for phenethyl desulfoglucosinolat (Klein *et al.*, 2006). In rice, *OsSOTs* have been classified into 7 subfamilies, with 25 genes containing the conserved PAPS-binding domain. Most *OsSOT* genes are specifically expressed in stigmas and anthers and are inducible by hormones such as indole-3-acetic acid (IAA). Additionally, eleven *OsSOT* genes are responsive to abiotic stresses, and their promoter regions contain cis-acting elements associated with drought and salt stress responses (Chen *et al.*, 2012). In tomato, the *SlSOT1* gene has been demonstrated to participate in salt stress responses. In Chinese cabbage (*Brassica rapa*), a total of 56 *BrSOT* genes have been identified, among which *Bra017370* and *Bra009300* are significantly upregulated under drought, salt stress, and abscisic acid (ABA) treatment, providing strong evidence that SOTs are widely involved in stress responses (Jin *et al.*, 2019). These studies collectively reveal the potential roles of the SOT gene family in plant responses to abiotic stresses. However, in pepper, systematic investigations on the family composition, structural characteristics, evolutionary relationships, and expression responses of SOT genes under high- and low-temperature stress are still scarce.

In current research, a comprehensive genome-wide identification of the SOT gene family in pepper was conducted based on available genomic data. Gene structure, conserved motifs, chromosomal localization, and phylogenetic relationships were systematically analyzed. Furthermore, transcriptome data were integrated to investigate the expression patterns of SOT genes under temperature stress conditions. This study aims to provide a theoretical basis for elucidating the potential roles of SOT genes in the molecular response mechanisms of pepper to temperature stress, and to offer valuable genetic resources for the molecular breeding of pepper varieties with enhanced tolerance to high and low temperatures.

Materials and Methods

Identification, gene structure and physicochemical property analysis of the SOT gene family in pepper: The whole-genome sequence, CDS sequences, protein sequences, and GFF gene information files for Zhangshugang were obtained from the Pepper Genomics Database (http://ted.bti.cornell.edu/cgi-bin/pepper/index). The pepper protein sequences were imported into the PFAM database (https://www.ebi.ac.uk/interpro/) to search for the SOT domain (PF00685), and potential members of the *CaSOT* gene family were identified using HMM models ($E<1e^{-5}$). All candidate sequences were further verified using Pfam and SMART databases to retain only genes with complete conserved domains. Redundant sequences and alternative splicing variants were removed, and only complete protein was retained for subsequent analysis. Simultaneously, known protein sequences of the SOT gene family from *Arabidopsis* were downloaded from the TAIR database (https://www.arabidopsis.org/) and aligned with the complete protein sequences of *Capsicum*. This yielded candidate SOT sequences with high sequence similarity. The intersection of the results from TBtools II (Chen *et al.*, 2023) Blastp and HMM analyses was then used to identify members of the SOT gene family in *Capsicum*, and false positives and duplicate sequences were filtered out through domain screening.

Conserved motifs of *CaSOT* transcription factor family members were predicted using the MEME website (https://meme-suite.org/meme/). Using NCBI's (https://www.ncbi.nlm.nih.gov) CD-Search, the search database was set to Pfam v35.0 – 19,638 PSSMs with a threshold of 0.01 to exclude false positives and duplicate sequences, yielding a structured results table, multiple sequence alignments, and functional annotation information. Using the Gene Structure View feature in TBtools, visualize the phylogenetic relationships, gene structures, and conserved motif characteristics of the *CaSOT* family. Utilize the Protein Parameter Calc tool in TBtools II to analyze information such as the number of amino acids, molecular weight, theoretical isoelectric point, stability index, aliphaticity index, and average hydrophilicity of proteins in the *CaSOT* gene family.

Conserved motif and phylogenetic analysis of CaSOT proteins: The genome sequences and annotation files of *Arabidopsis thaliana* and *Solanum tuberosum* were obtained from the Ensembl Plants database (https://plants.ensembl.org/) and the Sol Genomics Network (https://solgenomics.net/), respectively. Multiple sequence alignment of SOT proteins from *A. thaliana*, *C. annuum* and *S. tuberosum* was performed using MEGA 12.0 to ensure the conservation of functional domains. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method with default parameters. The resulting tree was visualized and annotated using TBtools. Bootstrap values were set to 1000 to assess tree reliability.

Synteny analysis within and between species: Collinearity analysis of SOT genes within *C. annuum* and among *A. thaliana*, *C. annuum*, and *S. tuberosum* was performed using the One Step MCScanX function in TBtools II. Syntenic gene pairs were identified using the File Merge for MCScanX tool, and the syntenic relationships were visualized using the Multiple Synteny Plot function.

Cis-Acting element analysis of promoters: Use the GFF3 Extract tool in TBtools II to extract the promoter sequence by mapping 2000 bp upstream of the start codon of *CaSOTs*. These promoter sequences were analyzed using the PlantCARE database, and the cis-acting regulatory elements were visualized using the BioSequence Viewer tool.

KEGG enrichment analysis of differentially expressed genes: Transcriptome data of *CaSOT* genes were obtained from the PepperHub database (http://lifenglab.hzau.edu.cn/PepperHub/index.php) (Liu *et al.*, 2017). We performed differential expression analysis using DESeq2 on transcriptomic data from high- (40°C) and low-temperature (10°C) stress treatments compared to the control group, Differentially expressed genes were identified using a threshold of $|\log_2\text{fold change } (\log_2FC)| \geq 1$ and p-value (padj) ≤ 0.05. KEGG pathway enrichment analysis was performed on the differentially expressed genes identified under high- and low-temperature stress, respectively, to investigate the major metabolic and signaling pathways involved in chili pepper under temperature stress.

Gene expression analysis: Based on transcriptome data from the PepperHub database, the average FPKM values of three biological replicates were calculated to analyze the expression patterns of *CaSOT* genes in pepper leaves. Using the TBtools software, a heatmap of gene expression patterns was constructed using $\log_2$-transformed expression levels [$\log_2$(FPKM + 1)] as the scale.

Plant materials, treatments, and expression analysis: Uniform, glossy pepper seeds were randomly selected and germinated in a growth chamber at 25°C until the seedlings developed 5–6 true leaves. The plants were then subjected to high- and low-temperature stress treatments by transferring them to growth chambers set at 40°C and 10°C, respectively. Young leaves were collected at three different timings: 0 h, 12 h, and 24 h post-treatment. Specific qRT-PCR primers were designed based on the coding sequence of the target pepper genes for subsequent expression level analysis. Total RNA was extracted from chili plants after high- and low-temperature treatments using the Super Total RNA Extraction Kit (Eastep, Beijing). The RNA was reverse-transcribed into cDNA using 5× Evo M-MLVRT Master Mix, and real-time quantitative PCR was performed using 2× ChamQ Universal SYBR qPCR Master Mix. Reaction system (20 μL): 10 μL Mix, 0.4 μL each of forward and reverse primers, 2 μL cDNA, 7.2 μL ddH_2O. Reaction program: initial incubation at 95°C for 2 min followed by 40 cycles of 95°C for 15 s, then 60°C for 15 s and 72°C for 15 s. Gene-specific qRT-PCR primers were designed (Table 1), and pepper ubiquitin was selected as the internal control gene (Wan *et al.*, 2011). The experiment was performed in triplicate. Statistical analysis of the data was conducted using GraphPad Prism and Excel software, and relative expression levels were calculated using the $2^{-\Delta\Delta Ct}$ method (Livak & Schmittgen, 2001). Differences in gene expression levels among groups were compared using one-way analysis of variance (one-way ANOVA).

