Plant Science 363 (2026) 112863

Contents lists available at ScienceDirect

Plant Science

journal homepage: www.elsevier.com/locate/plantsci

Genome-wide identification of trehalose-6-phosphate synthase genes in soybean and functional analysis of GmTPS12a in growth and stress response

Yuanyuan Liu [a,1], Yuxin Yao [a,1], Wei Deng [a], Ruotong Zhao [a], Jia Du [a], Hang Guo [a], Jing Xu [a], Shahid Ali [b], Aifen Yang [a,*], Houqing Zeng [a,*]

[a] College of Life and Environmental Sciences, Hangzhou Normal University, Hangzhou 311121, China
[b] Guangxi Key Laboratory of Agro-environment and Agro-products Safety, Key Laboratory of Crop Cultivation and Physiology, College of Agriculture, Guangxi University, Nanning 530004, China

ARTICLE INFO

Keywords:
Soybean
Trehalose
Trehalose-6-phosphate
TPS gene family
Abiotic stress
Salt stress
Seed germination
Flowering time

ABSTRACT

Trehalose-6-phosphate synthase (TPS) is an indispensable enzyme for the biosynthesis of trehalose-6-phosphate and trehalose, and plays a crucial role in plant growth and stress responses. However, the *TPS* gene family and its potential functions remain unclear in soybean (*Glycine max*). In this study, a comprehensive analysis of TPS family genes in the soybean genome identified 20 *GmTPS* genes, which were divided into two clades based on phylogenetic analysis. Gene structure and protein motif analyses showed that genes/proteins within the same clade share similar characteristics. Comparative genomics suggested that the expansion of *GmTPS* genes is mainly due to segmental duplication. Promoter analysis revealed that the *GmTPS* gene contains various hormonal and stress-responsive *cis*-acting regulatory elements. Transcriptome profiling indicated that 19 *GmTPS* genes exhibit differential expression across 15 tissues, and 11 genes respond to stresses such as salinity and drought. RT-qPCR confirmed their responses to salt or drought stress. Among them, *GmTPS12a*, which was induced by both salt and drought stresses, was selected for further functional characterization. Subcellular localization analysis revealed that GmTPS12a is mainly localized in the endoplasmic reticulum. Ectopic overexpression of *GmTPS12a* in *Arabidopsis* promoted flowering, consistent with the up- or down-regulation of flowering-related genes like *FT*, *SOC1*, and *FLC*. In addition, overexpression of *GmTPS12a* enhanced seed germination and cotyledon greening under salt and osmotic stresses, possibly by reducing sensitivity to abscisic acid. This study provides fundamental insights into the soybean TPS family genes and highlights the role of GmTPS12a in flowering regulation and abiotic stress responses.

1. Introduction

Trehalose is a non-reducing disaccharide composed of two α-glucose molecules, and it is widely distributed in bacteria, yeast, invertebrates, protozoa, and higher plants (Avonce et al. 2006). Trehalose is considered a potential osmotic and stress protectant and plays important roles in plant growth and stress responses. Beyond its metabolite role, trehalose and its derivatives also act as signaling molecules, regulating energy metabolism pathways and serving as a key component in sugar signaling in plants (Schluepmann et al. 2012; Paul et al. 2008). In most plants, trehalose is usually present at very low concentrations, but under certain conditions, its levels can increase significantly, protecting plants against stresses such as salt, drought, cold, and heat (Zhu et al. 2022; Zulfiqar et al. 2021; Yang et al. 2022; Sarkar and Sadhukhan, 2022; Ibrahim, 2025).

In plants, trehalose biosynthesis involves two steps. Firstly, trehalose-6-phosphate synthases (TPS) catalyze the conversion of uridine diphosphate glucose (UDPG) and glucose-6-phosphate (G6P) into trehalose-6-phosphate (T6P). Then, T6P is dephosphorylated by trehalose-6-phosphate phosphatases (TPP) to form trehalose (Vandesteene et al. 2010; Gómez et al. 2010; Shao et al. 2023). T6P, the immediate precursor of trehalose, is an important signaling metabolite

* Corresponding authors.
E-mail addresses: aifen_yang@hznu.edu.cn (A. Yang), zenghq@hznu.eud.cn (H. Zeng).
[1] Yuanyuan Liu and Yuxin Yao contributed equally to this study.

https://doi.org/10.1016/j.plantsci.2025.112863
Received 27 August 2025; Received in revised form 27 October 2025; Accepted 7 November 2025
Available online 12 November 2025

that regulates sucrose levels in plants (Ponnu et al. 2011). The sucrose-T6P nexus model indicates that T6P serves as a regulatory signal of sucrose status because fluctuations in sucrose concentration alter the activity of TPS and TPP proteins, which in turn increase or decrease T6P levels. These feedback mechanisms help maintain sucrose concentration within an optimal range (Lunn et al. 2006; Yadav et al. 2014). In source leaves, T6P primarily regulates sucrose levels by modulating its synthesis (Figueroa et al. 2016), while in sink organs, it controls sucrose utilization for growth, partly by inhibiting sucrose non-fermenting 1-related protein kinase 1 (SnRK1) (Baena-González and Lunn, 2020). By integrating sucrose availability with cellular responses, T6P influences developmental processes such as flowering, embryogenesis, and bud branching, establishing itself as a central signaling molecule in plant growth and development (Fichtner and Lunn, 2021; Wahl et al. 2013). T6P also regulates starch degradation and plays a vital role in overall carbohydrate metabolism (Martins et al. 2013; Schluepmann et al. 2003).

TPS is a plant-specific enzyme widely found in higher plants (Lunn, 2007). TPS family genes have been identified genome-widely in numerous plant species, including *Arabidopsis* (Vandesteene et al. 2010), rice (Liu et al. 2019), potato (Xu et al. 2017), *Medicago truncatula* (Song et al. 2021), alfalfa (*Medicago sativa*) (Guo et al. 2025), and cucumber (Dan et al. 2021). Phylogenetic analysis shows that TPS family members are classified into two distinct groups. In *Arabidopsis*, all the 11 TPS genes fall into Class I (AtTPS1-AtTPS4) and Class II (AtTPS5-AtTPS11) (Blázquez et al. 2002; Vandesteene et al. 2010). TPS proteins contain two conserved domains: a TPS-like domain and a TPP-like domain. In *Arabidopsis*, the Class I protein AtTPS1 has confirmed TPS activity, whereas Class II proteins lack both TPS and TPP activity and are thought to act as regulatory proteins (Blázquez et al. 2002; Ramon et al. 2009; Vogel et al. 2001; Vandesteene et al. 2010). In addition to AtTPS1, AtTPS2 and AtTPS4 were suggested to have TPS activity (Delorge et al. 2015). As a key enzyme in T6P biosynthesis, TPS plays a crucial role in plant development and stress responses (Yang et al. 2012; Lunn et al. 2014; Paul, 2007; Fichtner and Lunn, 2021). Class I *TPS* genes, such as *AtTPS1* and *OsTPS1*, have been shown to contribute to plant tolerance against abiotic stresses, such as salinity, low temperature, and drought (Avonce et al. 2004; Li et al. 2011). *AtTPS1* is also essential for embryo development and flowering, with its knockout leading to embryo lethality and delayed flowering by impairing the biosynthesis of T6P and downregulating the expression of flowering genes like *FLOWERING LOCUS T* (*FT*) (Gómez et al. 2010; Wahl et al. 2013). Overexpression of *Lilium* × *formolongi TPS3* and *TPS5* accelerates flowering in transgenic *Arabidopsis* by downregulating the expression of small RNA miR156 that is associated with developmental transition (Zhang et al. 2022). Functions of some class II *TPS* genes in stress tolerance and growth regulation have also been revealed. *AtTPS5* acts as a negative regulator of abscisic acid (ABA) signaling and regulates alginate content (Tian et al. 2019), while *AtTPS6* influences cellular morphology and contributes to plant growth and development (Chary et al. 2008). It has been proposed that T6P may participate in the regulation of ABA-mediated stomatal closure by involving the covalent modifications of nitrate reductase and SnRK2.6 (Tian et al. 2019; Lunn et al. 2014). Overexpression of *Brassica napus BnaC02.TPS8* increased the photosynthesis and seed yield, and promoted seed oil accumulation (Yuan et al. 2024). In rice, *OsTPS8* positively regulates salt stress tolerance (Vishal et al. 2019), while overexpression of wheat *TPS11* improved cold tolerance in *Arabidopsis* (Liu et al. 2019). Despite these findings, the physiological functions of most *TPS* genes in plants remain unclear. Elucidating their roles in plant growth, development, and stress resistance is essential for advancing our understanding of T6P and trehalose metabolism and for developing high-yield, stress-resistant crops.

Soybean (*Glycine max*) is a crucial crop that provides cooking oil and protein-rich food. However, it is vulnerable to various environmental stresses that can hinder growth and reduce yields (Wang et al. 2015; Du et al. 2023). *TPS* genes play important roles in growth regulation and stress responses, but the TPS gene family has not been identified in soybean. In this study, a comprehensive identification of TPS family genes in soybean was carried out, and their phylogenetic relationships, chromosomal location, collinearity, gene structure, conserved motif and domain, and *cis*-acting regulatory elements of promoters were analyzed. In addition, their tissue-specific expression profiles and expression patterns under environmental stresses were examined using published transcriptome data and reverse transcription quantitative polymerase chain reaction (RT-qPCR). A stress-responsive gene, *GmTPS12a,* was further selected for functional analysis through genetic transformation in *Arabidopsis*. GmTPS12a was localized to the endoplasmic reticulum (ER), and its ectopic overexpression enhanced resistance to salt and osmotic stress during seed germination and early seedling growth, while also promoting flowering in *Arabidopsis*. These comprehensive analyses of the TPS family genes provide fundamental information for further functional studies, and the functional characterization of GmTPS12a provides insights into developing soybean cultivars with improved growth and stress resistance through genetic engineering.

2. Materials and methods

2.1. Identification of TPS family genes in soybean

TPS family genes in the soybean genome were identified using the BLAST tool available on Soybase (https://www.soybase.org/)(E-value < $1*10^{-5}$). Protein sequences of *Arabidopsis* TPS members were used as queries for the search. The nucleotide and protein sequences of putative soybean TPS genes were retrieved from Soybase. Protein domains were analyzed by using the CD-Search module in the NCBI (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi). Subcellular localization was predicted using the WoLF PSORT program (http://wolfpsort.hgc.jp) (Horton et al. 2007). The MEME program (https://meme-suite.org/meme/tools/meme) was used to analyze conserved motifs in proteins (Bailey et al. 2009). The physical and chemical properties of soybean TPS proteins were calculated using the ProtParam tool in the ExPASY bioinformatics resource portal (Duvaud et al. 2021).

2.2. Phylogenetic analysis of the TPS family

TPS protein sequences of soybean, *Arabidopsis*, and rice were obtained from SoyBase (https://www.soybase.org/), TAIR (http://www.arabidopsis.org/), and RGAP (http://rice.uga.edu/), respectively. Protein sequences were aligned using ClustalW, and the phylogenetic tree was constructed using MEGA6.0 (Tamura et al. 2013). Bootstrap analysis with 1000 replicates was performed to evaluate the reliability of the branches.

2.3. Analyses of chromosome distribution, gene structure, and cis-acting regulatory elements

Using TBtools and the soybean genome annotation in Ensembl Plants, the gene structure and chromosomal distribution of the TPS gene family were analyzed, and a corresponding distribution map was generated. To investigate *cis*-regulatory elements, 2.0 kb promoter sequences upstream of the transcription start sites of *TPS* genes were extracted from the SoyBase database, and the distribution of *cis*-regulatory elements was mapped by using TBtools (Chen et al. 2020a).

2.4. Gene duplication, Ka/Ks value, and collinearity analysis

The One Step MCScanX program in TBtools was used to retrieve and analyze genomic annotation files, as well as to identify gene duplication and segmental replication events of *TPS* genes in the soybean genome. It was also used to determine the collinearity among family members, which was further visualized using the Circos program in TBtools (Chen et al. 2020a). Nonsynonymous substitution (Ka), synonymous

substitution (Ks), and their ratio (Ka/Ks) for collinear gene pairs were calculated by TBtools (Chen et al. 2020a). The Ka/Ks ratio was applied as a molecular indicator of nucleic acid evolution to determine whether *TPS* genes have undergone selective pressure (Hurst, 2002). Divergence time (T) was calculated using the formula: $T = Ks/(2 \times 6.1 \times 10^{-9}) \times 10^{-6}$ million years ago (MYA)(Lynch and Conery, 2000).

2.5. Expression profiling of TPS family genes

The expression levels of *TPS* genes in different soybean tissues were retrieved from the Soybean Expression Atlas (https://venanciogroup.uenf.br/cgibin/gmax_atlas-/index.cgi)(Machado et al. 2020). A heatmap of *TPS* gene expression was generated based on Log2 normalized RPKM values using TBtools (Chen et al. 2020a). Microarray and RNA sequencing datasets for soybean roots under various stresses were obtained from published studies, including salt stress (for 1 h, 6 h, and 12 h), dehydration stress (for 1 h and 6 h), cold stress (for 24 h), drought stress (for 6 days), and inoculation with pathogenic (FO40) and non-pathogenic (FO36) isolates of *Fusarium oxysporum* for 72 h (Shiu et al. 2012; Maruyama et al. 2011; Belamkar et al. 2014; Lanubile et al. 2015). Additionally, published datasets on the transcriptome response of rhizobia (*Bradyrhizobium japonicum*) in soybean root hairs were obtained and analyzed (Libault et al. 2010).

2.6. Plant material and growth conditions

Arabidopsis thaliana (Col-0) seeds were first sterilized by immersing them in 75 % ethanol for 5 min, rinsed with sterile water, and stratified at 4°C for two days. After stratification, the seeds were sown on 1/2 Murashige and Skoog (MS) medium (pH 5.7) containing 1 % agar and 1.5 % sucrose. Seven days after sowing, seedlings were transplanted from the MS medium to soil and grown in a chamber under a 16-hour light/8-hour dark cycle, with day and night temperatures of 22°C and 20°C, respectively.

