# Genome-wide analysis of sugar transporter genes in maize (*Zea mays* L.): identification, characterization and their expression profiles during kernel development (#88034)



First submission

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# Genome-wide analysis of sugar transporter genes in maize (*Zea mays* L.): identification, characterization and their expression profiles during kernel development

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Sugar transporters (STs) play a crucial role in the development of maize kernels. However, very limited information about STs in maize is known. In this study, sixty-eight *ZmST* genes were identified from the maize genome and classified into eight major groups based on phylogenetic relationship. Gene structure analysis revealed that members within the same group shared similar exon numbers. Synteny analysis indicated that *ZmSTs* underwent 15 segmental duplication events under purifying selection. Three-dimensional structure of ZmSTs demonstrated the formation of a compact helix bundle composed of 8-13 trans-membrane domains. Various development-related *cis*-acting elements, enriched in promoter regions, were correlated with the transcriptional response of *ZmSTs* during kernel development. Transcriptional expression profiles exhibited expression diversity of various *ZmST* genes in roots, stems, leaves, tassels, cobs, embryos, endosperms and seeds tissues. During kernel development, the expression of 24 *ZmST* genes was significantly upregulated in the early stage of grain filling. This upregulation coincided with the sharply increased grain-filling rate observed in the early stage. Overall, our findings shed light on the characteristics of *ZmST* genes in maize and provide a foundation for further functional studies.

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## Abstract

Sugar transporters (STs) play a crucial role in the development of maize kernels. However, very limited information about STs in maize is known. In this study, sixty-eight *ZmST* genes were identified from the maize genome and classified into eight major groups based on phylogenetic relationship. Gene structure analysis revealed that members within the same group shared similar

exon numbers. Synteny analysis indicated that *ZmSTs* underwent 15 segmental duplication events under purifying selection. Three-dimensional structure of ZmSTs demonstrated the formation of a compact helix bundle composed of 8-13 trans-membrane domains. Various development-related *cis*-acting elements, enriched in promoter regions, were correlated with the transcriptional response of *ZmSTs* during kernel development. Transcriptional expression profiles exhibited expression diversity of various *ZmST* genes in roots, stems, leaves, tassels, cobs, embryos, endosperms and seeds tissues. During kernel development, the expression of 24 *ZmST* genes was significantly upregulated in the early stage of grain filling. This upregulation coincided with the sharply increased grain-filling rate observed in the early stage. Overall, our findings shed light on the characteristics of *ZmST* genes in maize and provide a foundation for further functional studies.

## 1. Introduction

In higher plants, sugars, including monosaccharide and sucrose, play a crucial role in enhancing yield (Büttner, 2007; Julius et al., 2017). In many plant species, sucrose is synthesized in green organs (source) and transported over long distances through the phloem to heterotrophic organs (sink) (Van Bel, 2003). Upon reaching the sink organs, sucrose is either directly transported into sink cells or cleaved into monosaccharides by cell wall-bound invertases, which are subsequently taken up by the sink cells (Sherson et al., 2003). Extensive researches have established that the transport of sugars into sink cells is mediated by sugar transporters (STs), which facilitate the transport of both monosaccharides and sucrose (Noiraud et al., 2001; Büttner, 2007; Kühn and Grof, 2010).

Many sugar transporters, specifically those from major facilitator superfamily (MFS) and sugar will eventually be exported transporters (SWEET) family, have been identified in various species (Chen et al., 2012; Zheng et al., 2014). MFS is further divided into the monosaccharide transporter (MST) family and the sucrose transporter (SUT) family, with MST family exhibiting