Results

Identification and chromosomal localization of the *CaSOT* gene family: Based on SOT domain searches and sequence alignment with known members of the *AtSOT* gene family in *A. thaliana*, a total of 25 *CaSOT* genes were identified in the pepper genome. Subsequent bioinformatic analysis of the physicochemical properties of CaSOT proteins, including amino acid length, molecular weight, and isoelectric point (pI), revealed the following: The amino acid sequence length of CaSOT proteins ranged from 121 to 405 amino acids, with *CaSOT24* being the shortest and *CaSOT16* the longest. The molecular weight ranged from 14,218.32 to 46,301.26 Da, with *CaSOT24* having the lowest and *CaSOT16* the highest. The pI values ranged from 5.02 to 9.66, with *CaSOT25* exhibiting the lowest and *CaSOT21* the highest value (Table 2).

Chromosomal localization analysis of the *CaSOT* gene family revealed that the genes were most abundantly distributed on chromosome 11, which harbored a total of 11 members (*CaSOT1*, *CaSOT2*, *CaSOT3*, *CaSOT4*, *CaSOT5*, *CaSOT6*, *CaSOT7*, *CaSOT9*, *CaSOT10*, *CaSOT15*, and *CaSOT21*). In contrast, chromosome 4 contained only a single gene (*CaSOT13*), while no *CaSOT* genes were identified on chromosomes 5, 6, 7, 9, 10, or 12. On the other chromosomes, the number of *CaSOT* genes ranged from one to three. These results indicate a highly uneven distribution of *CaSOT* genes that is independent of chromosome length (Fig. 1).

Conserved motif distribution and classification of CaSOT proteins: To further investigate the sequence diversity of *CaSOTs*, we analyzed the composition of conserved motifs. According to the phylogenetic tree, the *CaSOT* gene family was classified into 4 subfamilies (I, II, III, and IV). Members within the same subfamily generally exhibited similar motif distribution patterns and gene structures, suggesting that they may share analogous functions. Significant evolutionary divergence was observed among different subfamilies. All family members contained 2 to 10 conserved motifs and the Sulfotransferase_1 domain. The majority of members possessed Motifs 1–4, while only a few contained Motif 10. The motif fragments (Motif 1–10) were primarily located within the Sulfotransferase_1 domain. Eight genes possessed UTR regions, whereas 17 genes lacked UTR regions (Fig. 2).

Secondary structure analysis of the 25 identified SOT proteins revealed that the structures were predominantly composed of alpha-helices, random coils, and extended strands, with the proportions of these components exhibiting certain similarities among members. Specifically, random coils constituted the highest proportion of the secondary structure in 22 genes, while alpha-helices constituted the highest proportion in the remaining 3 genes (Table 3).

Synteny and phylogenetic analysis of the *CaSOT* gene family across species: To insight the origin and evolutionary mechanisms of the *CaSOT* family in pepper, we performed an interspecies synteny analysis among *C. annuum*, *A. thaliana*, and *S. tuberosum* (Fig. 3). The results revealed that one *CaSOT* gene (4%) exhibited syntenic relationships with *A. thaliana*, while four *CaSOT* genes (16%) displayed syntenic relationships with *S. tuberosum*. Notably, one *CaSOT* gene displayed syntenic relationships with both species, indicating a high degree of conservation. The syntenic relationship between *CaSOT* genes and potato was stronger than that with *Arabidopsis*, which may be attributed to the fact that pepper and potato belong to the same *Solanaceae* family and are closely related compared to *Arabidopsis*.

Table1. Primer ssequences for qRT-PCR.

Gene code	Forward primers (5'–3')	Reverse primers (5'–3')
CaSOT18	CTTCCATCATCTGGCGCTCT	TGACGACGACAACCATGGAG
CaSOT22	CAAGGCAAAGTGGGCGATTC	GCTTTGGTTCTTGTCTGTGTCA
CaSOT6	CAAGGGCGTGACCGTTTATG	GCTGAATTTTGGGTTGCGCT
CaSOT2	GGTGGTGCCTTTTGGTCCAT	GCTGAAGTTTGGGTTGCACTT
CaSOT7	GTCAACAACAATTTCAAGCTCGTG	AGGGTTATGACCAAGTTCAAATTCA
CaSOT12	TCGGTATAGTTGAGAGTGGT	ATGTGTCACTCTTCAAGTCC
CaSOT14	CACATACTCTGGAGCGTTTA	CCCAACTTTTGCTTTGTGAT

Table 2. Physicochemical property analysis of SOT proteins.

Gene name	Sequence ID	Number of amino acid	Molecular weight	Theoretical pI	Instability index	Aliphatic index	Grand average of hydropathicity
CaSOT1	*Caz11g19190.1*	332.00	38783.67	8.35	39.52	82.80	-0.40
CaSOT2	*Caz11g19110.1*	354.00	41502.05	9.02	55.48	89.72	-0.38
CaSOT3	*Caz11g19170.1*	332.00	38682.33	6.11	46.02	84.49	-0.35
CaSOT4	*Caz11g19180.1*	333.00	38665.65	7.11	44.96	86.88	-0.23
CaSOT5	*Caz11g19120.1*	336.00	39452.02	7.20	49.15	75.98	-0.50
CaSOT6	*Caz11g19100.1*	332.00	38665.13	7.11	42.39	78.92	-0.42
CaSOT7	*Caz11g19200.1*	332.00	38927.73	6.84	45.87	82.44	-0.34
CaSOT8	*Caz03g16170.1*	329.00	38166.85	6.31	37.16	81.12	-0.30
CaSOT9	*Caz11g17590.1*	302.00	35222.51	7.67	33.41	81.62	-0.51
CaSOT10	*Caz11g17600.1*	304.00	35546.92	6.80	25.00	78.85	-0.46
CaSOT11	*Caz08g09880.1*	346.00	39664.69	7.10	42.66	80.58	-0.38
CaSOT12	*Caz01g08150.1*	338.00	39007.16	5.83	42.80	72.90	-0.54
CaSOT13	*Caz04g24500.1*	286.00	33644.57	6.22	49.72	74.93	-0.44
CaSOT14	*Caz03g09230.1*	361.00	41227.56	5.60	36.48	79.64	-0.43
CaSOT15	*Caz11g03080.1*	316.00	36896.26	5.87	40.81	77.75	-0.45
CaSOT16	*Caz03g08330.1*	405.00	46301.26	6.73	40.78	83.75	-0.20
CaSOT17	*Caz02g09450.1*	345.00	40190.99	5.57	35.26	85.07	-0.34
CaSOT18	*Caz02g09860.1*	337.00	38928.91	5.97	46.22	68.22	-0.53
CaSOT19	*Caz01g35740.1*	293.00	34334.15	6.50	43.36	71.50	-0.53
CaSOT20	*Caz03g09210.1*	299.00	34302.90	6.01	41.52	78.93	-0.50
CaSOT21	*Caz11g19130.1*	216.00	25305.27	9.66	47.37	82.08	-0.41
CaSOT22	*Caz03g09250.1*	169.00	19473.15	5.33	40.23	74.97	-0.44
CaSOT23	*Caz03g16150.1*	248.00	28996.97	6.15	45.01	73.87	-0.57
CaSOT24	*Caz03g16160.1*	121.00	14218.32	5.10	37.32	88.51	-0.25
CaSOT25	*Caz03g09220.1*	142.00	16212.30	5.02	38.51	74.79	-0.62

Table 3. Secondary structure analysis of SOT proteins.