Soybean seeds (Williams 82) were germinated at room temperature for 4 days, after which seedlings were transferred to a hydroponic culture system containing half-strength modified Hoagland nutrient solution (Zeng et al. 2019). Soybean plants were grown in a chamber under a 12-hour light/12-hour dark photoperiod, with temperatures of 28 °C during light and 25 °C during dark, and a light intensity of about 250 μmol m^{-2} s^{-1} . Twelve-day-old seedlings were then subjected to stress treatments. In the case of salt stress treatment, the roots of seedlings were submerged in nutrient solutions with 100 mM NaCl. As for the dehydration treatment, the plants were taken out of the hydroponic system and exposed to the air (Belamkar et al. 2014). Soybean roots were harvested six hours after salt or dehydration treatment for subsequent analyses.

2.7. RT-qPCR analysis

Total RNA was extracted using the Ultrapure RNA Kit (CWBIO, Beijing) following the manufacturer's guidelines. One μg of purified RNA was then reverse transcribed into cDNA using the HiScript III All-in-one RT SuperMix Perfect for qPCR kit (Vazyme, Nanjing). RT-qPCR was performed on a CFX96 Real-Time PCR System (Bio-Rad) with Taq Pro Universal SYBR qPCR Master Mix (Vazyme, Nanjing), as described previously (Chen et al. 2025). All qPCR reactions were run in triplicate and three biological replicates were used. Gene expression levels were normalized to *GmACTIN11* (Glyma.18G290800) for soybean, or to *AtACTIN2* (AT3G18780) for *Arabidopsis*. Primer sequences used for RT-qPCR analysis are listed in Table S1.

2.8. Identification of co-expressed genes of GmTPS12a and functional enrichment analysis

The ATTED-II v12 database (https://atted.jp/) was used to identify genes co-expressed with *GmTPS12a* in soybean. The top 100 co-expressed genes were selected for gene ontology (GO) and KEGG (Kyoto encyclopedia of genes and genomes) enrichment analyses using the David database (https://davidbioinformatics.nih.gov/). GO and KEGG categories with an adjusted P-value ≤ 0.05 were considered statistically significant.

2.9. Subcellular localization analysis

The coding sequence (CDS) of *GmTPS12a*, without the stop codon, was amplified and inserted into the pCAMBIA1300-GFP plasmid to generate the 35S:GmTPS12a-GFP construct through homologous recombination using the pEASY®-Basic Seamless Cloning and Assembly Kit (TransGen Biotech, Beijing). Agrobacterium cells (*Agrobacterium tumefaciens* GV3101) transformed with the plasmid were cultured in the LB liquid media at 28°C with shaking at 200 rpm for 24 h until the OD600 reached 0.5–0.6. The agrobacterium cells were then collected and suspended in infection solution (10 mM MES, 10 mM $MgCl_2$, 150 μM acetosyringone, pH = 5.6). Tobacco (*Nicotiana benthamiana*) leaves at the same growth stage were used for Agrobacterium infiltration using a syringe. The empty vector pCAMBIA1300-GFP was used as a control. After 12 h of infiltration, the injected tobacco leaves were collected for subcellular localization observation using a fluorescence confocal microscope (Zeiss, Germany). In addition, the 35S:GmTPS12a-GFP plasmid and an ER-mCherry marker plasmid were co-transformed into rice protoplasts by PEG-mediated transformation. After transformation, the protoplasts were incubated in the dark at 28°C for 12–16 h. Fluorescence was observed using a confocal laser scanning microscope (Zeiss, Germany).

2.10. Construction of GmTPS12a-overexpression plants

The recombinant plasmid 35S:GmTPS12a-GFP was transformed into Agrobacterium GV3101 and used to genetically transform wild-type (WT) *Arabidopsis* (Col-0) via the floral dip method (Clough and Bent, 1998). Transgenic T1 seeds were screened with 50 mg/L hygromycin, and T2 seeds exhibited a 3:1 segregation ratio for hygromycin resistance. These seeds were collected for further screening to identify homozygous lines. Homozygous lines overexpressing *GmTPS12a* were selected for phenotypic analyses.

2.11. Seed germination assay

Arabidopsis seeds were soaked in 75 % ethanol for 10 min and then rinsed twice with distilled water. They were sown on 1/2 MS agar medium containing ABA (0.5 μM), NaCl (140 mM), or mannitol (300 mM). After sowing, the seeds were kept at 4°C in the dark for 2 days to promote stratification. Subsequently, they were transferred to a growth chamber with a 16-hour light/8-hour dark cycle at 22°C/20°C for germination. At least 40 seeds were tested per treatment, with three biological replicates. The emergence of the radicle recorded germination, and cotyledon greening was measured by counting fully green cotyledons at specific time intervals.

2.12. Flowering time analysis

Flowering time was determined by recording the number of days to bolting and counting the total number of rosette leaves at this stage when the main stem reached approximately 0.5 cm in height (Dai et al. 2024). At least 15 plants were measured for each genotype.

3. Result

3.1. Identification and phylogenetic analysis of TPS family genes in soybean

To investigate the TPS gene family in soybean, the BLAST algorithm was adopted to search the soybean genome database with *Arabidopsis* TPS proteins as queries. A total of 20 genes encoding putative TPS proteins were identified (Table 1). The amino acid lengths of these TPS proteins ranged from 697 to 932, and their predicted molecular weight ranged from 78.72 to 105.39 kDa. The theoretical isoelectric point (pI) of the TPS proteins ranged from 5.36 to 7.9 (Table 1). The full lengths of soybean *TPS* genes vary from 2 to 16 kb, with exon numbers ranging from 2 to 17 and intron numbers from 1 to 16 (Table 1). *TPS* genes in the soybean genome were named according to their homologies in *Arabidopsis* genes, determined by phylogenetic analysis (Table 1; Fig. 1). Phylogenetic trees were constructed using TPS protein sequences from *Arabidopsis*, soybean, and rice to examine their evolutionary relationships. Based on this analysis, the *TPS* genes were classified into two subfamilies, Class I and Class II. Class I TPS genes were further divided into two subgroups (I-1, I-2), while Class II TPS genes were divided into five subgroups (II-1, II-2, II-3, II-4, and II-5) (Fig. 1). The number of TPS genes in each subgroup for *Arabidopsis*, soybean, and rice was as follows: I-1 (1, 5, 1), I-2 (3, 0, 0), II-1 (1, 2, 0), II-2 (1, 2, 2), II-3 (1, 2, 3), II-4 (3, 7, 2) and II-5 (1, 2, 3), respectively.

3.2. Gene and protein structures of soybean TPS proteins

Gene structure analysis showed that 20 TPS family genes were interrupted by one or more introns (Fig. 2A). The number of exons and introns differed between Class I and Class II genes. In Class I, all five members contain 16 introns and 17 exons, which are significantly more than that in Class II. Most Class II genes contain two introns and three exons except *GmTPS6a*, which has only one intron and two exons (Fig. 2A). To study the characteristic regions of soybean TPS proteins, conserved motifs were identified using MEME software. A total of 12 conserved motifs were detected (Fig. 2B). Motifs 1, 2, 4, 5, 6, 7, 9, 10, and 12 are present in all members, indicating that these nine motifs are highly conserved in soybean TPS proteins. Motif 3 was absent only in GmTPS11a, while motif 11 was absent only in GmTPS6a. Motif 8 was present in all Class II members but not in Class I, suggesting that motif 8 is a Class II TPS. Overall, soybean TPS proteins share identical or similar motif compositions, suggesting that they may perform similar functions. Domain prediction using the InterPro database showed that Class I TPS proteins contain a conserved α, α-trehalose-phosphate synthase (UDP-forming) domain (PLN03064), whereas Class II TPS proteins contain a conserved α, α-trehalose-phosphate synthase (UDP-forming) domain belonging to the PLN02205 superfamily (Fig. 2C). Furthermore, a 3D structural model predicted by SWISS-MODEL showed that soybean TPS proteins are mainly composed of α-helices. All models were in monomer form, with sequence similarities to their homologous templates greater than 70.1 %. The GMQE (global model quality estimate) values ranged from 0.77 to 0.89 (Fig. 3; Table S2), suggesting that the predicted 3D models are highly reliable.

3.3. Chromosomal localization and collinearity analyses of TPS family genes in soybean

To study the chromosomal location of *TPS* genes in soybean, their distribution was mapped using the soybean genome annotation file. Twenty *TPS* genes were unevenly distributed on 14 chromosomes: 1, 4, 7, 10, 15, 17, 18, and 20 each contained one *GmTPS* gene, while chromosomes 2, 5, 6, 8, 12, and 13 contained two *GmTPS* genes (Fig. S1). To investigate the amplification and evolution of soybean *TPS* genes, duplication events in the TPS family were analyzed. A total of 17 events were identified as whole-genome duplication (WGD)/segmental duplications (Fig. 4), indicating that such duplications have played an important role in the expansion of the *TPS* gene family. To explore divergence and selection, the Ks, Ka, and Ka/Ks ratios of the 17 duplicated gene pairs were calculated. The Ka/Ks ratios were all less than 1.0 (ranging from 0.051 to 0.341) (Table S3), suggesting that these *TPS* genes have been under strong purifying selection during evolution. The estimated divergence time of these duplication events ranged from 6.0 to 338.3 MYA, with an average of 74.6 MYA (Table S3).

3.4. cis-acting regulatory elements in promoters of soybean TPS genes

To explore the potential transcriptional regulatory mechanism of soybean *TPS* genes, *cis*-acting elements in their promoter regions were analyzed. Ten types of *cis*-acting elements related to hormone signaling or stress responses were identified, including ABA responsive element (ABRE), auxin signaling component ARF1 binding site (AuxRE), salicylic acid (SA)-responsive promoter element (SARE), dehydration and cold response element (DRE/CRT), stress-related CAMTA transcription factor

Table 1
Characteristics of TPS family genes identified in the soybean genome.

Locus name	Gene name	Physical location	Intron	Amino acid	Isoelectric point	MW (KDa)	Predicted subcellular localization[1]
Glyma.15G210100	*GmTPS1*	Chr15:32195637.32210054	16	915	7.19	91.95	cyto, chlo, nucl
Glyma.05G163600	*GmTPS2a*	Chr05: 35471465.35481277	16	920	6.54	104.58	pero, chlo, nucl
Glyma.08G120800	*GmTPS2b*	Chr08: 9331697.9339790	16	919	6.59	103.98	pero, chlo, nucl
Glyma.13G264600	*GmTPS3*	Chr13: 36759779.36771319	16	932	7.9	105.39	cyto, nucl, mito
Glyma.12G234200	*GmTPS4*	Chr12: 39365359.39377365	16	915	6.43	103.63	cyto, E.R., nucl
Glyma.10G270800	*GmTPS5a*	Chr10: 49300831.49306043	2	853	5.93	96.91	chlo, nucl, mito
Glyma.20G120300	*GmTPS5b*	Chr20: 36306759.36311703	2	852	5.91	96.74	chlo, nucl, mito
Glyma.02G086200	*GmTPS6a*	Chr02: 7512613.7516994	1	746	5.84	87.62	nucl, chlo, cysk
Glyma.07G172000	*GmTPS6b*	Chr07: 29897101.29901738	2	853	5.56	96.93	nucl, chlo, cyto
Glyma.06G275600	*GmTPS7a*	Chr06: 46653083.46658187	2	862	5.85	97.78	chlo, nucl, mito
Glyma.12G129800	*GmTPS7b*	Chr12: 14477192.14482275	2	862	5.85	97.93	chlo, nucl, cyto
Glyma.02G033500	*GmTPS8a*	Chr02: 3098851.3104160	2	860	6.47	97.43	cyto, nucl, cysk
Glyma.01G031900	*GmTPS8b*	Chr01: 3342396.3347771	2	860	6.75	97.38	nucl, cyto, chlo
Glyma.08G288600	*GmTPS9a*	Chr08: 40104690.40110059	2	861	5.75	96.85	nucl, cyto, chlo
Glyma.18G136200	*GmTPS9b*	Chr18: 19861177.19866466	2	861	5.83	96.97	nucl, cyto, chlo
Glyma.05G036100	*GmTPS10*	Chr05: 3177057.3183023	2	863	5.45	97.95	chlo, cyto, mito
Glyma.13G092500	*GmTPS11a*	Chr13: 20719315.20722074	2	697	5.74	78.72	chlo, cyto, nucl
Glyma.17G067800	*GmTPS11b*	Chr017: 5288105.5291690	2	855	5.89	90.96	chlo, cyto, nucl
Glyma.06G184200	*GmTPS12a*	Chr06: 15937184.15943930	2	865	5.71	98.02	nucl, cyto, chlo
Glyma.04G180900	*GmTPS12b*	Chr04: 44666299.44669352	2	865	5.36	94.77	nucl, cyto, chlo

[1] cyto: cytoplasm; chlo: chloroplast; nucl: nuclear; pero: peroxisomes; mito: mitochondrion; E.R.: endoplasmic reticulum; cysk: cytoskeleton. Only three predicted subcellular sites were listed.