greater diversity (Yan, 2013). The MST family members are classified into seven subfamilies, including sugar transporter proteins (STPs) that act as proton/sugar symporters for various monosaccharides (Büttner, 2007), polyol/monosaccharide transporters (PMTs) responsible for transporting monosaccharide and sugar alcohols on the plasma membrane (Noiraud et al., 2001), sugar facilitator proteins (SFPs) that export hexoses on vacuolar membrane (Yamada et al., 2010; Klemens et al., 2014), inositol transporters (INTs) that function as $H^+$/inositol symporters (Strobl et al., 2018), plastidic glucose translocators (pGlcTs) that export glucose into the cytosol (Cho et al., 2011), and two families of monosaccharide importers for sugar uptake in the tonoplast, namely tonoplastic sugar transporters (TSTs) and vacuolar glucose transporters (VGTs) (Aluri and Büttner, 2007; Cheng et al., 2018a; Cheng et al., 2018b). These eight families of MFS-type sugar transporters are ancient and present in both dicotyledonous and monocotyledonous plants (Lemoine, 2000; Johnson et al., 2006). The SWEET family, discovered in 2010, belongs to another superfamily and possesses seven transmembrane domains (Chen et al., 2010; Xuan et al., 2013). Due to these differences, the SWEET transporter family will not be discussed in this study.

Previous studies have shown the importance of *STs* in the transportation of sugars to sink tissues, which is crucial for crop yield and quality. In *Arabidopsis*, there are 62 identified *AtST* genes. Mutants of *AtSUC2*, which have decreased sucrose transport in the phloem, accumulate excessive starch in the leaves, leading to severe growth inhibition and reduced fertility (Gottwald et al., 2000; Gould et al., 2012). The expression of *AtSTP4* gradually increases during pollen development, with the highest level occurring in mature pollen (Truernit et al., 1996). *AtSTPs* are not been found in the female gametophyte or developing seeds (Büttner, 2010). *AtVGT1*, located on vacuolar membrane, plays an important role in flowering and seed germination by transporting glucose (Aluri and Büttner, 2007). *AtTMT1* and *AtTMT2* transport monosaccharides and sucrose into the vacuole (Schulz et al., 2011). Mutants of *Aterdl6* show increased vacuolar glucose levels and increased seed weight due to higher sugar, protein, and lipid levels (Poschet et

al., 2011).

In rice, *OsTMTs* transport glucose into vacuoles and contribute to sugar storage in vacuoles (Cho et al., 2010). *OsSUT1* is involved in long-distance sucrose transport, plant height, pollen vitality and seed germination. Mutants of *OsSUT1* exhibit a slight dwarf phenotype and complete infertility due to failed grain-filli(Hu et al., 2021; Sun et al., 2022; Wang et al., 2022). *OspGlcT2* is expressed in response to sugar and salt, indicating its role in salt stress tolerance (Deng et al., 2019). *OsSTP10* is induced by sucrose and fructose treatments in roots, but does not respond to hormone treatments. *OsSTP16* is highly expressed in flag leaf sheaths and responds rapidly to glucose and fructose (Deng et al., 2019). The *OsTMTs* in rice function similarly to *AtTMTs*, transporting monosaccharides into vacuoles (Cho et al., 2010).

Maize (*Zea mays* L.) is a significant global food crop with important economic and social value, as well as applications in the bioenergy industries (Tian et al., 2019). Additionally, it serves as an excellent model organism for genetic and genomic studies due to its high photosynthetic rate, availability of a reference genome and efficient transformation system (Schnable et al., 2009; Wang et al., 2020). *STs* not only play a role in sugar transport and allocation, but also have crucial impacts on plant yield and quality. However, compared to species like strawberry, pear, tomato and rice, limited research has been conducted on the *ST* gene family in maize. In this study, we performed a comprehensive search against the updated maize genome B73_RefGen_v5 and identified 68 *ZmST* genes. Through phylogenetic relationship, chromosome location, collinearity analysis, conservative structures and expression patterns analyses in maize, we found that *ZmSTs* play a significant role in sugar transportation and seed development. These results serve as valuable references for further research on *ZmSTs* and provide new genetic resources for the high-yield maize breeding.