Protein code	Alpha helix/%	Beta turn/%	Extended strand/%	Random coil/%
CaSOT1	39.16%	0.00%	10.84%	50.00%
CaSOT2	37.29%	0.00%	11.02%	51.69%
CaSOT3	39.76%	0.00%	9.04%	51.20%
CaSOT4	38.44%	0.00%	12.31%	49.25%
CaSOT5	39.88%	0.00%	11.31%	48.81%
CaSOT6	39.88%	0.00%	11.31%	48.81%
CaSOT7	38.55%	0.00%	11.75%	49.70%
CaSOT8	40.73%	0.00%	8.81%	50.46%
CaSOT9	37.75%	0.00%	11.26%	50.99%
CaSOT10	39.14%	0.00%	11.18%	49.67%
CaSOT11	40.46%	0.00%	10.69%	48.84%
CaSOT12	40.83%	0.00%	9.47%	49.70%
CaSOT13	41.61%	0.00%	12.94%	45.45%
CaSOT14	37.67%	0.00%	10.25%	52.08%
CaSOT15	42.41%	0.00%	9.49%	48.10%
CaSOT16	38.77%	0.00%	11.60%	49.63%
CaSOT17	35.94%	0.00%	11.59%	52.46%
CaSOT18	40.06%	0.00%	11.28%	48.66%
CaSOT19	37.20%	0.00%	9.56%	53.24%
CaSOT20	39.46%	0.00%	8.36%	52.17%
CaSOT21	31.02%	0.00%	12.50%	56.48%
CaSOT22	47.93%	0.00%	6.51%	45.56%
CaSOT23	40.73%	0.00%	13.71%	45.56%
CaSOT24	51.24%	0.00%	9.09%	39.67%
CaSOT25	46.48%	0.00%	8.45%	45.07%

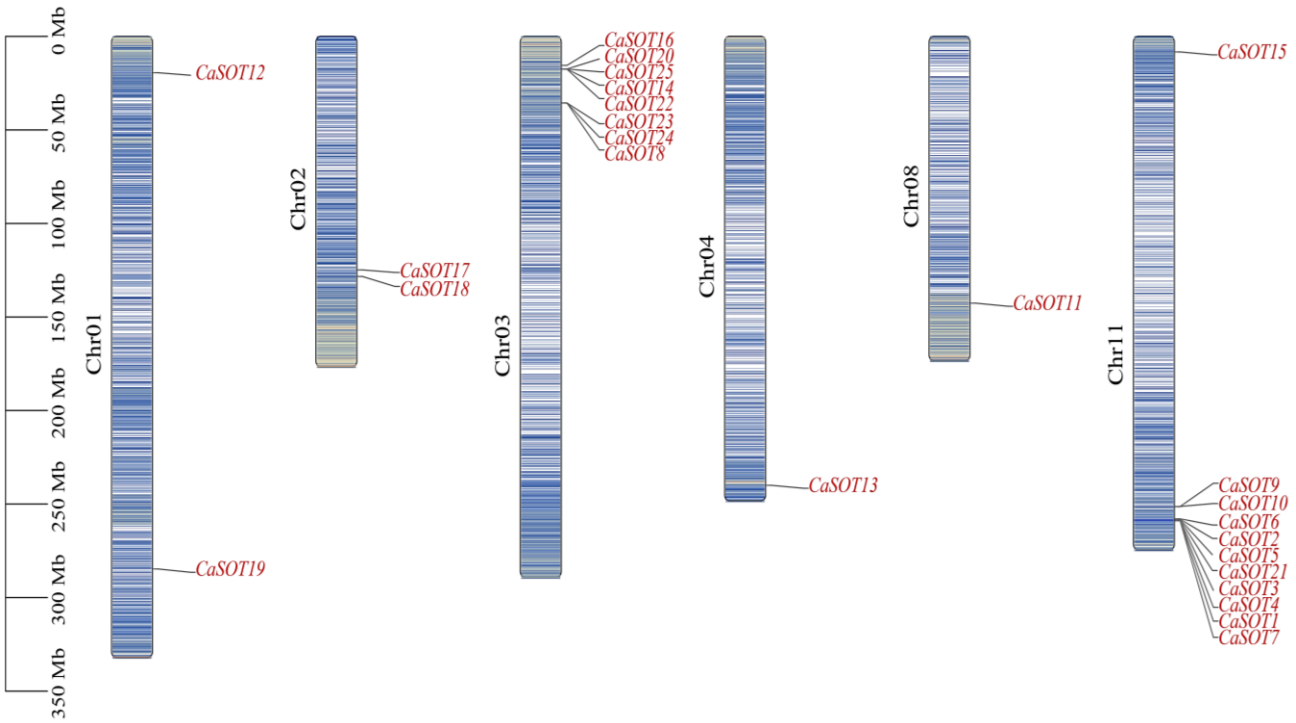

Fig. 1. Chromosomal map of *CaSOT* family genes in the pepper genome.

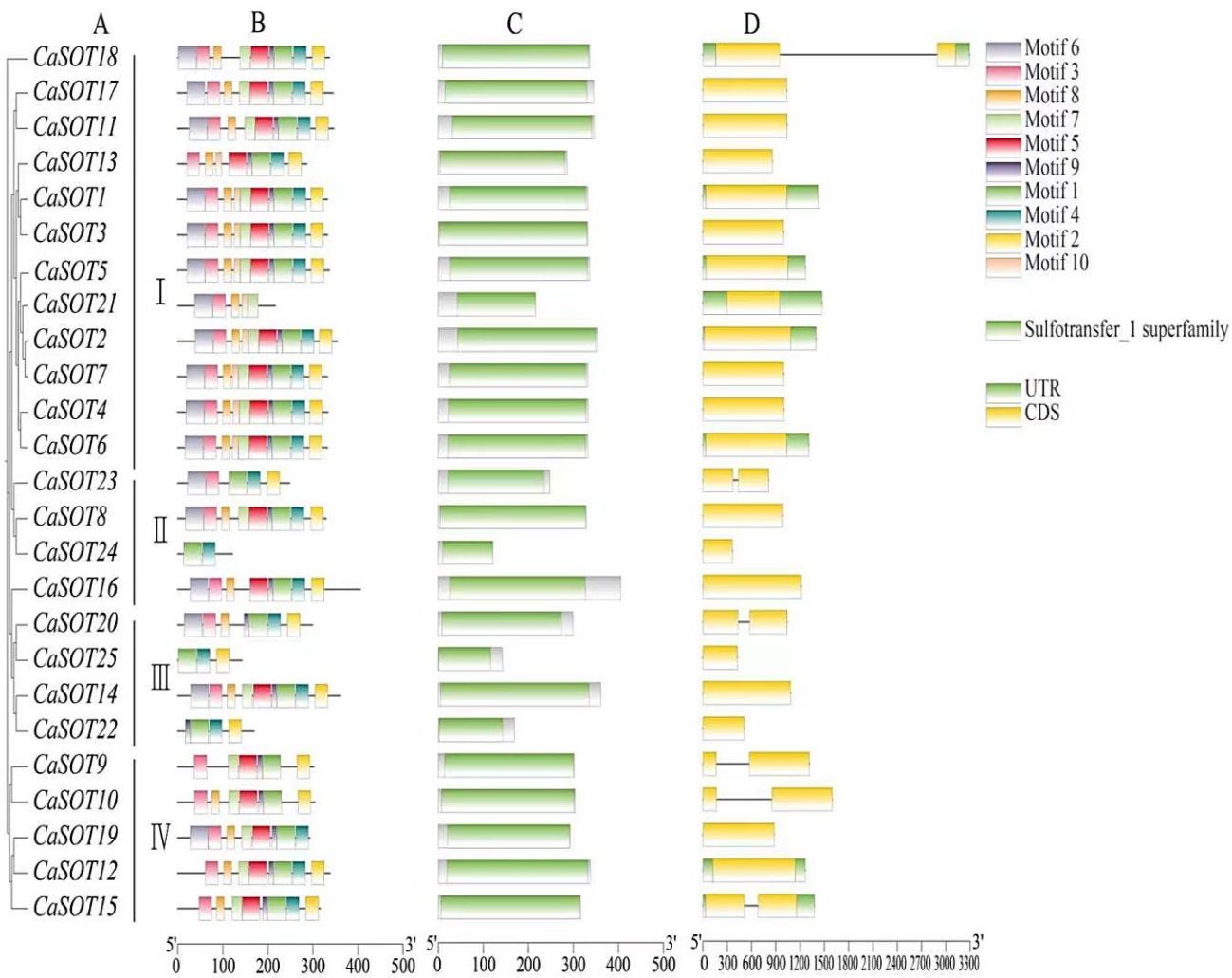

Fig. 2. A. Phylogenetic tree of the pepper *CaSOT* gene family; B. Distribution of conserved motifs; C. Analysis of protein domains; D. Conserved domain characterization.