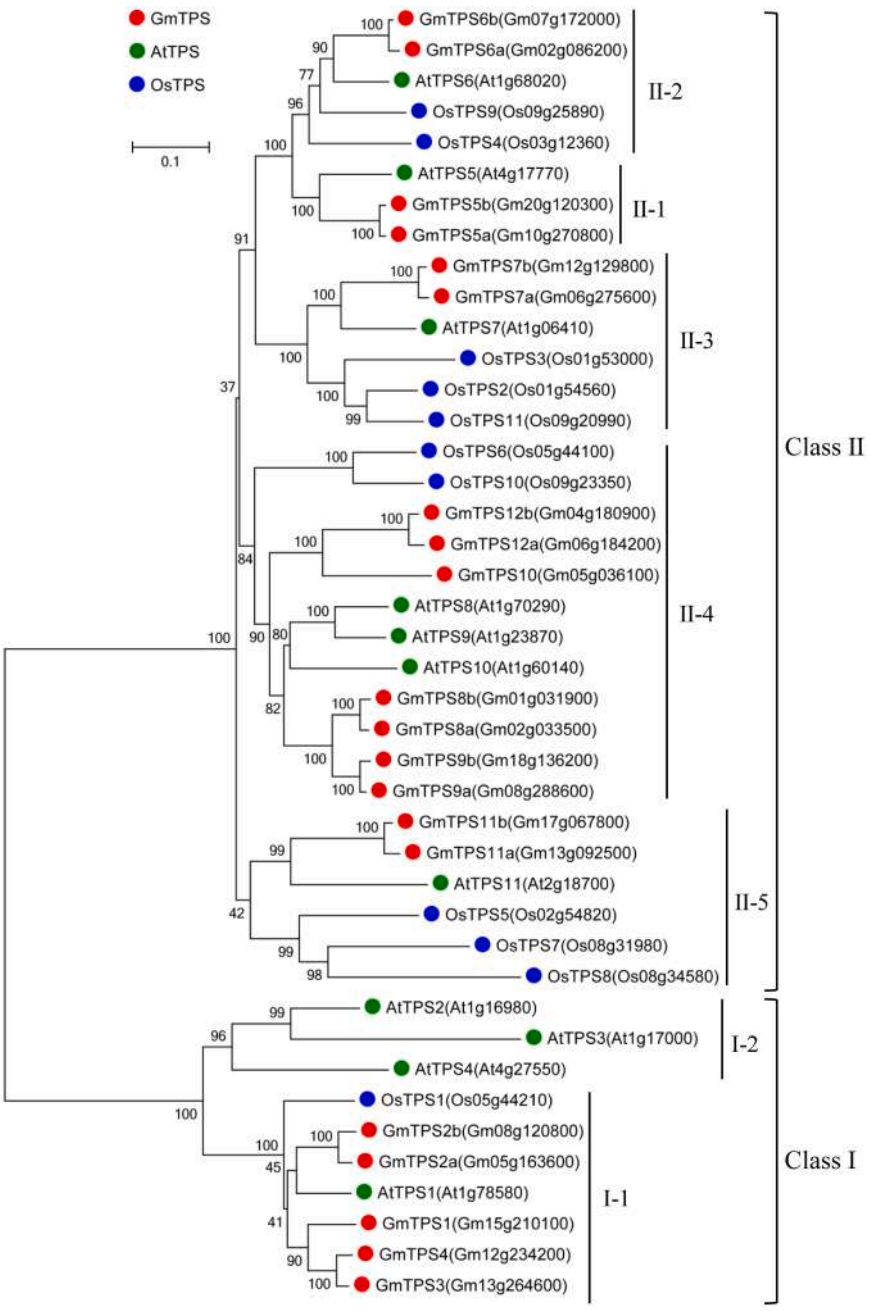

Fig. 1. Unrooted phylogenetic tree of TPS family proteins from soybean, *Arabidopsis,* and rice. Sequences were aligned using ClustalW, and the tree was built in MEGA6 using the neighbor-joining method with 1000 bootstrap replications. The scale bar shows sequence divergence, bootstrap values are indicated on branches, and proteins from each species are marked with colored circles.

binding site (CG-box), environmental signal response element (G-box), WRKY binding site (W-box), light-regulated gene expression (I-box), phosphate starvation signaling transcription factor PHR1 binding site (P1BS), and sulfur-responsive element (SURE) (Table S4). All *TPS* genes contained at least three types of *cis*-acting elements. Promoter of *GmTPS6a*, *GmTPS7a*, and *GmTPS7b*, carried eight types, while *GmTPS2b* and *GmTPS12a* carried seven (Table S5). Most of the *TPS* genes contained W-box and I-box elements, and more than half contained ABRE, SURE, and SARE elements (Fig. 5). The total number of *cis*-acting elements ranged from 4 to 24, with *GmTPS6a* having the most and *GmTPS8b* the fewest. Several elements were present in multiple copies; for example *GmTPS7b*, *GmTPS1*, *GmTPS6a*, and *GmTPS7a* contained 5 SARE, 6 SURE, 6 ABRE, and 6 CG-box elements, respectively (Fig. 5). The presence of these *cis*-acting elements indicates the potential responsiveness of *TPS* genes to hormones and stresses, as well as possible regulation by associated transcription factors.

3.5. *Expression profiling of soybean TPS genes in tissues*

The tissue expression profiling of TPS genes in soybean was analyzed by using RNA-seq datasets retrieved from the Soybean Expression Atlas. This dataset includes 15 different tissues, including leaves, shoots, callus, hypocotyls, seedlings, flowers, green pods, roots, root tips, nodules, embryos, seed coats, cotyledons, endosperm, and suspensors. Among the identified *TPS* genes, 19 showed differential expression patterns across tissues (Table S6), except for *GmTPS2a*, which may be weakly transcribed or expressed only under specific conditions. Several genes displayed tissue-specific expression patterns. For example,

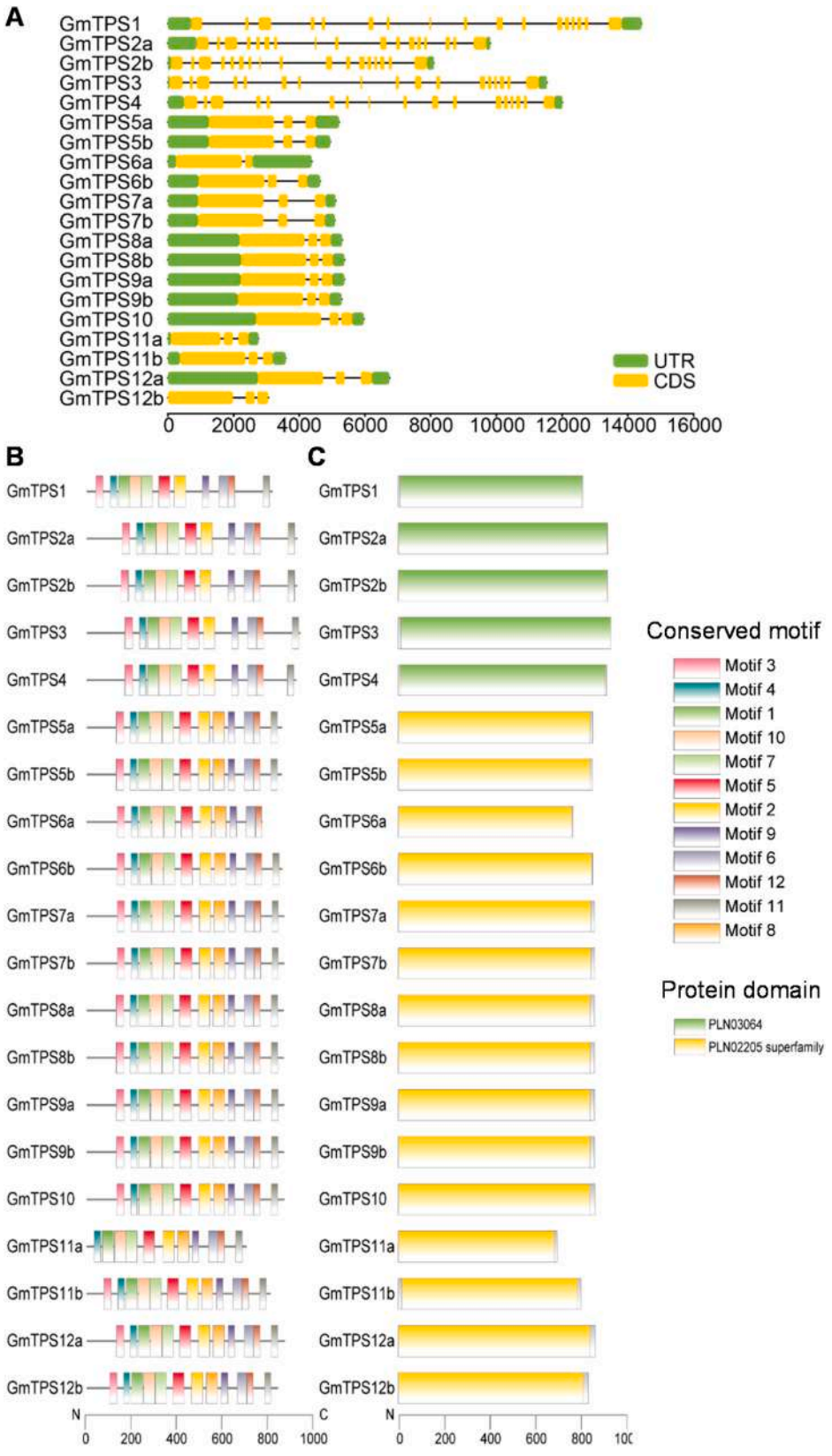

Fig. 2. Structural features of the 20 *TPS* genes in soybean, showing their gene structures (A), conserved motifs (B), and protein domains (C).

GmTPS2b was exclusively expressed in the embryo, while *GmTPS10* was predominantly expressed in the endosperm. Other genes were broadly expressed across multiple tissues. For example, *GmTPS6a*, *GmTPS6b*, *GmTPS7a*, and *GmTPS7b* are highly expressed in the endosperm, hypocotyl, seedling, and seed coat (Fig. 6). Expression of *TPS* genes in soybean root hairs following inoculation with *Bradyrhizobium japonicum* (12–48 h post-inoculation) was also examined using published transcriptome data. Nineteen *TPS* genes were detectable in root hairs

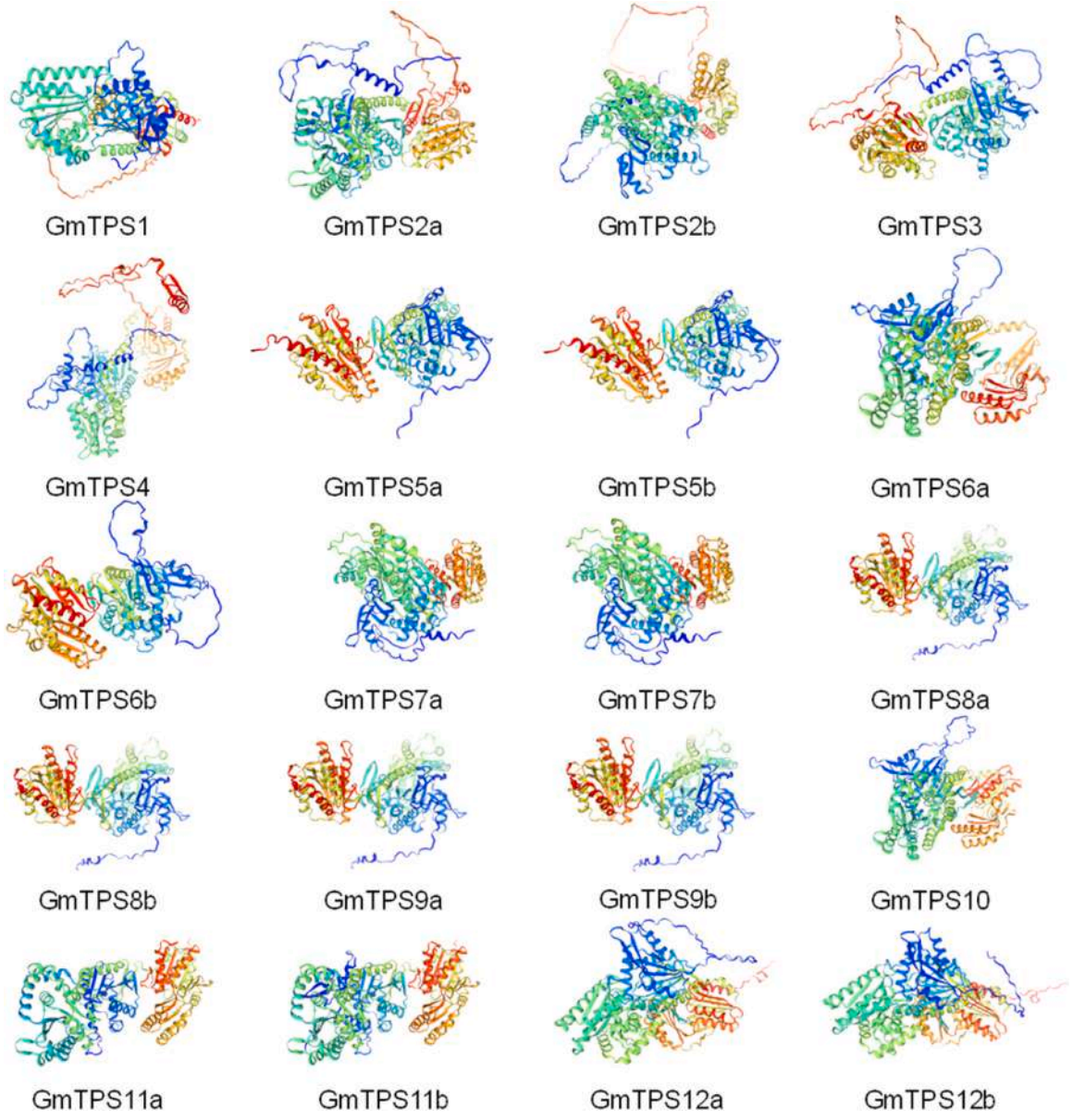

Fig. 3. Predicted three-dimensional ribbon models of soybean TPS proteins generated using SWISS-MODEL (https://swissmodel.expasy.org/).

regardless of rhizobial treatment. Although the inoculation of rhizobia did not significantly change their expression levels (Fig. S2; Table S7), some of these genes showed strong expression in root hairs with or without rhizobia inoculation, such as *GmTPS7a*, *GmTPS7b*, and *GmTPS11b*, suggesting their potential roles in root hair development.

3.6. Transcriptional responses of soybean TPS genes to stresses

By examining published transcriptome datasets, the expression patterns of soybean *TPS* genes were investigated under various stress conditions, including salinity, dehydration, cold, and inoculation with the fungal pathogen *F. oxysporum*. At least 15 *TPS* genes showed significant changes in response to one stress or multiple stresses (Fig. 7A). The expression levels of *GmTPS9a*, *GmTPS11a*, *GmTPS11b*, and *GmTPS12a* were upregulated under salt stress, whereas *GmTPS6a*, *GmTPS7a*, *GmTPS7b*, *GmTPS8b*, *GmTPS9a*, *GmTPS11a*, *GmTPS11b*, and *GmTPS12a* responded to dehydration (Fig. 7A). The expression levels of *GmTPS5b*, *GmTPS8a*, *GmTPS8b*, and *GmTPS9a* were repressed by pathogenic isolates of *F. oxysporum* (FO40), while *GmTPS10, GmTPS11a*, *GmTPS11b*, *GmTPS12a* and *GmTPS12b* were induced by FO40 inoculation (Fig. 7A). To validate the responsiveness of these *TPS* genes to abiotic stresses, six *TPS* genes (*GmTPS3*, *GmTPS6a*, *GmTPS9a*, *GmTPS11a*, *GmTPS11b* and *GmTPS12a*) were selected for expression analysis in soybean roots after salt (100 mM) and dehydration treatments at different time points (1, 3, 6, 12 h) using RT-qPCR. The expression of *GmTPS6a*, *GmTPS11a*, and *GmTPS11b* was significantly induced by both salt and dehydration across all time points. *GmTPS9a* and *GmTPS12a* were induced by dehydration, while *GmTPS3* was repressed after six hours of dehydration (Fig. 7B). These results confirmed the responsiveness of soybean *TPS* genes to stresses such as salt and dehydration.