## 2. Materials & Methods

### *2.1 Plant materials and growth condition*

The maize inbred line B73 was used in this study. B73 seeds were sterilized with mercuric

chloride and cultured in dd$H_2O$ at 28°C (light)/23°C (dark) with a 16 h light/8 h dark photoperiod (Li et al., 2013). After germination, the seedlings with uniformed growth were selected and moved into the field. Subsequently, various tissues and kernels at different days after pollination were collected for the analysis of *ZmST* expression levels.

*2.2 Identification and characterization of ST proteins in maize*

To investigate putative *ST* genes in *Zea mays*, two methods were employed. First, 62 AtST sequences in *Arabidopsis* were downloaded and used to perform a BLAST search against the *Zea mays* genome obtained from maizeGDB (https://www.maizegdb.org/) with default parameters (Long et al., 2021). Additionally, the Hidden Markov Model (HMM) profiles of the Sugar_tr domain (PF00083), MFS-1 (PF07690) and MFS-2 (PF13347) were obtained from Pfam (http://pfam.xfam.org/) and utilized for HMMER 3.0 searches against the potential ST proteins in maizeGDB (Prakash et al., 2017; Mistry et al., 2018). All potential ZmST proteins were determined on NCBI (https://www.ncbi.nlm.nih.gov/cdd/) and SMART (https://smart.embl.de/).

*2.3 Comparison of the numbers of ST gene families in different plants*

*ST* genes from various plants, including *Arabidopsis* (Büttner, 2007), rice (Deng et al., 2019), tomato (Reuscher et al., 2014), pear (Li et al., 2015), strawberry (Liu et al., 2020), grape (Afoufa-Bastien et al., 2010), Longan (Fang et al., 2020), and apple (Wei et al., 2014) were analyzed to compare the number of *ST*s across different plant species.

*2.4 Chromosomal location, Collinearity and duplication event analyses*

The chromosomal locations of *ZmST* genes on chromosomes and chromosome synteny were performed by TBtools (Chen et al., 2020). Gene duplication analyses were conducted as previously described. The ratio of non-synonymous substitution rate (Ka) to synonymous substitution rate (Ks) was calculated by TBtools.

*2.5 Phylogenetic tree analysis of STs from different plants*

Amino acid sequences of STs from *Zea mays*, *Arabidopsis thaliana* and *Oryza sativa* were used to create a phylogenetic tree. The phylogenetic tree was constructed by MEGA 7.0 software

using neighbor-joining (NJ) phylogenetic method with 1000 bootstrap replications (Kumar et al., 2016).

*2.6 Gene structure, conserved motif and domain analyses*

The gene structure of *ZmST* genes was analyzed by TBtools software. The conserved motifs of ZmST proteins were analyzed with MEME (http://memesuite.org/tools/meme) (Bailey et al., 2009). The maximum number of predicted motifs was set to 15. The final graph was presented by TBtools.

*2.7 Expression heatmap of transcriptome*

RNA-Seq datasets from different tissues were acquired from maizeGDB to analyze the expression profiles of the *ZmST* genes (Stelpflug et al., 2015). Ten tissues from maize vegetative development to reproductive development stages were used to identify tissue specificity of *ZmST* genes. The expression data of *ST*s was visualized using the TBtools.

*2.8 RNA extraction and qRT-PC*

RNAs from B73 materials were extracted by RNAprep Pure Plant Kit (TIANGEN Biotech Co., Ltd) according to the manufacturer's instruction. About 1-2 μg of RNA was using to reverse transcribe with HiScript® III All-in-one RT SuperMixreverse kit reagents (Vazyme Biotech Co., Ltd). The qRT-PCR was performed with primers listed in Supplemental Table S1, with *ZmACTIN1* as an internal reference. qPCR was run on CFX96TM real-time system (Bio-Rad, Germany), with ChamQ Universal SYBR qPCR Master Mix (Vazyme Biotech Co., Ltd), as previously described (Fang et al., 2023). Finally, the calculation method for *ZmST* genes expression level was proposed by Livak and Schmittgen (Livak and Schmittgen, 2001).