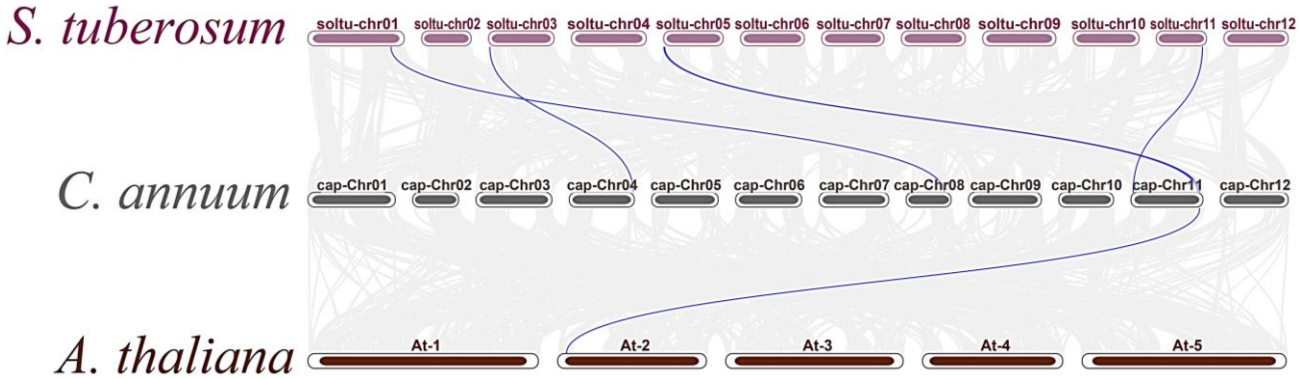

Fig. 3. Collinearity analysis among *C. annuum*, *A. thaliana* and *S. tuberosum*. Collinear gene pairs are marked with gray lines, while collinear pairs associated with *CaSOT* genes are highlighted in blue. Red triangles indicate the chromosomal locations of *CaSOT* genes.

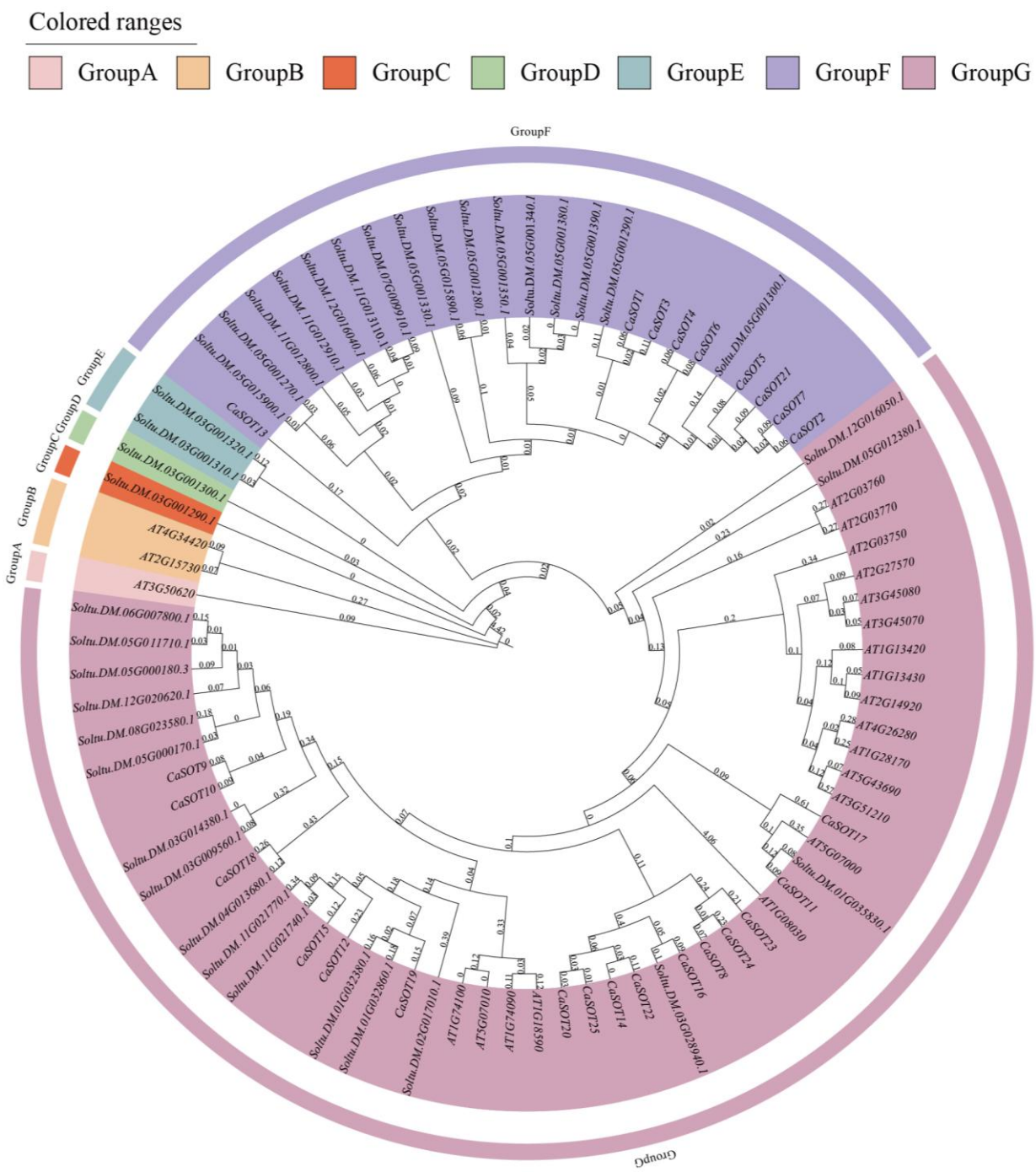

Fig. 4. Phylogenetic tree of the SOT gene family in *C. annuum*, *A. thaliana* and *S. tuberosum*. Numbers represent the branch lengths.

To investigate the phylogenetic relationships of the pepper *CaSOT* gene family, we constructed a phylogenetic tree using the identified 25 CaSOT proteins, 22 AtSOT proteins, and 37 StSOT proteins. Based on this analysis, SOT proteins were classified into 6 groups (Group A–G). Phylogenetic analysis revealed significant differences in the number of members among the various subfamilies. Group A contained only a single SOT member from *Arabidopsis*, and Group B exclusively comprised SOT members from *Arabidopsis*. Group C, Group D and Group E consisted solely of potato SOT members, which may result from gene loss or functional redundancy. In contrast, Group F and Group G encompassed SOT family members from *Arabidopsis*, pepper, and potato. Notably, Group G contained the largest number of SOT members from all three species, which could be associated with specific gene duplication events or expansion of the gene family (Fig. 4).