3.7. Subcellular localization and co-expression genes of GmTPS12a

We selected the stress-responsive *GmTPS12a* for further analysis. Subcellular localization showed that the green fluorescence signal of the GmTPS12a-GFP fusion protein was detected in the ER. The 35S: GmTPS12a-GFP construct was co-transformed with an ER marker protein (ER-rk CD3–959) into rice protoplasts. The green fluorescence signal of GmTPS12a-GFP overlapped with the red fluorescence signal of the ER marker-mCherry fusion protein (Fig. 8 A). These results indicated

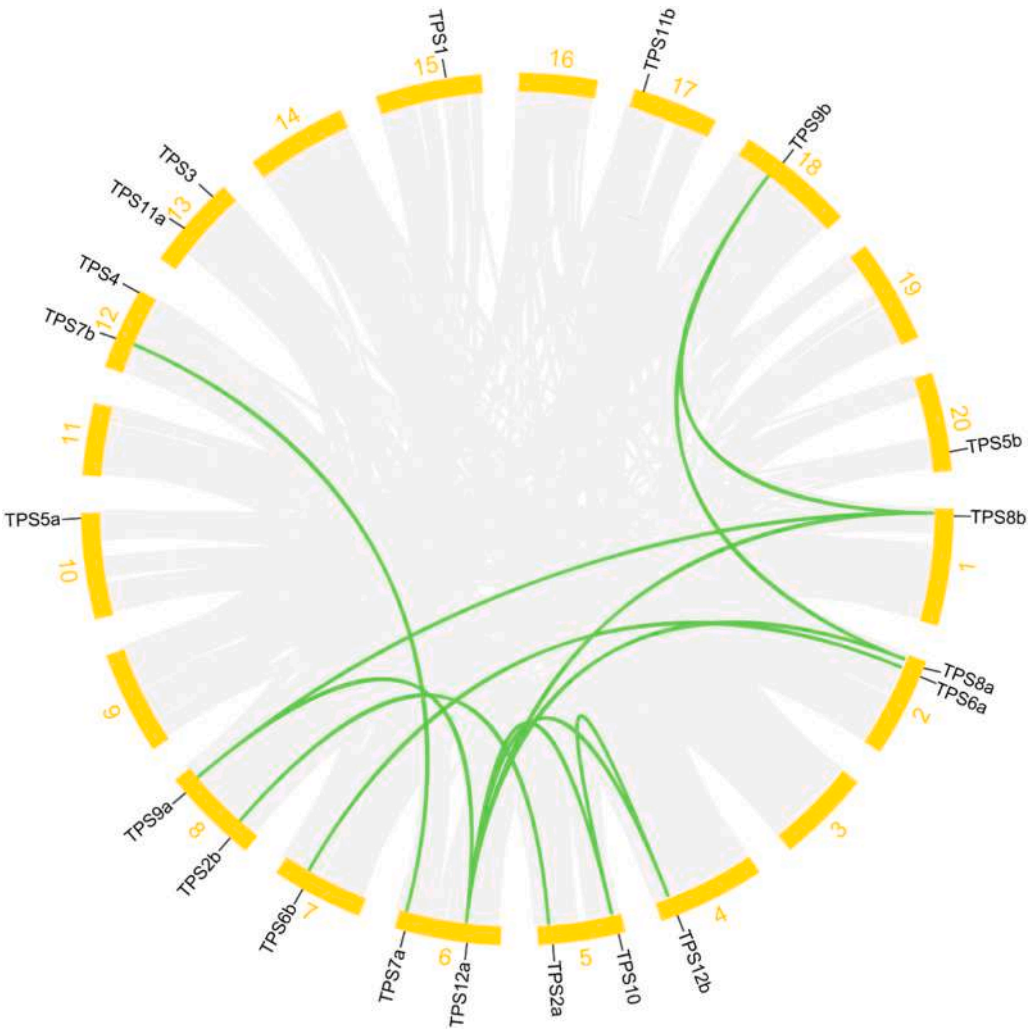

Fig. 4. Syntenic analysis of TPS family genes in the soybean genome. The yellow circle represents the 20 soybean chromosomes, labeled with Arabic numerals. Gene names mark their chromosome positions, and the green lines indicate syntenic relationships among TPS genes.

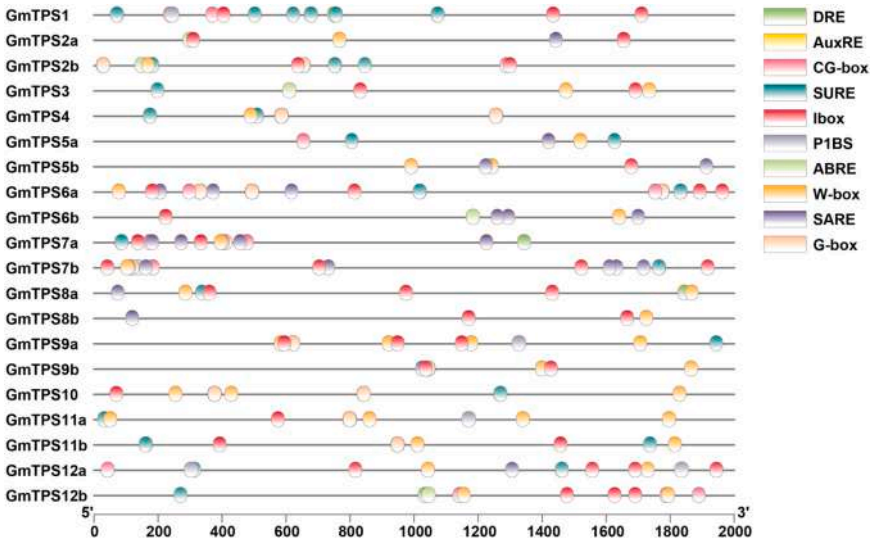

Fig. 5. Distribution of stress- and phytohormone-related *cis*-acting regulatory elements in 2.0 kb promoter regions upstream of transcription start sites of soybean TPS family genes.

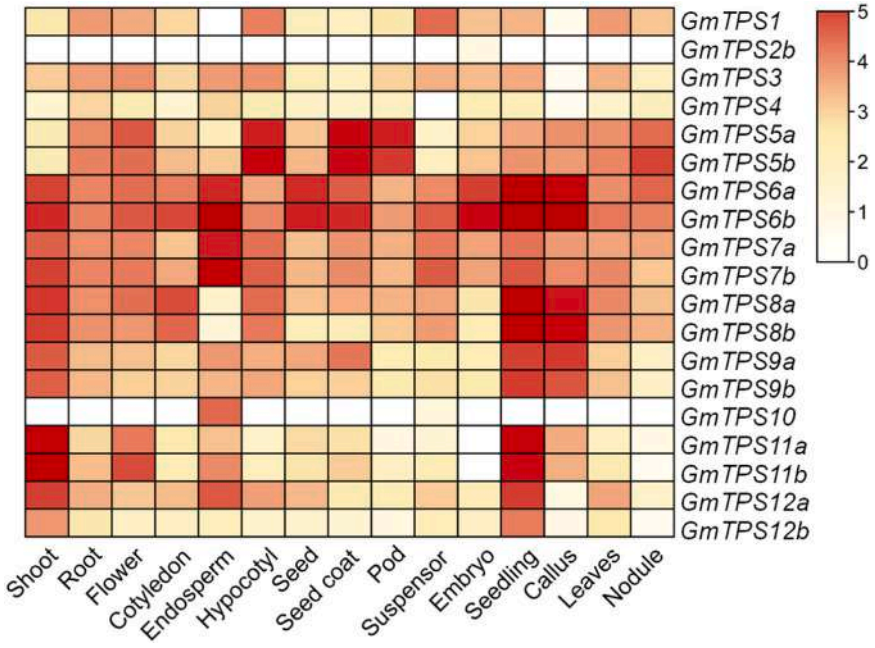

Fig. 6. Tissue-specific expression heatmap of soybean trehalose-6-phosphate synthase genes. The analysis is based on Illumina transcriptome data with log2-transformed TPM (transcripts per million mapped reads) values shown as a color gradient (red indicates higher expression, white indicates no expression).

that GmTPS12a is localized to the ER.

To gain a broader understanding of the regulatory network associated with GmTPS12a, the STRING database was used to predict its interacting proteins. The predicted protein-protein interaction (PPI) network consisted of 11 nodes, including three TPP proteins, a trehalose-related protein, a K-box domain-containing protein, and a bHLH transcription factor (Fig. S3). This suggests that the bHLH transcription factor may regulate *GmTPS12a* and may function together with other proteins, such as TPP proteins. In addition, a co-expression analysis of *GmTPS12a* was performed using the ATTED-II database. Among the top 100 co-expressed genes, several genes encoded transcription factors (*bZIP104*, *bZIP105*, *HSF09,* and *CAMTA4*) and sugar transporters like *STP16* and *SWEET29* (Table S8). GO and KEGG pathway enrichment analyses were carried out to explore their potential functions. Fourteen GO terms related to biological processes were significantly enriched, including trehalose biosynthetic process, regulation of starch metabolism, and AMP metabolism (Table S9). Six KEGG pathways were significantly enriched, such as starch and sucrose metabolism, and nucleotide metabolism (Table S9). These findings suggest that GmTPS12a may participate in these processes or pathways through cooperation with its co-expressed genes.

3.8. Overexpression of GmTPS12a promotes flowering in Arabidopsis

To investigate the physiological function of *GmTPS12a*, ectopic *GmTPS12a*-overexpression transgenic *Arabidopsis* were generated. RT-qPCR analysis confirmed the overexpression of *GmTPS12a* in these lines (Fig. 8B). Compared to WT plants, transgenic plants overexpressing *GmTPS12a* exhibited accelerated flowering, as revealed by fewer days to bolting and a reduced number of rosette leaves at bolting (Fig. 9A, B). To explore the underlying molecular mechanism, the expression of several flowering-related genes, including *FT*, *TWIN SISTER OF FT* (*TSF*), *SUPPRESSOR OF CONSTANS OVEREXPRESSION 1* (*SOC1*), *FLOWERING LOCUS C* (*FLC*), *SPL3* (*SQUAMOSA promoter binding protein-like 3*), *SPL4*, and *SPL5* (Quiroz et al. 2021), was examined in leaves by RT-qPCR. Expression of *FT*, *TSF*, *SOC1*, *SPL3*, *SPL4* and *SPL5* were significantly upregulated, while the flowering repressor *FLC* was downregulated in *GmTPS12a*-overexpressing plants (Fig. 9C). These results were consistent with the early flowering phenotype of *GmTPS12a*-overexpression plants, suggesting that GmTPS12a may promote flowering by regulating T6P and trehalose biosynthesis and, in turn, modulating the expression of flowering-related genes.

3.9. Overexpression of GmTPS12a improves seed germination and early seedling development in Arabidopsis under salt and osmotic stresses

Since *GmTPS12a* responds to both salt and dehydration stresses, its potential role in these stress responses was further examined by examining the seed germination rate and cotyledon greening rate of *GmTPS12a*-overexpressing plants under salt, mannitol, and ABA treatments. Under normal conditions, the seed germination and cotyledon greening rates of *GmTPS12a*-overexpressing plants were comparable to those of WT. Under both salt stress (140 mM NaCl) and osmotic stress (300 mM mannitol), the seed germination and cotyledon greening rates of *GmTPS12a*-overexpressing lines (#8 and #11) were significantly higher than those of WT (Fig. 10). In line #10, the seed germination rate was similar to that of WT under treatments of salt and mannitol, but the cotyledon greening rate was significantly higher (Fig. 10). Because ABA plays a crucial role in salt and osmotic stress responses, seed germination and cotyledon greening rates of *GmTPS12a*-overexpressing plants were also tested under ABA treatment. Both the seed germination rate and cotyledon greening rate of *GmTPS12a*-overexpressing plants were significantly higher than those of WT under ABA treatment (Fig. 10), suggesting that GmTPS12a functions in regulating seed germination and early seedling growth under salt and osmotic stresses in an ABA-dependent manner. The expression of several ABA-responsive genes was also examined. Interestingly, the expression of ABA/abiotic stress-induced genes such as *ABI4, RD26*, *P5CS1*, and *COR15a* (Liu et al. 2023a; Fujita et al. 2004; Zeng et al. 2022) was down-regulated in the *GmTPS12a*-overexpression plants (Fig. 10D), suggesting that ABA signaling is repressed by *GmTPS12a* overexpression. Interestingly, the expression of ABA-responsive *RAB18* was up-regulated around two folds in the *GmTPS12a*-overexpression lines (Fig. 10D), suggesting that *RAB18* may be up-regulated by the internal T6P or other sugars.