*2.9 Cis-acting regulatory elements analysis in the ZmST gene promoters*

The promoter regions of *ZmSTs* were obtained with TBtools software for promoter analysis. The *cis*-acting regulatory elements were identified by PlantCARE (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) and presented with TBtools.

## 3. Results

*3.1 Sixty-eight ZmSTs are identified in maize genom*

In this study, a total of 68 amino acid sequence encoding putative ST proteins were identified. Their physicochemical properties, including gene ID, protein size, molecular weight (MW), isoelectric point (*p*I), the grand averages of hydropathicity (GRAVY), and localization prediction, were characterized (Table 1). The molecular weight of ZmST proteins ranged from 41.83 kDa (ZmSFP1) to 80.96 kDa (ZmTST3), while the isoelectric points ranged from 4.72 (ZmTST3) to 9.82 (ZmSTP12) (Table 1). The grand averages of hydropathicity for all ST proteins indicated their hydrophobic nature. Subcellular localization analysis revealed that all ZmST proteins were located in the cell membrane (Table 1, Table S2).

*3.2 ZmST proteins are divided into eight groups*

A Neighbor-Joining tree with 199 STs, including 68 ZmSTs from maize, 62 AtSTs from *Arabidopsis*, and 69 OsSTs from rice, was constructed (Fig. 1). The phylogenetic tree suggested that the sugar transporters in maize were classified into eight groups. Among them, the VGT clade consisted of ZmVGT1 and ZmVGT2, while the STP clade contained ZmSTP1 to ZmSTP20. Additionally, the PMT, SFP, SUT, INT, pGlcT and TST clades included 16, 11, 7, 4, 4, and 4 members, respectively, and were annotated as ZmPMT1 to ZmPMT16, ZmSFP1 to ZmSFP11, ZmSUT1 to ZmSUT7, ZmINT1 to ZmINT4, ZmpGlcT1 to ZmpGlcT4, ZmTST1 to ZmTST4 (Fig. 1). Furthermore, phylogenetic analysis showed that there were some closely related orthologous STs between maize and rice, implying the existence of a set of ancestral *ST* genes before the divergence of the two species (Fig. 1).

Additionally, a comparison of the numbers of different *ST* groups among maize, *Arabidopsis*, rice, tomato, pear, woodland strawberry, grape, longan, and apple was carried out (Table 2). The results revealed that STP and PMT were the largest clades in maize, consistent with previous findings in rice, pear, and apple. Conversely, in *Arabidopsis*, tomato, strawberry, grape, and longan, the largest clades were STP and SFP.

*3.3 Segmental duplication events are observed in ZmSTs*

To investigate features of the *ZmSTs* gene family, we analyzed the chromosome distribution of each *ZmST* gene. Our findings revealed that the *ZmST* genes were located on all 10 chromosomes of maize (Fig. 2A). Chromosome 1 had the highest number of *ZmST* genes, including *ZmPMT1*, *ZmPMT2*, *ZmPMT5*, *ZmPMT7*, *ZmSFP1*, *ZmSFP2*, *ZmSTP2*, *ZmSTP3*, *ZmSTP8*, *ZmSTP13*, *ZmTST1*, *ZmSUT1*, and *ZmSUT3*. Chromosome 2 contained *ZmPMT3*, *ZmPMT4*, *ZmPMT8*, *ZmPMT10*, *ZmSTP11*, *ZmSTP15*, and *ZmSTP20*. *ZmpGlcT1*, *ZmSFP3*, *ZmSTP10*, and *ZmSUT2* were located on chromosome 3. Chromosome 4 harbored *ZmpGlcT4*, *ZmPMT14*, *ZmPMT15*, *ZmPMT16*, *ZmSTP5*, *ZmSTP6*, *ZmSTP9*, *ZmTST4*, and *ZmSUT6*. Chromosome 5 contained *ZmSTP18*, *ZmTST2*, *ZmTST3*, *ZmVGT1*, *ZmVGT2*, *ZmSUT4*, and *ZmSUT5*. Chromosome 6 had *ZmSFP6*, *ZmSFP7*, and *ZmSFP8* genes, while chromosome 7 contained *ZmINT2*, *ZmINT3*, *ZmpGlcT3*, *ZmPMT6*, *ZmPMT9*, *ZmPMT11*, *ZmSTP1*, *ZmSTP4*, *ZmSTP14*, *ZmSTP16*, and *ZmSTP17*. Chromosome 8 harbored *ZmpGlcT2*, *ZmSFP4*, *ZmSFP5*, *ZmSFP9*, *ZmSFP10*, and *ZmSFP11*. Chromosome 9 contained *ZmSTP7*, *ZmSTP12*, and *ZmSU7*, while chromosome 10 harbored *ZmINT1*, *ZmINT4*, *ZmPMT12*, *ZmPMT13*, and *ZmSTP19* (Fig. 2A). Notably, all members of the *ZmVGT* group are located on chromosome 5, and four members of the *ZmINT* group are evenly distributed on chromosomes 7 and 10. Chromosome 6 only contained three *ZmSFP* members. Aside from these observations, the distribution of other *ZmST* genes on maize chromosomes was uneven.