Cis-acting element analysis of *CaSOT* promoters: Analysis of the 2000 bp upstream promoter regions of *CaSOT* genes revealed the presence of multiple hormone-responsive and stress-related cis-acting elements (Fig. 5). Notably, low-temperature-responsive elements such as MYB and DRE core motifs, the abscisic acid-responsive element ABRE, and high-temperature stress-responsive elements such as STRE and MYB were widely distributed across the promoter regions of most *CaSOT* genes. These findings indicate that the *CaSOT* family is broadly involved in plant temperature stress responses and hormone signal transduction, representing an important gene family for the regulation of stress tolerance and growth and development in plants.

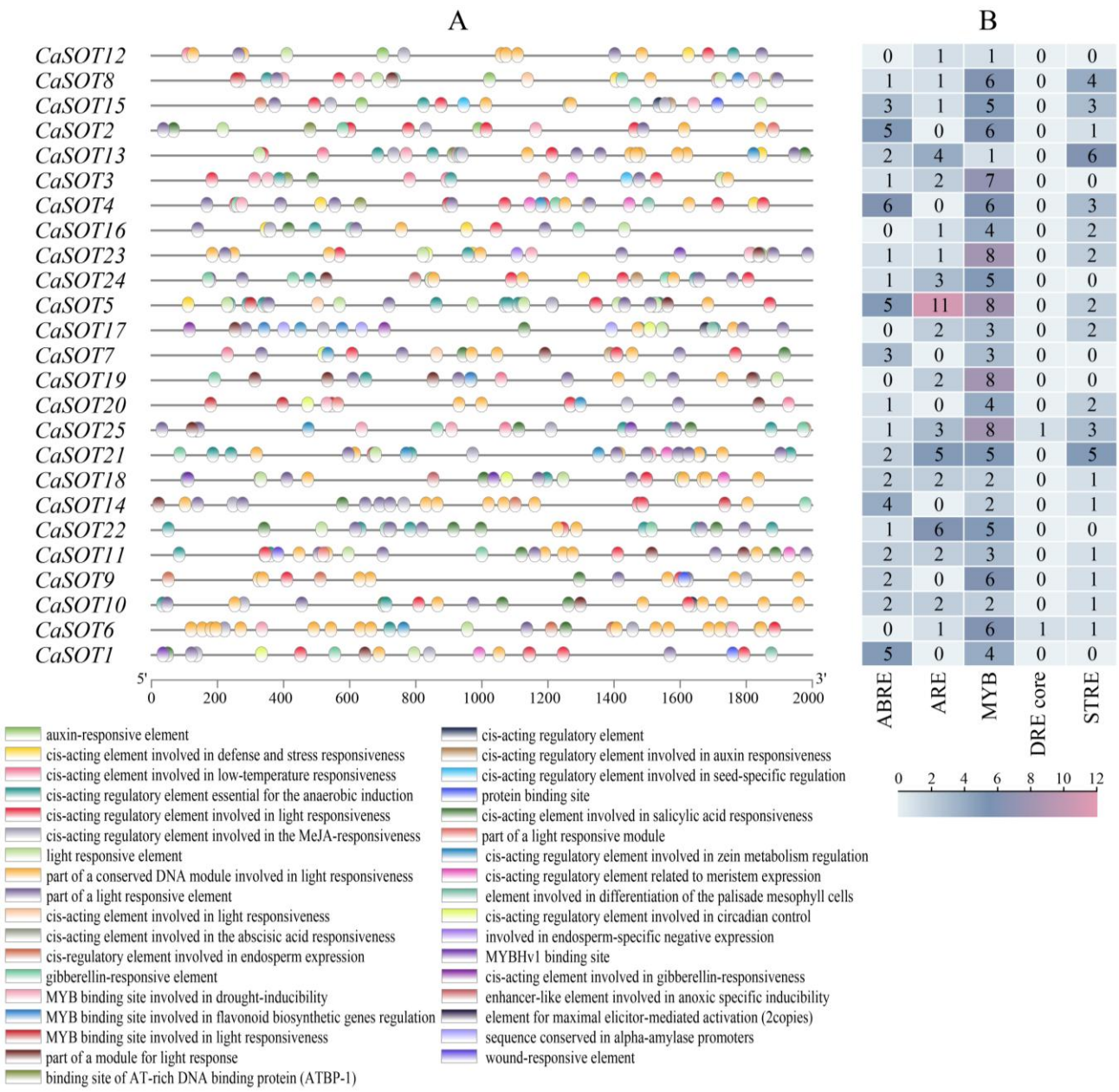

Fig. 5. A .Analysis of upstream cis-acting elements of the *CaSOTs* gene family in pepper; B. Classification and quantity of cis-acting elements in the promoters of the *CaSOTs* gene family in pepper. The redder the color, the greater the number of elements; the bluer the color, the fewer the elements.

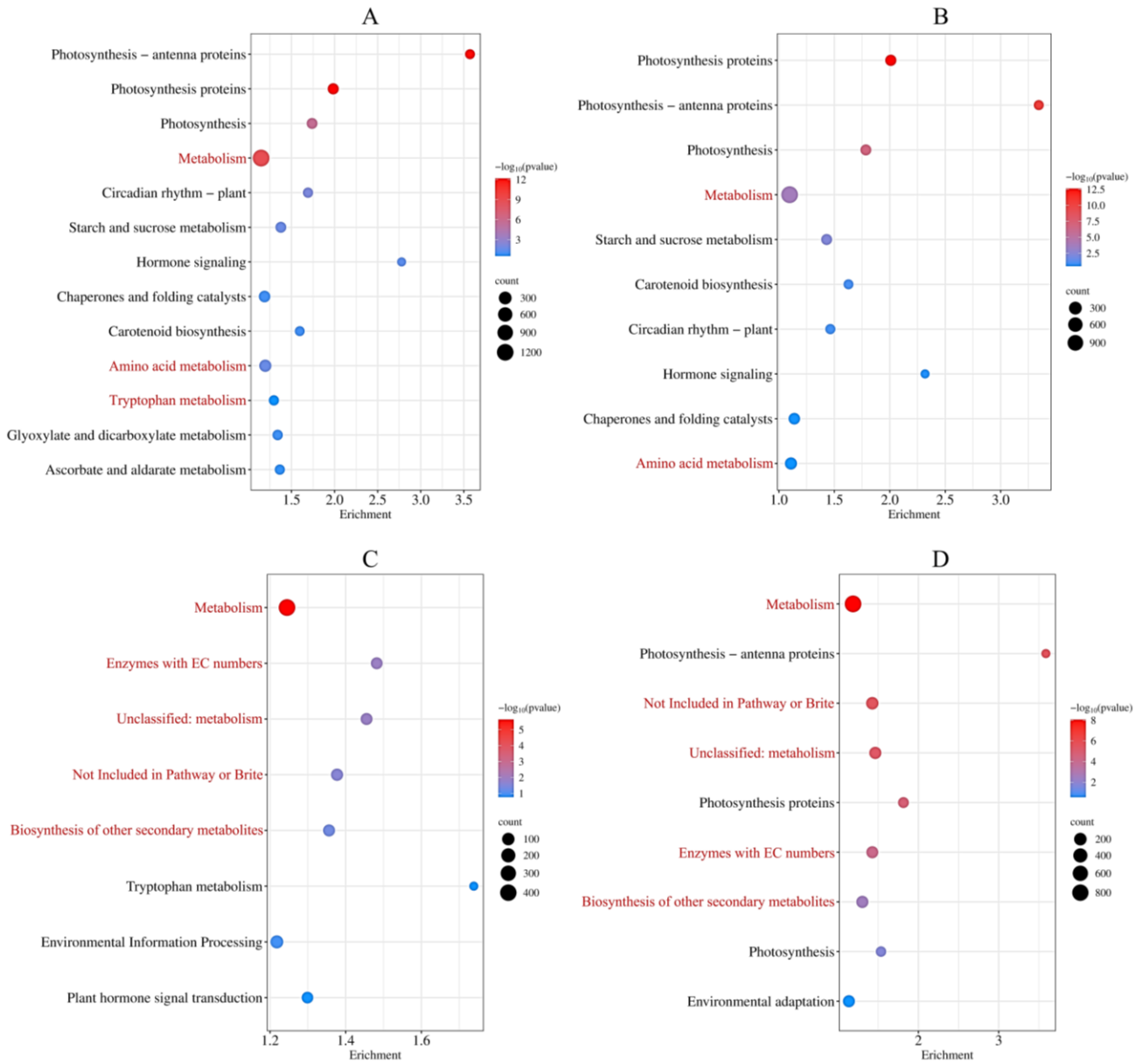

Fig. 6. KEGG Enrichment Analysis of the SOT Gene Family in Pepper.
A. Under high temperature for 12 h; B. Under high temperature for 24 h; C. Under low temperature for 12 h; D. Under low temperature for 24 h. Pathways marked in red represent significant enrichment of the gene family members