4. Discussion

Trehalose and T6P play vital roles in plant growth, development, and

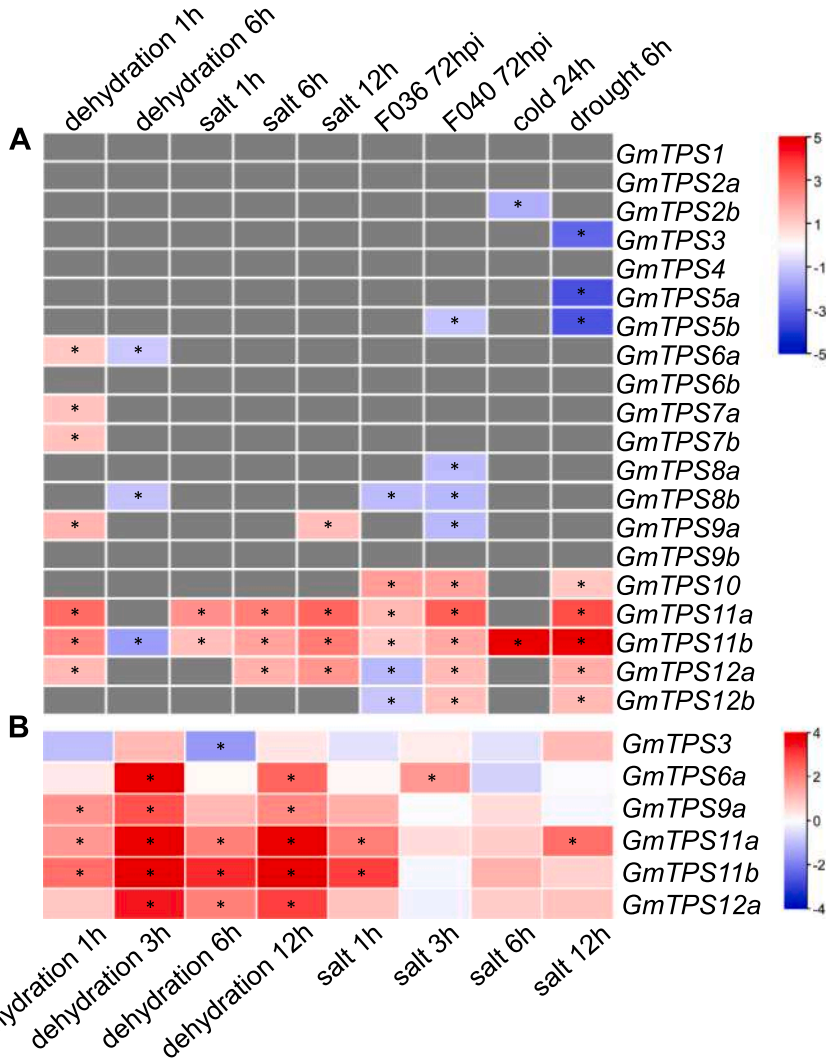

Fig. 7. Transcriptional responses of soybean TPS family genes to environmental stresses. (A) Heatmap showing expression profiling of soybean *TPS* genes under various stresses based on transcriptome data. (B) Expression profiles of six *TPS* genes in soybean roots in response to salt and dehydration stresses were analyzed by RT-qPCR. Color intensity represents log2-transformed fold change, with red indicating induction, blue indicating repression, and gray indicating no significant change. Significantly differentially expressed genes (fold change > 2, P-value < 0.05) are marked with an asterisk.

responses to environmental stresses (Fichtner and Lunn, 2021; Sarkar and Sadhukhan, 2022; Fernandez et al. 2010). TPS is critical for the biosynthesis of T6P and trehalose (Lunn et al. 2014). In this study, we identified 20 TPS genes in soybean, distributed across 14 chromosomes (Fig. S1). The number of TPS genes in soybean is about 1.8 times higher than in *Arabidopsis* and rice (both with 11 genes). This expansion may be explained by two whole-genome duplication events that occurred approximately 59 and 13 million years ago (Schmutz et al. 2010). Phylogenetic analysis showed that soybean TPS genes have a close evolutionary relationship with those in *Arabidopsis* and rice (Fig. 1), indicating that *TPS* genes are highly conserved in both monocots and dicots. Based on this analysis, the soybean *TPS* gene family can be grouped into two subfamilies (Class I and Class II), consistent with reports in *Arabidopsis*, common bean, peanut, and grapevine (Morabito et al. 2021; Vandesteene et al. 2010; Zhong et al. 2024; Liu et al. 2023b). All Class I *GmTPS* genes contained 17 exons, while 15 Class II *GmTPS* genes contained three exons, except for *GmTPS6a* (Fig. 2A), suggesting the genes within the same subfamily share a similar structure. Genes from other families, such as TPP proteins and sugar transporters, also tend to share similar structures within the same phylogenetic clade or subfamily (Shao et al. 2023; Guo et al. 2024). Furthermore, soybean TPS proteins within the same clade displayed similar structural features and contained conserved motifs or domains (Fig. 2B–C), supporting the idea that these genes are evolutionarily related and may share similar physiological and biochemical functions.

Gene duplication is a major driving force in the evolution of genomes and genetic systems (Gu et al. 2003). Gene families can form through four main mechanisms: whole-genome duplication (WGD), tandem duplication, chromosomal segmental duplication, and retrotransposition (Panchy et al. 2016). Among these, segmental duplication and tandem duplication are considered the primary contributors to the expansion of gene families in plants (Cannon et al. 2004; Zhu et al. 2014). Gene duplication events can also lead to the development of new functions (Panchy et al. 2016). In the soybean TPS family, 17 gene pairs were likely generated by WGD or segmental duplication, with duplication events estimated to have occurred between 5.99 and 338.29 MYA; no tandem duplication was detected (Fig. 4; Table S3). Almost all of these gene pairs (16/17) had Ka/Ks ratios below 1.0, indicating that they have been subjected to strong purifying selection (Hurst, 2002). Thus, segmental duplication has played a key role in the expansion of TPS family genes in soybean, consistent with observations in other plants such as common bean and peanut (Zhong et al. 2024; Liu et al. 2023b).

The biological function of a protein is influenced by its subcellular

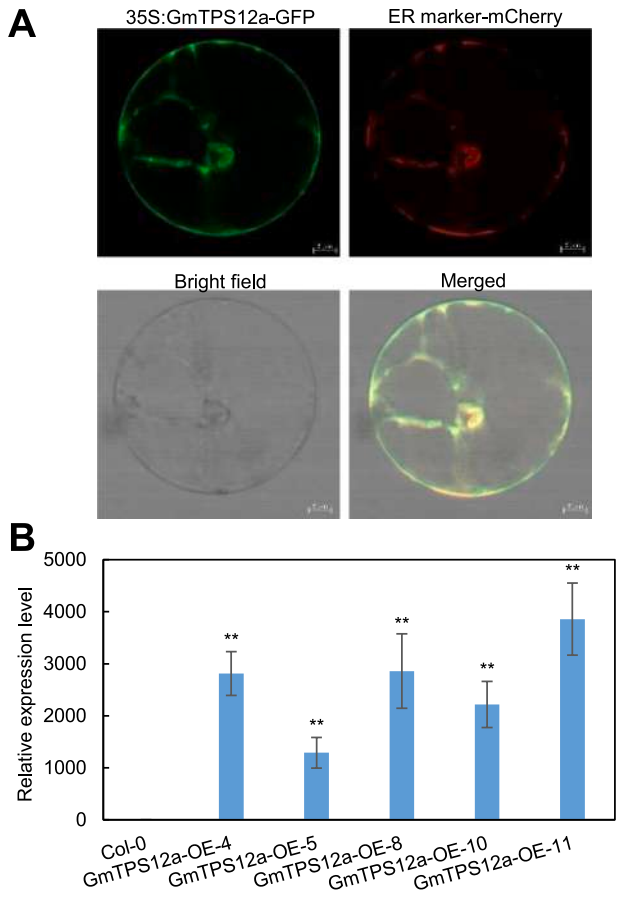

Fig. 8. (A) Subcellular localization of GmTPS12a. The 35S:GmTPS12a-GFP construct was transformed into rice protoplasts along with an endoplasmic reticulum marker mCherry fusion. Fluorescence signals from GFP, mCherry, bright-field, and merged images are shown. Scale bars = 5 μm. (B) Relative transcript levels of *GmTPS12a* in five independent overexpression lines (OE-4, OE-5, OE-8, OE-10, OE-11) measured by RT-qPCR. Data represent mean ± standard deviation of three replicates. Asterisks indicate significant differences (** P-value < 0.01, Student's *t*-test).

location. GmTPS proteins were predicted to be located in various sites, including the nucleus, cytoplasm, chloroplast, and peroxisomes (Table 1), suggesting diverse cellular functions. TPS proteins have also been reported to localize in different subcellular compartments. For example, *Arabidopsis* AtTPS1 is mainly localized in the nucleus (Fichtner et al. 2020), At TPS5 in the ER (Tian et al. 2019), and rapeseed BnaC02. TPS8 in the cytosol (Yuan et al. 2024). In addition, the function of a gene depends on when and where it is transcribed. Tissue expression profiling showed that TPS family genes were differentially expressed across tissues and development stages (Fig. 6), highlighting their functional diversity throughout soybean growth. Notably, *GmTPS2a* was not detected in soybean tissues and may be expressed only under specific conditions. Interestingly, many duplicated gene pairs, such as *GmTPS6a/GmTPS6b*, *GmTPS8a/GmTPS8b*, and *GmTPS9a/GmTPS9b*, displayed similar tissue expression patterns. For example, *GmTPS5a/GmTPS5b* was highly expressed in the hypocotyl, seed coat, and pod; *GmTPS6a/GmTPS6b* in endosperm and callus, and *GmTPS11a/GmTPS11b* in shoot and seedling. These similarities suggest that duplicated genes may perform overlapping or redundant physiological functions during soybean growth and development. However, despite their similar spatial expression patterns, duplicated genes could respond differently to environmental stresses and may play diverse roles in stress adaptation.

Cis-acting elements in promoters play a key role in the spatial and temporal regulation of gene expression. Under stress conditions, transcription factors bind to specific *cis*-acting elements to regulate the expression of downstream stress-responsive genes (Liu et al. 2013). The promoter regions of GmTPS family genes contain numerous hormone- and environment-responsive elements, such as ABA, SA, and auxin response elements, as well as elements responsive to light, dehydration, cold, phosphate, and sulfur (Fig. 5). More than half of the *GmTPS* genes contained light-responsive I-box elements, sulfur-responsive SURE elements, abscisic acid-responsive ABRE elements, SA-responsive SARE elements, ethylene-responsive G-box elements, and W-box motif (Table S5). The ABRE element is associated with abiotic stresses such as drought, salt stress, and low temperature (Gómez-Porras et al. 2007). The W-box, recognized by WRKY transcription factors, plays an important role in plant abiotic stress responses (Chen et al. 2012). The type and abundance of *cis*-acting elements suggest *TPS* genes may respond to both hormones and environmental stresses. *TPS* genes in other plants have also been reported to contain diverse *cis*-acting elements in their promoters (Xu et al. 2017; Liu and Zhou, 2022). Analyzing of published transcriptome data revealed that the expression of most soybean *TPS* genes (80 %) was strongly affected by stresses like cold, drought, salt, or inoculation of isolates of *F. oxysporum* (Fig. 7A). The expression of some *TPS* genes like *GmTPS3*, *GmTPS6a*, *GmTPS9a*, *GmTPS11a*, *GmTPS11b*, and *GmTPS12a* were confirmed to be induced or repressed by salt and dehydration stresses by RT-qPCR (Fig. 7B). However, the molecular mechanisms underlying the regaultion of *TPS* genes under stress and their specific roles in stress responses remain to be investigated.

GmTPS12a, which was up-regulated by salt and dehydration stresses (Fig. 7), was selected for further functional study. In *Arabidopsis,* seed germination and early seedling growth are typically inhibited by salt and mannitol-mediated osmotic stress. Interestingly, seed germination and cotyledon greening rates of *GmTPS12a*-overexpressing plants were significantly higher than those of WT under salt and mannitol treatments (Fig. 10). Under ABA treatment, *GmTPS12a*-overexpressing plants also showed higher seed germination and cotyledon greening rates compared with WT (Fig. 10), suggesting that GmTPS12a acts as a negative regulator of ABA response. Consistent with this, ectopic expression of *AtTPS1* in *Arabidopsis* leads to ABA-insensitive phenotypes (Avonce et al. 2004), whereas functional knockdown of *AtTPS1* causes hypersensitiveness of seed germination to ABA (Gómez et al. 2010). Similarly, AtTPS5, a Class II TPS in *Arabidopsis*, has been shown to regulate ABA signaling negatively (Tian et al. 2019). GmTPS12a was found to be localized in the ER (Fig. 8 A), which is similar to AtTPS5 (Tian et al. 2019), suggesting they may have similar biological functions. Further studies are required to investigate its exact role in the ER. These findings indicate that GmTPS12a may promote seed germination and early seedling growth under salt and drought conditions by repressing ABA responses. Supporting this, the expression of several ABA-induced genes, including *ABI4, RD26*, *P5CS1*, and *COR15a* (Fujita et al. 2004; Chen et al. 2020b; Zeng et al. 2022) was downregulated in *GmTPS12a*-overexpressing *Arabidopsis* seedlings (Fig. 10D). It has been suggested that T6P plays a possible role in ABA-mediated stomatal regulation by involving the covalent modifications of nitrate reductase and SnRK2.6 (Tian et al. 2019; Lunn et al. 2014). But further studies are still needed to reveal the underlying linkage between TPS and ABA signaling. TPS genes in other plants have also been implicated in stress adaptation (Yang et al. 2024; Li et al. 2011; Zou et al. 2024). For example, overexpression of *OsTPS1* enhanced tolerance to salt, low temperature, and drought in rice (Li et al. 2011), while *OsTPS8* overexpression improved salt tolerance (Vishal et al. 2018). In poplar, overexpression of *PagTPS1* and *PagTPS10* increased drought tolerance, possibly by increasing trehalose content in both poplar and *Arabidopsis* (Yang et al. 2024). Similarly, overexpression of an N-terminal truncated *IbTPS1* gene improved drought and salt tolerance in sweet potato (Zou et al. 2024). The upregulation of *TPS* genes is thought to promote the accumulation of osmoprotective compounds and activate the antioxidant system by mediating the biosynthesis of T6P and trehalose (Eh et al. 2024). Taken together, our results