Gene duplications are important for the expansion of gene families (Cannon et al., 2004; Konrad et al., 2011). Collinear analysis showed that 15 gene pairs, *ZmpGlcT1* and *ZmpGlcT2*, *ZmPMT7* and *ZmPMT9*, *ZmPMT7* and *ZmPMT10*, *ZmPMT8* and *ZmPMT9*, *ZmSFP3* and *ZmSFP4*, *ZmSFP6* and *ZmSFP9*, *ZmSTP5* and *ZmSTP18*, *ZmSTP5* and *ZmSTP19*, *ZmSTP5* and *ZmSTP20*, *ZmSTP18* and *ZmSTP19*, *ZmSTP18* and *ZmSTP20*, *ZmSTP19* and *ZmSTP20*, *ZmSUT1* and *ZmSUT3*, *ZmSUT1* and *ZmSUT7*, *ZmSUT3* and *ZmSUT7*, have undergone segmental duplication events (Fig. 2B). Further analysis revealed that all the Ka/Ks values of the

*ST* gene pairs were less than 1, indicating that the duplication events occurred under purifying selection (Fig. 2B, Table 3).

*3.4 ZmST gene structures are highly conserved*

The gene structures play crucial roles in the evolution and functional diversification of multiple gene families (Lei et al., 2020). The structural analysis of *ZmST*s indicated that all *ZmST* genes, except *ZmSTP6*, harbored at least one intron. Several genes, namely *ZmSFP1*, *ZmSFP2*, *ZmSFP3*, *ZmSFP4*, *ZmSFP5*, *ZmSFP6*, *ZmSFP7*, *ZmSFP8*, *ZmSFP9*, *ZmSFP10*, *ZmSFP11*, *ZmpGlcT1*, *ZmpGlcT2*, *ZmpGlcT3*, *ZmpGlcT4*, *ZmVGT1*, *ZmVGT2*, *ZmSUT1*, *ZmSUT4* and *ZmSUT7* contained more than ten introns. However, genes within the same group usually had a similar number of exons (Fig. 3A, B). *ZmSFP1* gene had 19 exons, whereas *ZmSTP6* had only one exon, which indicated that the exons gain and loss occurred during the evolution of *ZmST* gene family. The structures of *ZmSTs* in duplication pairs, such as *ZmpGlcT1* and *ZmpGlcT2*, *ZmPMT7* and *ZmPMT10*, *ZmSFP3* and *ZmSFP4*, *ZmSTP5* and *ZmSTP19*, *ZmSTP5* and *ZmSTP20*, *ZmSUT1* and *ZmSUT7*, were highly similar.