KEGG enrichment analysis: Under high- and low-temperature stress, a large number of differentially expressed genes were identified in pepper, indicating that pepper can respond to temperature stress through significant transcriptional reprogramming. KEGG enrichment analysis revealed that the differentially expressed genes were significantly enriched in multiple pathways, including amino acid metabolism, tryptophan metabolism, secondary metabolite biosynthesis, and plant hormone signal transduction. Notably, several members of the *CaSOT* gene family (e.g., *CaSOT14*, *CaSOT22*, *CaSOT12*) were primarily enriched in pathways such as amino acid metabolism, tryptophan metabolism, and secondary metabolite biosynthesis (Fig. 6).

Tissue-specific expression analysis of *CaSOT* genes: Transcriptome data revealed that the expression of *CaSOT* genes exhibited tissue specificity. Analysis of expression patterns across different time points showed that under high-temperature stress, a small subset of *CaSOT* genes displayed an upward expression trend in functional leaves at the vegetative growth stage, whereas the majority of *CaSOT* genes exhibited a downward trend. Temporal differences in *CaSOT* expression were observed: *CaSOT6* showed an up-regulated expression pattern, while *CaSOT7*, *CaSOT12* and *CaSOT2* were down-regulated. Under low-temperature stress, the expression of most *CaSOT* genes trended downward. Specifically, the expression levels of *CaSOT6* and *CaSOT18* in young leaf tissue samples decreased progressively with prolonged stress duration, whereas *CaSOT22* and *CaSOT14* exhibited an up-regulated expression (Fig. 7).

Expression profiles of *CaSOT* genes under temperature stress: The expression levels of 5 members of the pepper *CaSOT* gene family under high- and low-temperature

stress were analyzed using qRT-PCR. The results showed that under high-temperature stress, *CaSOT6* expression in leaves was significantly upregulated ($p<0.05$), increasing to 1.38-fold at 12 h and 1.78-fold at 24 h; *CaSOT7* expression was highly significantly downregulated ($p<0.01$), decreasing to 0.51-fold at 12 h and 0.16-fold at 24 h; *CaSOT2* expression was also highly significantly downregulated ($p<0.01$), dropping to 0.49-fold at 12 h and 0.38-fold at 24 h; and *CaSOT12* expression was significantly downregulated ($p<0.05$), falling to 0.38-fold at 12 h and 0.39-fold at 24 h. Under low-temperature stress, *CaSOT6* expression was significantly downregulated ($p<0.05$), decreasing to 0.39-fold at 12 h and 0.26-fold at 24 h; *CaSOT18* expression was highly significantly downregulated ($p<0.01$), dropping to 0.28-fold at 12 h and 0.22-fold at 24 h; *CaSOT22* expression was highly significantly upregulated ($p<0.01$), rising to 3.62-fold at 12 h and 3.17-fold at 24 h; and *CaSOT14* expression was also highly significantly upregulated ($p<0.01$), increasing to 2.98-fold at 12 h and 1.56-fold at 24 h (Fig. 8).

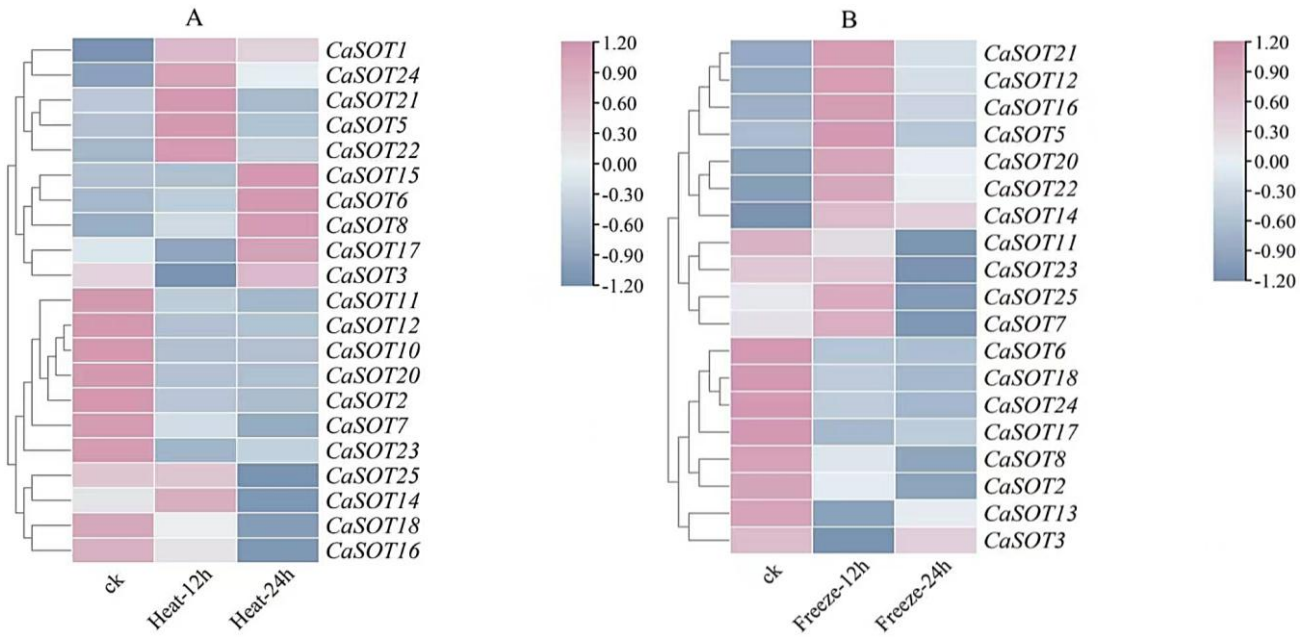

Fig. 7. A. Heatmap of the expression of *CaSOTs* genes in leaves under high temperature stress; B. Heatmap of the expression of *CaSOTs* genes in leaves under low temperature stress. Expression data were normalized using $\log_2$ transformation. Red indicates high expression, and blue indicates low expression.