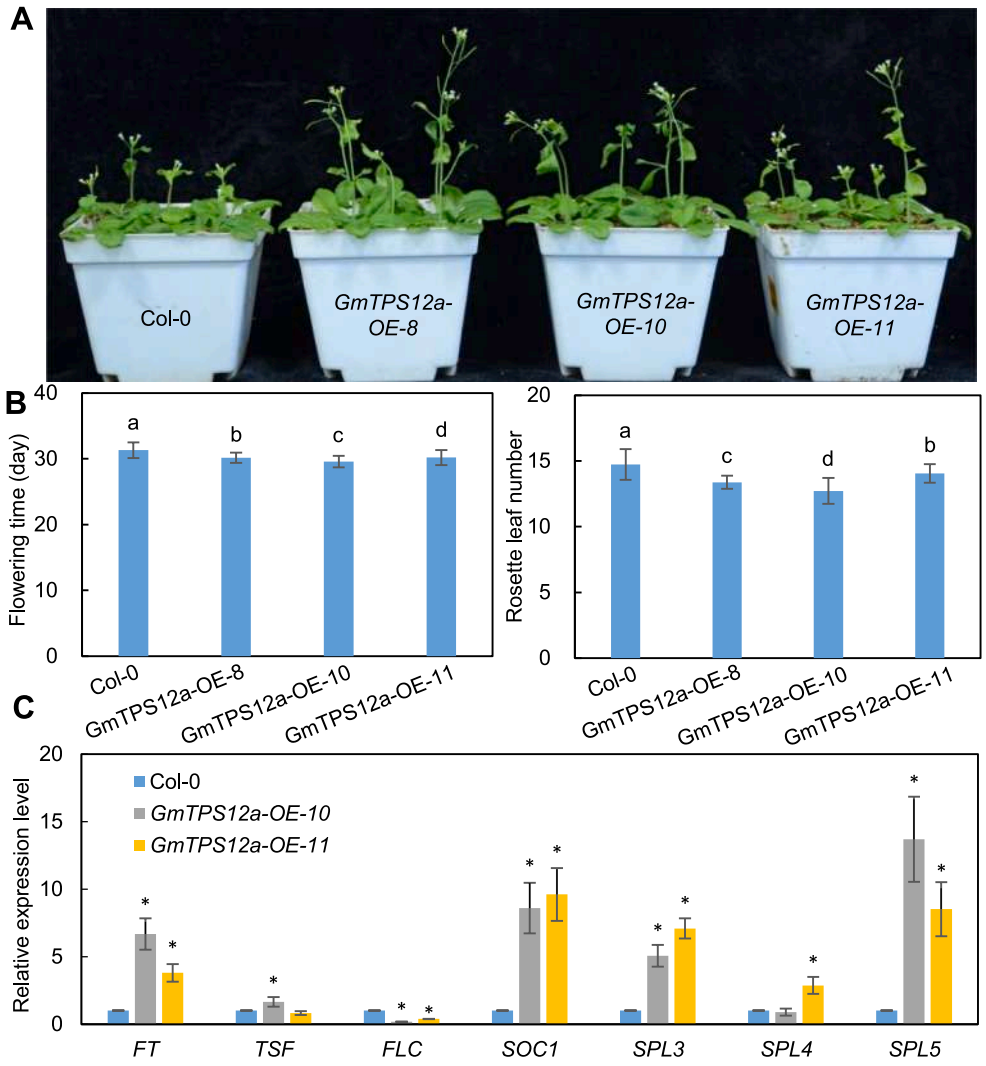

Fig. 9. Flowering phenotype and expression of flowering-related genes in *GmTPS12a*-overexpressing plants. (A) Early flowering phenotype of *GmTPS12a*-overexpressing plants under long-day (LD) conditions. Photo was taken after growth for 44 days under LD conditions. (B) Days to bolting and rosette leaf number at bolting (n = 20, ±SD). (C) Expression levels of flowering-related genes in leaves of two-week-old *GmTPS12a*-overexpressing plants. Gene expression data is the mean ± standard deviation of three replicates. Different letters indicate a significant difference at $P < 0.05$ (Student's *t*-test). An asterisk indicates a significant difference (*P-value < 0.05, Student's *t*-test).

indicate that *GmTPS12a* plays a positive role in enhancing seed germination and early seedling growth under salt and osmotic stresses in *Arabidopsis*. Further studies using transgenic and knockout soybean lines will be required to calarify its role in T6P and trehalose biosynthesis and stress adaptation.

Plant flowering time is controlled by various factors, including internal signals such as sugars, hormones, and age (Maple et al. 2024; Quiroz et al. 2021). Here, we found that overexpression of *GmTPS12a* promotes flowering in *Arabidopsis* (Fig. 9). In the flowering regulation pathway, floral activators FT and SOC1 promote the transcription of floral meristem identity genes involved in floral organ formation, while the floral repressor FLC inhibits the expression of *FT* and *SOC1* (Maple et al. 2024; Quiroz et al. 2021). *SPL3*, *SPL4*, and *SPL5* are post-transcriptionally regulated by miR156, an age-dependent small RNA that is repressed by sugars like sucrose and glucose (Teotia and Tang, 2015; Yu et al. 2013). *Arabidopsis TPS1* also plays a pivotal role in flowering time regulation through the T6P pathway (Wahl et al. 2013). The *tps1* knockdown mutant shows delayed flowering in *Arabidopsis* (van Dijken et al. 2004), and the expression of flowering time genes such as *FT*, *SPL3*, *SPL4*, and *SPL5* is repressed in *TPS1*-deficient plants (Wahl et al. 2013). In this study, the expression of flowering-related genes like *FT*, *TSF*, *SOC1*, *SPL3*, *SPL4*, and *SPL5* was increased by *GmTPS12a*-overexpression, while the expression of *FLC* was repressed in *Arabidopsis* leaves (Fig. 9C). These results suggest that GmTPS12a promotes flowering by influencing the expression of these key flowering-related genes. It is also known that T6P functions as a sensitive proxy of sucrose levels in plants (Figueroa et al. 2016) and acts as a potent inhibitor of SnRK1 (Blanford et al. 2024), a central regulator of energy balance that controls growth, development, and stress responses (Sanagi et al. 2025). Proteins in the Class II TPS subfamily generally lack TPS enzymatic activity (Ramon et al. 2009; Delorge et al. 2015). However, *Arabidopsis* Class II TPS AtTPS8 has been shown to interact with SnRK1 and function as a negative regulator of SnRK1 (Van Leene et al. 2022). Similarly, overexpression of the Class II TPS *BnaC02.TPS8* in rapeseed increased TPS activity and promoted sucrose and trehalose accumulation in leaves (Yuan et al. 2024). Interestingly, TPS family members may also form TPS protein complexes to regulate T6P biosynthesis (Zang et al. 2011). GmTPS12a belongs to the Class II TPS subfamily. Whether its functions in flowering time regulation and stress responses are associated with T6P/trehalose biosynthesis or related to the alternation of sucrose and other sugars remains to be determined.

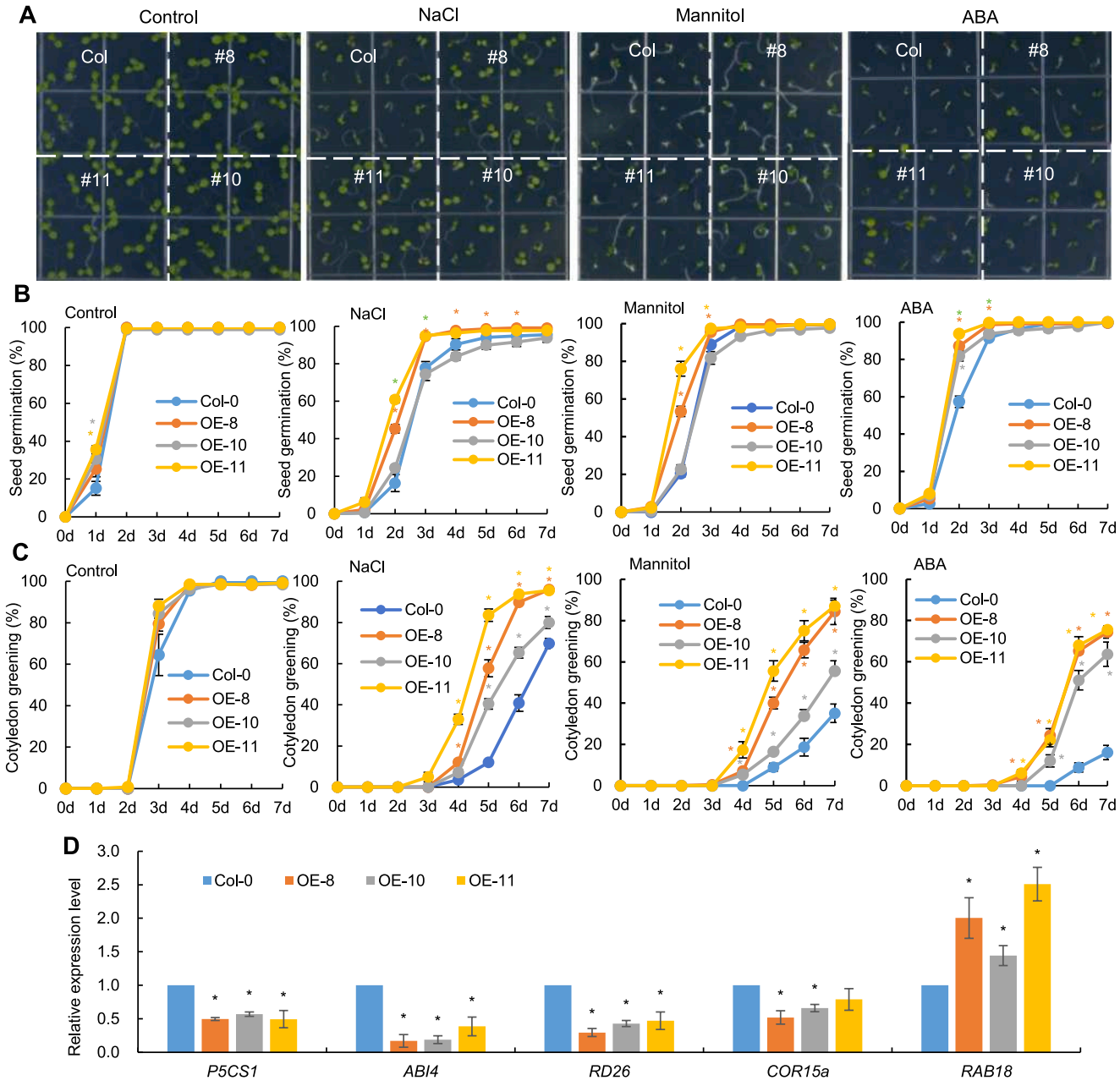

Fig. 10. Seed germination and cotyledon greening of *GmTPS12a*-overexpression plants under salt, mannitol, and ABA treatments. (A) Seedling growth phenotypes under salt (140 mM), mannitol (300 mM), and ABA (0.5 μM) treatment. (B) Seed germination rates under the same treatments. (C) Cotyledon greening rates under the same treatments. Seeds were germinated and grown for 7 days. Values represent means ± SD of three replicates, with at least 40 seeds per replicate. (D) Expression levels of selected ABA/abiotic stress-responsive genes in *GmTPS12a*-overexpression plants. Gene expression data is the mean ± standard deviation of three replicates. Asterisk indicates significant difference compared with Col-0 (* $P < 0.05$, Student's *t*-test).

5. Conclusion

In this study, 20 TPS family genes were identified in the soybean genome. WGD/segmental duplication played an important role in their evolution. Promoter analysis revealed diverse hormone- and stress-responsive elements. Expression profiling showed distinct patterns across tissues and under diverse stress conditions. Among them, *GmTPS12a* was responsive to salt and drought stresses, and its over-expression enhanced resistance to salt and osmotic stresses during seed germination and early seedling growth. Overexpression of *GmTPS12a* also promoted flowering by altering the expression of flowering-related genes. This may be the first Class II TPS family gene playing dual roles in regulating plant flowering time and stress resistance. Overall, this study provides a systematic analysis of the TPS gene family in soybean and highlights the role of *GmTPS12a* in stress tolerance and flowering regulation. Further investigation of *TPS* genes will aid in developing soybean cultivars with improved yield and stress resilience through targeted modulation of their expression or activity.

Author contributions

Houqing Zeng conceived the study. Yuanyuan Liu, Yuxin Yao, Wei Deng, Ruotong Zhao, Jia Du and Hang Guo performed the bioinformatic analyses and the experiments. Yuanyuan Liu, Jing Xu, Aifen Yang, and Houqing Zeng analyzed the data. Houqing Zeng, Yuanyuan Liu, Yuxin Yao, and Shahid Ali wrote the manuscript. All the authors approved the

manuscript.

CRediT authorship contribution statement

Hang Guo: Methodology, Formal analysis. **Jia Du:** Investigation, Formal analysis. **Shahid Ali:** Writing – review & editing. **Jing Xu:** Methodology, Formal analysis. **Houqing Zeng:** Writing – review & editing, Writing – original draft, Validation, Supervision, Formal analysis, Conceptualization. **Aifen Yang:** Project administration, Investigation. **Yuxin Yao:** Writing – original draft, Formal analysis, Data curation. **Yuanyuan Liu:** Investigation, Formal analysis, Data curation. **Ruotong Zhao:** Investigation, Data curation. **Wei Deng:** Formal analysis, Data curation.

Funding

This work was supported by the National Natural Science Foundation of China (grant No. 32372802) and Zhejiang Provincial College Students' Science and Technology Innovation Project.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: Houqing Zeng reports financial support was provided by National Natural Science Foundation of China. If there are other authors, they declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.plantsci.2025.112863.

Data availability

Data will be made available on request.

References

Avonce, N., Leyman, B., Mascorro-Gallardo, J.O., Van Dijck, P., Thevelein, J.M., Iturriaga, G., 2004. The arabidopsis trehalose-6-P synthase AtTPS1 gene is a regulator of glucose, abscisic acid, and stress signaling. Plant Physiol. 136 (3), 3649–3659. https://doi.org/10.1104/pp.104.052084.

Avonce, N., Mendoza-Vargas, A., Morett, E., Iturriaga, G., 2006. Insights on the evolution of trehalose biosynthesis. BMC Evol. Biol. 6, 109. https://doi.org/10.1186/1471-2148-6-109.

Baena-González, E., Lunn, J.E., 2020. SnRK1 and trehalose 6-phosphate - two ancient pathways converge to regulate plant metabolism and growth. Curr. Opin. Plant Biol. 55, 52–59. https://doi.org/10.1016/j.pbi.2020.01.010.

Bailey, T.L., Boden, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., Ren, J., Li, W.W., Noble, W.S., 2009. MEME SUITE: tools for motif discovery and searching. Nucleic Acids Res. 37 (Web Server), W202–W208. https://doi.org/10.1093/nar/gkp335.

Belamkar, V., Weeks, N.T., Bharti, A.K., Farmer, A.D., Graham, M.A., Cannon, S.B., 2014. Comprehensive characterization and RNA-Seq profiling of the HD-Zip transcription factor family in soybean (*Glycine max*) during dehydration and salt stress. BMC Genom. 15, 950. https://doi.org/10.1186/1471-2164-15-950.

Blanford, J., Zhai, Z., Baer, M.D., Guo, G., Liu, H., Liu, Q., Raugei, S., Shanklin, J., 2024. Molecular mechanism of trehalose 6-phosphate inhibition of the plant metabolic sensor kinase SnRK1. Sci. Adv. 10 (20), eadn0895. https://doi.org/10.1126/sciadv.adn0895.