We used the MEME online tool to predict the potentially conserved motifs of 68 ZmSTs. Among the 15 distinct motifs identified, only motif 6 was present in all ZmST proteins (Fig. 3C, D, Table S3). Notably, significant differences were observed in the conserved motifs between the SUT subfamily and MST subfamilies, despite their similar function in sugar transport (Fig. 3C, D). Motifs 1, 2, 4 and 5 were present in all 61 MST proteins, but were absent in SUT proteins, while motifs 13 and 15 existed in all 7 SUT proteins but not in MST proteins. These findings indicated that motifs 1, 2, 4 and 5 may be critical for the function of MST subfamilies, while motifs 13 and 15 may be necessary for the function of SUT subfamily. This may be due to functional differences between MST subfamilies (monosaccharide transport) and SUT subfamily (sucrose transport). Although all MST proteins contained motifs 1, 2, 4 and 5, the specific types and numbers of motifs varied among each subfamily. Motif 3 was absent in VGT subfamily and ZmSTP3 protein. Motif 7 was not present in any members of the SFP subfamily but was

observed in the VGT subfamily. Motif 12 was absent only in ZmINT3 and ZmpGlcT4. Motif 14 was only observed in STP subfamily. Similar motif structures were observed in gene pairs such as *ZmpGlcT1* and *ZmpGlcT2*, *ZmPMT7* and *ZmPMT9*, *ZmPMT7* and *ZmPMT10*, *ZmPMT8* and *ZmPMT9*, *ZmSFP3* and *ZmSFP4*, *ZmSFP6* and *ZmSFP9*, *ZmSTP5* and *ZmSTP18*, *ZmSTP5* and *ZmSTP20*, *ZmSTP18* and *ZmSTP20*, as well as *ZmSUT1* and *ZmSUT7*.

*3.5 ZmST proteins were predicted to form a compact helix bundle*

In order to explore the potential roles of ZmST proteins, we predicted their conserved domains and 3D models of all ZmSTs with NCBI-CDD and Swiss-model, respectively. All ZmST proteins contained a conserved MFS domain, which facilitated the transportation of various substrates (including sugars, ions, nucleosides, amino acids and so on) through the cytoplasm or inner membrane, except SUT clade. This SUT clade comprises a conserved GPH_sucrose superfamily domain, which might export sucrose from photosynthetic sources to the phloem or import sucrose into sucrose sinks (Fig.4). Furthermore, 3D prediction demonstrated that all the ZmSTs were folded into 8-13 transmembrane domains, and then formed a compact helix bundle (Fig. 5, Fig. S1). However, it's worth noting that most members within the same group exhibited a similar 3D structure. For instance, the ZmTST subfamily members displayed a unique central loop, composed of approximately 320 amino acids, which connected to the predicted transmembrane domains, a feature absence in all other sugar transporters (Fig. 5).

*3.6 ZmST promoters contain the cis-acting elements for light, phytohormone, stress and development.*

The regulatory *cis*-elements are the binding sites for transcription factors, carrying information to regulate the gene expression in biological pathways. Thus, we extracted the promoter regions of 68 *ZmST* genes and examined their *cis*-acting elements using PlantCARE database 5.0. A total of sixty-six *cis*-elements were identified and categorized into four main groups: photoresponse, hormonal response, stress response and development. Among these *cis*-