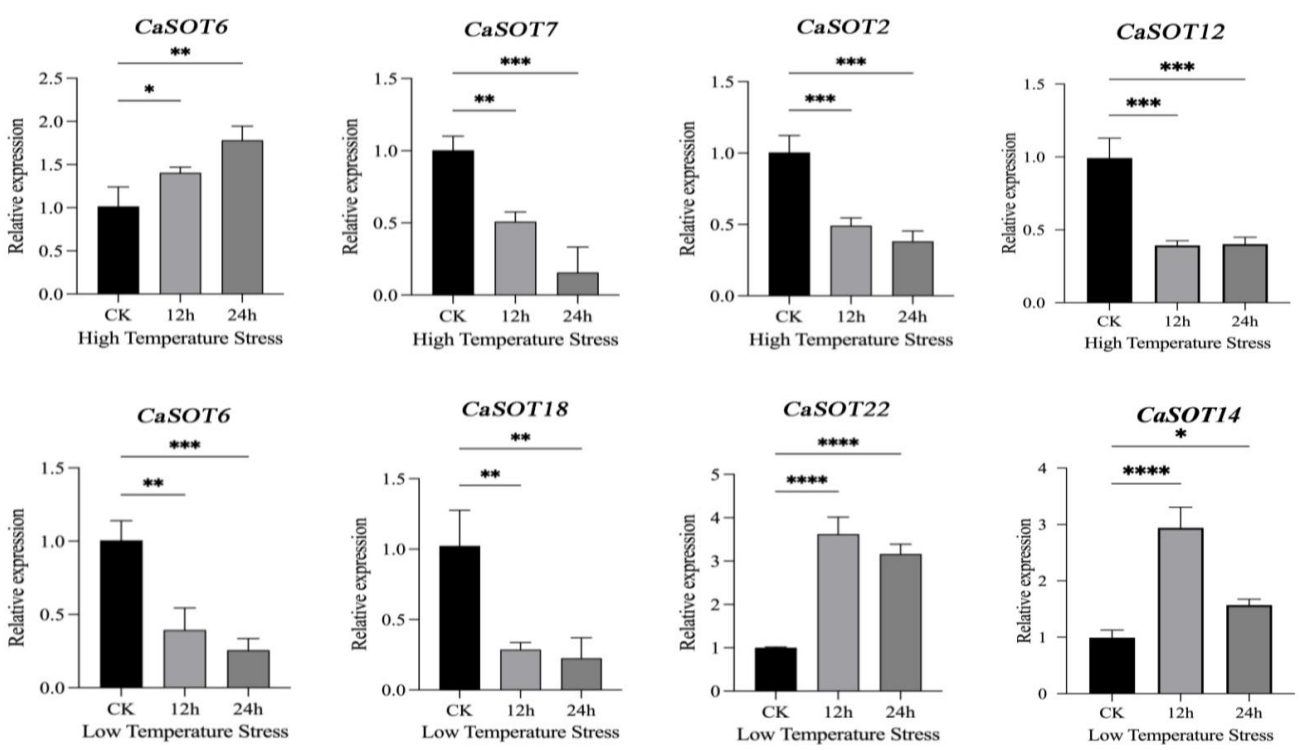

Fig. 8. Expression patterns of *CaSOTs* under high- and low-temperature stress.
*, **, ***, **** Significant at 5%, 1%, 0.1% and 0.01% probability level, respectively.

Discussion

In current study, we identified a total of 25 SOT genes, which exhibited distinct expression patterns under various abiotic stresses. Compared with model plants such as *Arabidopsis*, the SOT gene family in pepper shows a relatively higher degree of diversity (Han *et al.*, 2025), which may be attributed to species-specific gene duplication events during evolution. The variation in transcriptional expression of *CaSOT* genes is likely closely associated with their roles in adaptive regulation. Previous studies indicated that some SOT genes in potato originated from gene duplication events (Zang *et al.*, 2009), further supporting the importance of duplication in the expansion of this gene family. Conserved motif and domain analyses revealed that most CaSOT proteins contain the typical Sulfotransferase_1 domain and key catalytic sites. Moreover, members within the same phylogenetic subfamily shared highly similar gene structures and motif compositions, indicating functional conservation. However, notable differences in sequence length and structure were observed among certain *CaSOT* members, suggesting that these genes may have evolved new functions or regulatory mechanisms during pepper adaptation to specific thermo environments. Notably, most functional regions of potato SOT proteins are also located within the Sulfotransferase_1 domain (Faraji *et al.*, 2021). Given that both pepper and potato belong to the Solanaceae family, this finding supports the evolutionary conservation of SOT genes among Solanaceous crops (Wang *et al.*, 2008).

Promoter analysis showed that *CaSOT* genes contained more than 10 types of stress and hormone-responsive cis-elements, including MYB and DRE core elements associated with low-temperature response, ABRE elements related to abscisic acid signaling, and STRE and MYB elements involved in high-temperature and general stress responses (Ge *et al*., 2022). These results indicate that *CaSOT* genes may respond to both high-temperature stresses and low-temperature stresses. Phylogenetic analysis further revealed that *CaSOT11* and *CaSOT17* clustered with *AtSOT14* from *Arabidopsis*, suggesting that these genes may catalyze the sulfation of hydroxyjasmonic acid and thereby regulate jasmonic acid activity in response to temperature stress (Klein & Papenbrock, 2004). KEGG enrichment analysis revealed that the *CaSOT* gene family was significantly enriched in pathways related to amino acid metabolism, tryptophan metabolism, and secondary metabolite biosynthesis. This suggests that their core function may be to mediate the synthesis of secondary metabolites, thereby exerting antioxidant, osmoregulatory, and stress defense roles, which likely represent an important route for pepper to respond to temperature stress. Meanwhile, the high enrichment of this gene family in enzyme functional categories and unclassified metabolic pathways indicated that they were extensively involved in enzymatic reactions and possess unexplored metabolic functions. By regulating the synthesis and transformation of specific secondary metabolites and participating in diverse metabolic processes, SOT genes play crucial roles in the response of pepper to temperature stress.

Earlier studies show that some *StSOT* genes in potato are involved in abscisic acid-related metabolic processes, while genes such as *Bra017370*, *Bra009300*, and *Bra027880* in Chinese cabbage are significantly induced by ABA treatment (Jin *et al*., 2019). ABA plays a crucial role in temperature stress responses (Nakashima *et al*., 2014; Zhang *et al*., 2022), the presence of ABRE cis-elements in several *CaSOT* promoters suggests that these genes may act as key regulators in the temperature stress response network in pepper. To further investigate the functional roles of *CaSOT* genes under temperature stress, qRT-PCR analysis was performed. Results demonstrated that *CaSOT* genes exhibited significant differential expression under both high and low-temperature conditions. Specifically, under high-temperature stress, *CaSOT6* was significantly upregulated, whereas *CaSOT7* and *CaSOT2* were significantly downregulated. Under low-temperature stress, *CaSOT6* and *CaSOT18* were significantly downregulated, while *CaSOT22* exhibited a transient upregulation followed by a decline. Under low-temperature stress, both *CaSOT22* and *CaSOT14* showed an expression trend of initial increase followed by a decrease. It is speculated that these two genes can rapidly respond to cold stress and transiently activate the stress signaling pathway, and their molecular regulatory mechanisms deserve further functional validation. Expression analyses demonstrated that *CaSOT* genes responded differently to high- and low-temperature stress, with some genes showing opposite expression trends under different conditions. These findings imply that *CaSOT* genes may participate in complex regulatory networks involved in temperature adaptation. These findings were consistent with transcriptome data, suggesting that these genes play important roles in temperature stress responses. These findings lay a theoretical foundation for further functional validation of SOT genes and provide valuable clues for exploring their interactions with other stress-related genes in improving temperature tolerance in pepper. This study was conducted using a single pepper variety (Zhangshugang) and did not include resistant or susceptible control varieties, nor a recovery treatment group. Therefore, it cannot fully distinguish between stress-adaptive responses and injury-related gene expression. Additionally, the sampling time points were limited to 0, 12, and 24 h, which are suitable for capturing early stress response patterns but do not cover the full temporal dynamics of temperature adaptation. These limitations will be addressed in future studies by incorporating appropriate control varieties, a recovery time course, and additional sampling time points. It should be noted that KEGG enrichment analysis is inherently limited by the existing annotation databases, which may not fully capture the functional diversity of uncharacterized or species-specific genes, particularly those involved in complex metabolic networks.