Blázquez, M.A., Santos, E., Flores, Cl, Martínez-Zapater, J.M., Salinas, J., Gancedo, C., 2002. Isolation and molecular characterization of the Arabidopsis TPS1 gene, encoding trehalose-6-phosphate synthase. Plant J. 13 (5), 685–689. https://doi.org/10.1046/j.1365-313x.1998.00063.x.

Cannon, S.B., Mitra, A., Baumgarten, A., Young, N.D., May, G., 2004. The roles of segmental and tandem gene duplication in the evolution of large gene families in Arabidopsis thaliana. BMC Plant Biol. 4 (1), 10. https://doi.org/10.1186/1471-2229-4-10.

Chary, S.N., Hicks, G.R., Choi, Y.G., Carter, D., Raikhel, N.V., 2008. Trehalose-6-phosphate synthase/phosphatase regulates cell shape and plant architecture in Arabidopsis. Plant Physiol. 146 (1), 97–107. https://doi.org/10.1104/pp.107.107441.

Chen, C., Chen, H., Zhang, Y., Thomas, H.R., Frank, M.H., He, Y., Xia, R., 2020a. TBtools: an integrative toolkit developed for interactive analyses of big biological data. Mol. Plant 13 (8), 1194–1202. https://doi.org/10.1016/j.molp.2020.06.009.

Chen, H., Du, J., Wang, Y., Chao, K., Wang, Z., Ali, S., Zeng, H., 2025. Transcription factors PHR1 and PHR1-like 1 regulate ABA-mediated inhibition of seed germination and stomatal opening in Arabidopsis. Plant Sci. 352, 112389. https://doi.org/10.1016/j.plantsci.2025.112389.

Chen, K., Li, G.J., Bressan, R.A., Song, C.P., Zhu, J.K., Zhao, Y., 2020b. Abscisic acid dynamics, signaling, and functions in plants. J. Integr. Plant Biol. 62 (1), 25–54. https://doi.org/10.1111/jipb.12899.

Chen, L., Song, Y., Li, S., Zhang, L., Zou, C., Yu, D., 2012. The role of WRKY transcription factors in plant abiotic stresses. Biochimica et Biophysica Acta (BBA) Gene Regulatory Mechanisms 1819 (2), 120–128. https://doi.org/10.1016/j.bbagrm.2011.09.002.

Clough, S.J., Bent, A.F., 1998. Floral dip: a simplified method for Agrobacterium-mediated transformation of Arabidopsis thaliana. Plant J. 16 (6), 735–743. https://doi.org/10.1046/j.1365-313x.1998.00343.x.

Dai, S.H., Chen, H.Y., Shi, Y.T., Xiao, X.L., Xu, L., Qin, C., Zhu, Y.Y., Yi, K.K., Lei, M.G., Zeng, H.Q., 2024. PHOSPHATE1-mediated phosphate translocation from roots to shoots regulates floral transition in plants. J. Exp. Bot. 75 (16), 5054–5075. https://doi.org/10.1093/jxb/erae222.

Dan, Y., Niu, Y., Wang, C., Yan, M., Liao, W., 2021. Genome-wide identification and expression analysis of the trehalose-6-phosphate synthase (TPS) gene family in cucumber (Cucumis sativus L.). PeerJ 9, e11398. https://doi.org/10.7717/peerj.11398.

Delorge, I., Figueroa, Carlos M., Feil, R., Lunn, John E., Van Dijck, P., 2015. Trehalose-6-phosphate synthase 1 is not the only active TPS in Arabidopsis thaliana. Biochem. J. 466 (2), 283–290. https://doi.org/10.1042/BJ20141322.

Du, H.P., Fang, C., Li, Y.R., Kong, F.J., Liu, B.H., 2023. Understandings and future challenges in soybean functional genomics and molecular breeding. J. Integr. Plant Biol. 65 (2), 468–495. https://doi.org/10.1111/jipb.13433.

Duvaud, S., Gabella, C., Lisacek, F., Stockinger, H., Ioannidis, V., Durinx, C., 2021. Expasy, the Swiss Bioinformatics Resource Portal, as designed by its users. Nucleic Acids Res. 49 (W1), W216–W227. https://doi.org/10.1093/nar/gkab225.

Eh, T.-J., Jiang, Y., Jiang, M., Li, J., Lei, P., Ji, X., Kim, H.-I., Zhao, X., Meng, F., 2024. The role of trehalose metabolism in plant stress tolerance. :S2090-1232(2024)00603-00609 J. Adv. Res.. https://doi.org/10.1016/j.jare.2024.12.025.

Fernandez, O., Béthencourt, L., Quero, A., Sangwan, R.S., Clément, C., 2010. Trehalose and plant stress responses: friend or foe? Trends Plant Sci. 15 (7), 409–417. https://doi.org/10.1016/j.tplants.2010.04.004.

Fichtner, F., Lunn, J.E., 2021. The role of trehalose 6-phosphate (Tre6P) in plant metabolism and development. Annu. Rev. Plant Biol. 72 (1), 737–760. https://doi.org/10.1146/annurev-arplant-050718-095929.

Fichtner, F., Olas, J.J., Feil, R., Watanabe, M., Krause, U., Hoefgen, R., Stitt, M., Lunn, J. E., 2020. Functional features of TREHALOSE-6-PHOSPHATE SYNTHASE1, an essential enzyme in Arabidopsis. Plant Cell 32 (6), 1949–1972. https://doi.org/10.1105/tpc.19.00837.

Figueroa, C.M., Feil, R., Ishihara, H., Watanabe, M., Kölling, K., Krause, U., Höhne, M., Encke, B., Plaxton, W.C., Zeeman, S.C., Li, Z., Schulze, W.X., Hoefgen, R., Stitt, M., Lunn, J.E., 2016. Trehalose 6-phosphate coordinates organic and amino acid metabolism with carbon availability. Plant J. 85 (3), 410–423. https://doi.org/10.1111/tpj.13114.

Fujita, M., Fujita, Y., Maruyama, K., Seki, M., Hiratsu, K., Ohme-Takagi, M., Tran, L.-S.P., Yamaguchi-Shinozaki, K., Shinozaki, K., 2004. A dehydration-induced NAC protein, RD26, is involved in a novel ABA-dependent stress-signaling pathway. Plant J. 39 (6), 863–876. https://doi.org/10.1111/j.1365-313X.2004.02171.x.

Gómez-Porras, J.L., Riaño-Pachón, D.M., Dreyer, I., Mayer, J.E., Mueller-Roeber, B., 2007. Genome-wide analysis of ABA-responsive elements ABRE and CE3 reveals divergent patterns in Arabidopsis and rice. BMC Genom. 8 (1), 260. https://doi.org/10.1186/1471-2164-8-260.

Gómez, L.D., Gilday, A., Feil, R., Lunn, J.E., Graham, I.A., 2010. AtTPS1-mediated trehalose 6-phosphate synthesis is essential for embryogenic and vegetative growth and responsiveness to ABA in germinating seeds and stomatal guard cells. Plant J. 64 (1), 1–13. https://doi.org/10.1111/j.1365-313X.2010.04312.x.

Gu, Z., Steinmetz, L.M., Gu, X., Scharfe, C., Davis, R.W., Li, W.H., 2003. Role of duplicate genes in genetic robustness against null mutations. Nature 421 (6918), 63–66. https://doi.org/10.1038/nature01198.

Guo, H., Guan, Z., Liu, Y., Chao, K., Zhu, Q., Zhou, Y., Wu, H., Pi, E., Chen, H., Zeng, H., 2024. Comprehensive identification and expression analyses of sugar transporter genes reveal the role of GmSTP22 in salt stress resistance in soybean. Plant Physiol. Biochem. 216, 109095. https://doi.org/10.1016/j.plaphy.2024.109095.

Guo, W., Sun, Y., Chai, J., Liu, L., Li, J., Guo, R., Guo, C., 2025. Genome-wide identification of the trehalose-6-phosphate synthase gene family in alfalfa (Medicago sativa L.) reveals involvement of MsTPS16 in tolerance to saline–alkali stress. Gene 963, 149609. https://doi.org/10.1016/j.gene.2025.149609.

Horton, P., Park, K.J., Obayashi, T., Fujita, N., Harada, H., Adams-Collier, C.J., Nakai, K., 2007. WoLF PSORT: protein localization predictor. Nucleic Acids Res. 35 (Web Server), W585–W587. https://doi.org/10.1093/nar/gkm259.

Hurst, L.D., 2002. The Ka/Ks ratio: diagnosing the form of sequence evolution. Trends Genet. 18 (9), 486–487. https://doi.org/10.1016/s0168-9525(02)02722-1.

Ibrahim, E.A., 2025. Review: Trehalose and its role in plant adaptation to salinity stress. Plant Sci. 357, 112533. https://doi.org/10.1016/j.plantsci.2025.112533.

Lanubile, A., Muppirala, U.K., Severin, A.J., Marocco, A., Munkvold, G.P., 2015. Transcriptome profiling of soybean (Glycine max) roots challenged with pathogenic

and non-pathogenic isolates of Fusarium oxysporum. BMC Genom. 16 (1), 1089. https://doi.org/10.1186/s12864-015-2318-2.
Li, H.W., Zang, B.S., Deng, X.W., Wang, X.P., 2011. Overexpression of the trehalose-6-phosphate synthase gene OsTPS1 enhances abiotic stress tolerance in rice. Planta 234 (5), 1007–1018. https://doi.org/10.1007/s00425-011-1458-0.
Libault, M., Farmer, A., Brechenmacher, L., Drnevich, J., Langley, R.J., Bilgin, D.D., Radwan, O., Neece, D.J., Clough, S.J., May, G.D., Stacey, G., 2010. Complete transcriptome of the soybean root hair cell, a single-cell model, and its alteration in response to Bradyrhizobium japonicum infection. Plant Physiol. 152 (2), 541–552. https://doi.org/10.1104/pp.109.148379.
Liu, J.H., Peng, T., Dai, W., 2013. Critical cis-acting elements and interacting transcription factors: key players associated with abiotic stress responses in plants. Plant Mol. Biol. Report. 32 (2), 303–317. https://doi.org/10.1007/s11105-013-0667-z.
Liu, K., Zhou, Y., 2022. Genome-wide identification of the trehalose-6-phosphate synthase gene family in sweet orange (Citrus sinensis) and expression analysis in response to phytohormones and abiotic stresses. PeerJ 10, e13934. https://doi.org/10.7717/peerj.13934.
Liu, W., Thapa, P., Park, S.-W., 2023a. RD29A and RD29B rearrange genetic and epigenetic markers in priming systemic defense responses against drought and salinity. Plant Sci. 337, 111895. https://doi.org/10.1016/j.plantsci.2023.111895.
Liu, W., Zhang, H.-H., Long, Z.-X., Chi, X.-N., Wang, Y.-P., 2023b. Identification, evolutionary relationship analysis of the trehalose-6-phosphate synthase (TPS) gene family in common bean (phaseolus vulgaris) and their expression in response to cold stress. J. Plant Growth Regul. 43 (1), 323–340. https://doi.org/10.1007/s00344-023-11087-9.
Liu, X., Fu, L.S., Qin, P., Sun, Y.L., Liu, J., Wang, X.N., 2019. Overexpression of the wheat trehalose 6-phosphate synthase 11 gene enhances cold tolerance in Arabidopsis thaliana. Gene 710, 210–217. https://doi.org/10.1016/j.gene.2019.06.006.
Lunn, J.E., 2007. Gene families and evolution of trehalose metabolism in plants. Funct. Plant Biol. 34 (6), 550–563. https://doi.org/10.1071/fp06315.
Lunn, J.E., Delorge, I., Figueroa, C.M., Van Dijck, P., Stitt, M., 2014. Trehalose metabolism in plants. Plant J. 79 (4), 544–567. https://doi.org/10.1111/tpj.12509.
Lunn, J.E., Feil, R., Hendriks, J.H.M., Gibon, Y., Morcuende, R., Osuna, D., Scheible, W. R., Carillo, P., Hajirezaei, M.R., Stitt, M., 2006. Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADPglucose pyrophosphorylase and higher rates of starch synthesis in Arabidopsis thaliana. Biochem. J. 397 (1), 139–148. https://doi.org/10.1042/BJ20060083.
Lynch, M., Conery, J.S., 2000. The evolutionary fate and consequences of duplicate genes. Science 290 (5494), 1151–1155. https://doi.org/10.1126/science.290.5494.1151.
Machado, F.B., Moharana, K.C., Almeida-Silva, F., Gazara, R.K., Pedrosa-Silva, F., Coelho, F.S., Grativol, C., Venancio, T.M., 2020. Systematic analysis of 1298 RNA-Seq samples and construction of a comprehensive soybean (Glycine max) expression atlas. Plant J. 103 (5), 1894–1909. https://doi.org/10.1111/tpj.14850.
Maple, R., Zhu, P., Hepworth, J., Wang, J.-W., Dean, C., 2024. Flowering time: from physiology, through genetics to mechanism. Plant Physiol. 195 (1), 190–212. https://doi.org/10.1093/plphys/kiae109.
Martins, M.C.M., Hejazi, M., Fettke, J., Steup, M., Feil, R., Krause, U., Arrivault, S., Vosloh, D., Figueroa, C.M., Ivakov, A., Yadav, U.P., Piques, M., Metzner, D., Stitt, M., Lunn, J.E., 2013. Feedback inhibition of starch degradation in arabidopsis leaves mediated by trehalose 6-phosphate. Plant Physiol. 163 (3), 1142–1163. https://doi.org/10.1104/pp.113.226787.
Maruyama, K., Todaka, D., Mizoi, J., Yoshida, T., Kidokoro, S., Matsukura, S., Takasaki, H., Sakurai, T., Yamamoto, Y.Y., Yoshiwara, K., Kojima, M., Sakakibara, H., Shinozaki, K., Yamaguchi-Shinozaki, K., 2011. Identification of cis-acting promoter elements in cold- and dehydration-induced transcriptional pathways in arabidopsis, rice, and soybean. DNA Res. 19 (1), 37–49. https://doi.org/10.1093/dnares/dsr040.
Morabito, C., Secchi, F., Schubert, A., 2021. Grapevine TPS (trehalose-6-phosphate synthase) family genes are differentially regulated during development, upon sugar treatment and drought stress. Plant Physiol. Biochem. 164, 54–62 doi:1016/j.plaphy.2021.04.032.
Panchy, N., Lehti-Shiu, M., Shiu, S.-H., 2016. Evolution of gene duplication in plants. Plant Physiol. 171 (4), 2294–2316. https://doi.org/10.1104/pp.16.00523.
Paul, M., 2007. Trehalose 6-phosphate. Curr. Opin. Plant Biol. 10 (3), 303–309. https://doi.org/10.1016/j.pbi.2007.04.001.
Paul, M.J., Primavesi, L.F., Jhurreea, D., Zhang, Y., 2008. Trehalose metabolism and signaling. Annu. Rev. Plant Biol. 59, 417–441. https://doi.org/10.1146/annurev.arplant.59.032607.092945.
Ponnu, J., Wahl, V., Schmid, M., 2011. Trehalose-6-phosphate: connecting plant metabolism and development. Front. Plant Sci. 2, 70.
Quiroz, S., Yustis, J.C., Chávez-Hernández, E.C., Martínez, T., Sanchez, M.P., Garay-Arroyo, A., Álvarez-Buylla, E.R., García-Ponce, B., 2021. Beyond the genetic pathways, flowering regulation complexity in arabidopsis thaliana. Int. J. Mol. Sci. 22 (11). https://doi.org/10.3390/ijms22115716.
Ramon, M., De Smet, I., Vandesteene, L., Naudts, M., Leyman, B., Van Dijck, P., Rolland, F., Beeckman, T., Thevelein, J.M., 2009. Extensive expression regulation and lack of heterologous enzymatic activity of the Class II trehalose metabolism proteins from Arabidopsis thaliana. Plant Cell Environ. 32 (8), 1015–1032. https://doi.org/10.1111/j.1365-3040.2009.01985.x.
Sanagi, M., Rolland, F., Sato, T., 2025. New insights into nutrient- and drought-responsive flowering. J. Exp. Bot., eraf090 https://doi.org/10.1093/jxb/eraf090.
Sarkar, A.K., Sadhukhan, S., 2022. Imperative role of trehalose metabolism and trehalose-6-phosphate signaling on salt stress responses in plants. Physiol. Plant. 174 (1), e13647. https://doi.org/10.1111/ppl.13647.
Schluepmann, H., Berke, L., Sanchez-Perez, G.F., 2012. Metabolism control over growth: a case for trehalose-6-phosphate in plants. J. Exp. Bot. 63 (9), 3379–3390.
Schluepmann, H., Pellny, T., van Dijken, A., Smeekens, S., Paul, M., 2003. Trehalose 6-phosphate is indispensable for carbohydrate utilization and growth in Arabidopsis thaliana. Proc. Natl. Acad. Sci. 100 (11), 6849–6854. https://doi.org/10.1073/pnas.1132018100.
Schmutz, J., Cannon, S.B., Schlueter, J., Ma, J., Mitros, T., Nelson, W., Hyten, D.L., Song, Q., Thelen, J.J., Cheng, J., Xu, D., Hellsten, U., May, G.D., Yu, Y., Sakurai, T., Umezawa, T., Bhattacharyya, M.K., Sandhu, D., Valliyodan, B., Lindquist, E., Peto, M., Grant, D., Shu, S., Goodstein, D., Barry, K., Futrell-Griggs, M., Abernathy, B., Du, J., Tian, Z., Zhu, L., Gill, N., Joshi, T., Libault, M., Sethuraman, A., Zhang, X.C., Shinozaki, K., Nguyen, H.T., Wing, R.A., Cregan, P., Specht, J., Grimwood, J., Rokhsar, D., Stacey, G., Shoemaker, R.C., Jackson, S.A., 2010. Genome sequence of the palaeopolyploid soybean. Nature 463 (7278), 178–183. https://doi.org/10.1038/nature08670.
Shao, W., Zhang, X., Zhou, Z., Ma, Y., Chu, D., Wang, L., Yang, Y., Du, L., Du, Y., Du, J., Zhao, Q., 2023. Genome- and transcriptome-wide identification of trehalose-6-phosphate phosphatases (TPP) gene family and their expression patterns under abiotic stress and exogenous trehalose in soybean. BMC Plant Biol. 23 (1), 641. https://doi.org/10.1186/s12870-023-04652-7.
Shiu, S.H., Le, D.T., Nishiyama, R., Watanabe, Y., Tanaka, M., Seki, M., Ham, L.H., Yamaguchi-Shinozaki, K., Shinozaki, K., Tran, L.S.P., 2012. Differential gene expression in soybean leaf tissues at late developmental stages under drought stress revealed by genome-wide transcriptome analysis. PLoS One 7 (11), e49522. https://doi.org/10.1371/journal.pone.0049522.
Song, J., Mao, H., Cheng, J., Zhou, Y., Chen, R., Zeng, L., Li, H., Wang, Y., 2021. Identification of the trehalose-6-phosphate synthase gene family in Medicago truncatula and expression analysis under abiotic stresses. Gene 787, 145641. https://doi.org/10.1016/j.gene.2021.145641.
Tamura, K., Stecher, G., Peterson, D., Filipski, A., Kumar, S., 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol. Biol. Evol. 30 (12), 2725–2729. https://doi.org/10.1093/molbev/mst197.
Teotia, S., Tang, G., 2015. To bloom or not to bloom: role of MicroRNAs in plant flowering. Mol. Plant 8 (3), 359–377. https://doi.org/10.1016/j.molp.2014.12.018.
Tian, L., Xie, Z., Lu, C., Hao, X., Wu, S., Huang, Y., Li, D., Chen, L., 2019. The trehalose-6-phosphate synthase TPS5 negatively regulates ABA signaling in Arabidopsis thaliana. Plant Cell Rep. 38 (8), 869–882. https://doi.org/10.1007/s00299-019-02408-y.
van Dijken, A.J., Schluepmann, H., Smeekens, S.C., 2004. Arabidopsis trehalose-6-phosphate synthase 1 is essential for normal vegetative growth and transition to flowering. Plant Physiol. 135 (2), 969–977. https://doi.org/10.1104/pp.104.039743.
Van Leene, J., Eeckhout, D., Gadeyne, A., Matthijs, C., Han, C., De Winne, N., Persiau, G., Van De Slijke, E., Persyn, F., Mertens, T., Smagghe, W., Crepin, N., Broucke, E., Van Damme, D., Pleskot, R., Rolland, F., De Jaeger, G., 2022. Mapping of the plant SnRK1 kinase signalling network reveals a key regulatory role for the class II T6P synthase-like proteins. Nat. Plants 8 (11), 1245–1261. https://doi.org/10.1038/s41477-022-01269-w.
Vandesteene, L., Ramon, M., Le Roy, K., Van Dijck, P., Rolland, F., 2010. A single active trehalose-6-p synthase (TPS) and a family of putative regulatory TPS-like proteins in arabidopsis. Mol. Plant 3 (2), 406–419. https://doi.org/10.1093/mp/ssp114.
Vishal, B., Krishnamurthy, P., Ramamoorthy, R., Kumar, P.P., 2018. OsTPS8 controls yield-related traits and confers salt stress tolerance in rice by enhancing suberin deposition. N. Phytol. 221 (3), 1369–1386.
Vishal, B., Krishnamurthy, P., Ramamoorthy, R., Kumar, P.P., 2019. OsTPS8 controls yield-related traits and confers salt stress tolerance in rice by enhancing suberin deposition. N. Phytol. 221 (3), 1369–1386. https://doi.org/10.1111/nph.15464.
Vogel, G., Fiehn, O., Jean-Richard-dit-Bressel, L., Boller, T., Wiemken, A., Aeschbacher, R.A., Wingler, A., 2001. Trehalose metabolism in Arabidopsis: occurrence of trehalose and molecular cloning and characterization of trehalose-6-phosphate synthase homologues. J. Exp. Bot. 52 (362), 1817–1826. https://doi.org/10.1093/jexbot/52.362.1817.
Wahl, V., Ponnu, J., Schlereth, A., Arrivault, S., Langenecker, T., Franke, A., Feil, R., Lunn, J.E., Stitt, M., Schmid, M., 2013. Regulation of flowering by trehalose-6-phosphate signaling in Arabidopsis thaliana. Science 339 (6120), 704–707. https://doi.org/10.1126/science.1230406.
Wang, F., Chen, H.W., Li, Q.T., Wei, W., Li, W., Zhang, W.K., Ma, B., Bi, Y.D., Lai, Y.C., Liu, X.L., Man, W.Q., Zhang, J.S., Chen, S.Y., 2015. GmWRKY27 interacts with GmMYB174 to reduce expression of GmNAC29 for stress tolerance in soybean plants. Plant J. 83 (2), 224–236. https://doi.org/10.1111/tpj.12879.
Xu, Y.C., Wang, Y.J., Mattson, N., Yang, L., Jin, Q.J., 2017. Genome-wide analysis of the Solanum tuberosum (potato) trehalose-6-phosphate synthase (TPS) gene family: evolution and differential expression during development and stress. BMC Genom. 18 (1), 926. https://doi.org/10.1186/s12864-017-4298-x.
Yadav, U.P., Ivakov, A., Feil, R., Duan, G.Y., Walther, D., Giavalisco, P., Piques, M., Carillo, P., Hubberten, H.-M., Stitt, M., Lunn, J.E., 2014. The sucrose–trehalose 6-phosphate (Tre6P) nexus: specificity and mechanisms of sucrose signalling by Tre6P. J. Exp. Bot. 65 (4), 1051–1068. https://doi.org/10.1093/jxb/ert457.
Yang, H.L., Liu, Y.J., Wang, C.L., Zeng, Q.Y., 2012. Molecular evolution of trehalose-6-phosphate synthase (TPS) gene family in populus, arabidopsis and rice. PloS One 7 (8), e42438. https://doi.org/10.1371/journal.pone.0042438.
Yang, Y., Wang, C., Liang, Y., Xiao, D., Fu, T., Yang, X., Liu, J., Wang, S., Wang, Y., 2024. PagTPS1 and PagTPS10, the trehalose-6-phosphate synthase genes, increase trehalose content and enhance drought tolerance. Int. J. Biol. Macromol. 279, 135518. https://doi.org/10.1016/j.ijbiomac.2024.135518.

Yang, Y., Yao, Y., Li, J., Zhang, J., Zhang, X., Hu, L., Ding, D., Bakpa, E.P., Xie, J., 2022. Trehalose alleviated salt stress in tomato by regulating ros metabolism, photosynthesis, osmolyte synthesis, and trehalose metabolic pathways. Front. Plant Sci. 13, 772948. https://doi.org/10.3389/fpls.2022.772948.

Yu, S., Cao, L., Zhou, C.M., Zhang, T.Q., Lian, H., Sun, Y., Wu, J.Q., Huang, J.R., Wang, G.D., Wang, J.W., 2013. Sugar is an endogenous cue for juvenile-to-adult phase transition in plants. Elife 2, e00269. https://doi.org/10.7554/eLife.00269.

Yuan, P., Zhou, G.L., Yu, M.Z., Hammond, J.P., Liu, H.J., Hong, D.F., Cai, H.M., Ding, G. D., Wang, S.L., Xu, F.S., Wang, C., Shi, L., 2024. Trehalose-6-phosphate synthase 8 increases photosynthesis and seed yield in Brassica napus. Plant J. 118 (2), 437–456. https://doi.org/10.1111/tpj.16617.

Zang, B., Li, H., Li, W., Deng, X.W., Wang, X., 2011. Analysis of trehalose-6-phosphate synthase (TPS) gene family suggests the formation of TPS complexes in rice. Plant Mol. Biol. 76 (6), 507–522. https://doi.org/10.1007/s11103-011-9781-1.

Zeng, H., Wu, H., Wang, G., Dai, S., Zhu, Q., Chen, H., Yi, K., Du, L., 2022. Arabidopsis CAMTA3/SR1 is involved in drought stress tolerance and ABA signaling. Plant Sci. 319, 111250. https://doi.org/10.1016/j.plantsci.2022.111250.

Zeng, H., Zhang, X., Ding, M., Zhang, X., Zhu, Y., 2019. Transcriptome profiles of soybean leaves and roots in response to zinc deficiency. Physiol. Plant 167 (3), 330–351. https://doi.org/10.1111/ppl.12894.

Zhang, Q., Zhang, M., Zhao, Y.-Q., Hu, H., Huang, Y.-X., Jia, G.-X., 2022. Identification of trehalose-6-phosphate synthase (TPS)-coding genes involved in flowering induction of Lilium× formolongi. Plant Physiol. Biochem. 171, 84–94. https://doi.org/10.1016/j.plaphy.2021.12.025.

Zhong, C., He, Z., Liu, Y., Li, Z., Wang, X., Jiang, C., Kang, S., Liu, X., Zhao, S., Wang, J., Zhang, H., Zhao, X., Yu, H., 2024. Genome-wide identification of TPS and TPP genes in cultivated peanut (Arachis hypogaea) and functional characterization of AhTPS9 in response to cold stress. Front. Plant Sci. 14, 1343402. https://doi.org/10.3389/fpls.2023.1343402.

Zhu, F., Li, M., Sun, M., Jiang, X., Qiao, F., 2022. Plant hormone signals regulate trehalose accumulation against osmotic stress in watermelon cells. Protoplasma 259 (5), 1351–1369. https://doi.org/10.1007/s00709-021-01715-0.

Zhu, Y., Wu, N., Song, W., Yin, G., Qin, Y., Yan, Y., Hu, Y., 2014. Soybean (Glycine max) expansin gene superfamily origins: segmental and tandem duplication events followed by divergent selection among subfamilies. BMC Plant Biol. 14 (1), 93. https://doi.org/10.1186/1471-2229-14-93.

Zou, X., Wang, S., Cheng, Q., Yu, H., Yang, Z., Wang, Y., Yang, Y., Liang, X., Yang, D., Kim, H.S., Jia, X., Li, L., Kwak, S.-S., Wang, W., 2024. N-terminal truncated trehalose-6-phosphate synthase 1 gene (ΔNIbTPS1) enhances the tolerance of sweet potato to abiotic stress. Plant Physiol. Biochem. 214, 108917. https://doi.org/10.1016/j.plaphy.2024.108917.

Zulfiqar, F., Chen, J., Finnegan, P.M., Younis, A., Nafees, M., Zorrig, W., Hamed, K.B., 2021. Application of trehalose and salicylic acid mitigates drought stress in sweet basil and improves plant growth. Plants 10 (6), 1078. https://doi.org/10.3390/plants10061078.