Conclusion

This study identified a total of 25 SOT genes in chili peppers. Most CaSOT proteins contain the typical Sulfotransferase_1 domain. The *CaSOT* genes were found to contain MYB and DRE core response elements associated with low temperatures, the ABRE response element associated with abscisic acid, and the STRE and MYB stress response elements associated with high temperatures, indicating that *CaSOTs* may respond to both high- and low-temperature stresses. Under high-temperature stress, the expression of *CaSOT6* was significantly upregulated in leaves, while the expression of

CaSOT7, *CaSOT12* and *CaSOT2* was significantly down regulated in leaves; Under low-temperature stress, the expression levels of *CaSOT6* and *CaSOT18* in leaves were significantly downregulated, while the expression level of *CaSOT22* and *CaSOT14* in young leaves showed an initial increase followed by a decrease. This suggests that these genes may play a key role in the response to both high- and low-temperature stress.

Acknowledgement

This work was supported by the following projects: the National Key Research and Development Program of China (Grant 2023YFD1600201 to F.L.).

Conflict of Interest: The authors declare no competing interests.

Author's Contributions: Y.M; contributed to the conceptualization and design of the study, as well as data collection, analysis, and interpretation. Y.M contributed to the statistical analysis; Y.M interpretation of the data. All authors have reviewed and approved the final version of the manuscript.

References

Chen, C., Y. Wu, J. Li, X. Wang, Z. Zeng, J. Xu, Y. Liu, J. Feng, H. Chen, Y. He and R. Xia. 2023. Tbtools-ii: A "one for all, all for one" bioinformatics platform for biological big-data mining. *Mol. Plant,* 16(11): 1733-1742.

Chen, R., Y. Jiang, J. Dong, X. Zhang, H. Xiao, Z. Xu and X. Gao. 2012. Genome-wide analysis and environmental response profiling of sot family genes in rice (*Oryza sativa*). *Genes Genom.,* 34(5): 549-560.

Faraji, S., P. Heidari, H. Amouei, E. Filiz, Abdullah and P. Poczai. 2021. Investigation and computational analysis of the sulfotransferase (sot) gene family in potato (*Solanum tuberosum*): Insights into sulfur adjustment for proper development and stimuli responses. *Plants,* 10: 2597.

Ge, W., M. Luo, H. Sun, B. Wei, X. Zhou, Q. Zhou and S. Ji. 2022. The camyb340 transcription factor induces chilling injury in post-harvest bell pepper by inhibiting fatty acid desaturation. *Plant J.,* 111(3): 800-818.

Guo, W.L., R.G. Chen, Z.H. Gong, Y.X. Yin and D.W. Li. 2013. Suppression subtractive hybridization analysis of genes regulated by application of exogenous abscisic acid in pepper plant (*Capsicum annuum* L.) leaves under chilling stress. *PLoS One,* 8: e66667.

Han, S., Z. Chen, Q. Liu, Y. Ding, J. Wang, H. Liu, J. Zou, Z. Hong, H. Zhang, W. Yang, L. Zhang, H. Liu and M. Yuan. 2025. Identification and evolution of the plant sulfotransferase family. *BMC Genom.,* 26: 895.

Hirschmann, F., F. Krause and J. Papenbrock. 2014. The multi-protein family of sulfotransferases in plants: Composition, occurrence, substrate specificity, and functions. *Front. Plant Sci.,* 5: 556.

Jin, L., N. Ouyang, Y. Huang, C. Liu and Y. Ruan. 2019. Genome-wide analysis of sulfotransferase genes and their responses to abiotic stresses in chinese cabbage (*Brassica rapa* L.). *PLoS One,* 14: e0221422.

Klein, M. and J. Papenbrock. 2004. The multi-protein family of arabidopsis sulphotransferases and their relatives in other plant species. *J. Exp. Bot.,* 55(404): 1809-1820.

Klein, M., M. Reichelt, J. Gershenzon and J. Papenbrock. 2006. The three desulfoglucosinolate sulfotransferase proteins in arabidopsis have different substrate specificities and are differentially expressed. *The FEBS Journal,* 273: 122-136.

Lee, S.G., S.K. Kim, H.J. Lee, H.S. Lee and J.H. Lee. 2018. Impact of moderate and extreme climate change scenarios on growth, morphological features, photosynthesis, and fruit production of hot pepper. *Ecology and Evolution,* 8: 197-206.

Liu, F., H. Yu, Y. Deng, J. Zheng, M. Liu, L. Ou, B. Yang, X. Dai, Y. Ma, S. Feng, S. He, X. Li, Z. Zhang, W. Chen, S. Zhou, R. Chen, M. Liu, S. Yang, R. Wei, H. Li, F. Li, B. Ouyang and X. Zou. 2017. Pepperhub, an informatics hub for the chili pepper research community. *Mol. Plant,* 10(8): 1129-1132.

Livak, K.J. and T.D. Schmittgen. 2001. Analysis of relative gene expression data using real-time quantitative pcr and the 2(-delta delta c(t)) method. *Methods,* 25(4): 402-408.

Mistry, R., D.P. Byrne, D. Starns, I.L. Barsukov, E.A. Yates and D.G. Fernig. 2024. Polysaccharide sulfotransferases: The identification of putative sequences and respective functional characterisation. *Essays Biochem.,* 68: 431-447.

Nakashima, K., K. Yamaguchi-Shinozaki and K. Shinozaki. 2014. The transcriptional regulatory network in the drought response and its crosstalk in abiotic stress responses including drought, cold, and heat. *Front. Plant Sci,* 5: 170.

Negishi, M., L.G. Pedersen, E. Petrotchenko, S. Shevtsov, A. Gorokhov, Y. Kakuta and L.C. Pedersen. 2001. Structure and function of sulfotransferases. *Arch. Biochem. Biophys.,* 390: 149-157.

Rao, M.J. and B. Zheng. 2025. The role of polyphenols in abiotic stress tolerance and their antioxidant properties to scavenge reactive oxygen species and free radicals. *Antioxidants,* 14(1):

Wan, H., W. Yuan, M. Ruan, Q. Ye, R. Wang, Z. Li, G. Zhou, Z. Yao, J. Zhao, S. Liu and Y. Yang. 2011. Identification of reference genes for reverse transcription quantitative real-time pcr normalization in pepper (*Capsicum annuum* L.). *Biochem Biophys Res Commun*., 416(1-2): 24-30.

Wang, Y., A. Diehl, F. Wu, J. Vrebalov, J. Giovannoni, A. Siepel and S.D. Tanksley. 2008. Sequencing and comparative analysis of a conserved syntenic segment in the solanaceae. *Genetics*, 180(1): 391-408.

Wawrzynska, A. and A. Sirko. 2024. Sulfate availability and hormonal signaling in the coordination of plant growth and development. *Int. J. Mol. Sci*., 25: 3978.

Zang, Y.X., H.U. Kim, J.A. Kim, M.H. Lim, M. Jin, S.C. Lee, S.J. Kwon, S.I. Lee, J.K. Hong, T.H. Park, J.H. Mun, Y.J. Seol, S.B. Hong and B.S. Park. 2009. Genome-wide identification of glucosinolate synthesis genes in brassica rapa. *FEBS J.,* 276: 3559-3574.

Zhang, J., L. Liang, Y. Xie, Z. Zhao, L. Su, Y. Tang, B. Sun, Y. Lai and H. Li. 2022. Transcriptome and metabolome analyses reveal molecular responses of two pepper (*Capsicum annuum* L.) cultivars to cold stress. *Front. Plant Sci.,* 13: 819630.

Zhang, X., X. Ma, S. Wang, S. Liu and S. Shi. 2024. Physiological and genetic aspects of resistance to abiotic stresses in *Capsicum* species. *Plants,* 13: 3013.