elements, thirty were related to light responsive pathway, indicating their role in responding to light, which aligned with the function of sugar transporters in the distribution of photosynthetic products. Additionally, fourteen were associated with hormone response, eleven with abiotic and biotic stress response, and eleven with plant growth and development (Fig. 6). All promoters of *ZmSTs* had the same number of CGTCA-motif and TGACG-motif, and most *ZmSTs* were regulated by abscisic acid and methyl-jasmonate. *ZmSUT4* and *ZmSFP9* had seven and nine CCGTCC-boxes in their promoters, respectively, indicating their potential involvement in meristem-specific activation. The GCN4_motif was present in the promoters of *ZmINT2*, *ZmPMT8*, *ZmPMT16*, *ZmSFP2*, *ZmSFP6*, *ZmSFP8*, *ZmSTP4*, *ZmSTP6*, *ZmSTP9*, *ZmSTP17*, *ZmTST3*, *ZmSUT4* and *ZmSUT5*, suggesting their potential role in endosperm expression. The RY-element was found in the promoters of *ZmINT2*, *ZmPMT8*, *ZmPMT16*, *ZmSFP2*, *ZmSFP6*, *ZmSFP8*, *ZmSTP4*, *ZmSTP6*, *ZmSTP9*, *ZmSTP17*, *ZmTST3*, *ZmSUT5* and *ZmSUT7*, indicating their potential involvement in seed-specific regulation. The most abundant *cis*-elements in *ZmST* promoter regions were G-box, ABRE and STRE, implying that *ZmSTs* may participate in maize's growth, development and response to light, hormones and stress.

### *3.7 ZmSTs exhibit distinctive expression profiles across ten different tissues in maize*

We downloaded the transcriptome data for *ZmST* genes and generated an expression pattern map using data from ten different tissues: young leaves, mature leaves, old leaves, roots, stems, tassels, cobs, embryos, endosperms, and seeds. Our analysis revealed that nineteen *ZmST* genes (*ZmINT1*, *ZmpGlcT1*, *ZmpGlcT2*, *ZmpGlcT3*, *ZmpGlcT4*, *ZmPMT4*, *ZmPMT13*, *ZmSFP4*, *ZmSFP5*, *ZmSFP7*, *ZmSFP9*, *ZmSTP16*, *ZmTST1*, *ZmTST2*, *ZmTST4*, *ZmVGT1*, *ZmVGT2*, *ZmSUT2* and *ZmSUT4*) showed constitutive expression (log2 (FPKM+1) $\geq$ 1), Among these genes, *ZmpGlcT1*, *ZmpGlcT2*, *ZmpGlcT4*, *ZmSFP5*, *ZmTST1*, *ZmTST2*, *ZmTST4*, *ZmSUT2* and *ZmSUT4* showed the highest expression levels across all tested tissues and organs (log2(FPKM+1) $\geq$ 3) (Fig. 7). On the other hand, eleven *ZmST* genes (*ZmINT3*, *ZmPMT14*, *ZmPMT15*, *ZmSFP2*, *ZmSFP3*, *ZmSTP6*, *ZmSTP9*, *ZmSTP17*, *ZmSTP18*, *ZmSTP19* and

285 *ZmSUT3*) showed no expression across all tested tissues and organs (Fig. 7). Furthermore, we
286 observed that two *ZmST* genes (*ZmPMT1* and *ZmPMT7*), four genes (*ZmPMT3*, *ZmPMT6*,
287 *ZmSTP5*, and *ZmSTP14*), and four genes (*ZmSTP10*, *ZmSTP11*, *ZmTST3*, and *ZmSUT6*)
288 exhibited specific expression only in old leaves, roots, and tassels, respectively, with hardly
289 detected expression in other organs. Additionally, we found that *ZmSTP* and *ZmPMT* groups
290 displayed high expression in vegetative organs, whereas *ZmPMT* group members were scarcely
291 detected in developing seeds. However, *ZmTST* group members showed higher transcription
292 level in the developing seeds (Fig. 7).

293 *3.8 Most sugar transporter genes showed a notable upregulation during the early stage of grain*
294 *filling*

295 As shown in Fig. 7, the diverse expression patterns of *ZmST* genes during stages of embryo,
296 endosperm, and seed development suggested a significant role of *ZmSTs* in maize kernel
297 development. To explore the probable functions of *ZmST* genes, we randomly selected 24 *ZmST*
298 genes representing eight groups and executed qRT-PCR analysis across embryo and endosperm
299 developmental stages as well as seed maturity stages. *ZmpGlcT2*, *ZmSFP5*, *ZmSTP3*, *ZmTST1*,
300 *ZmTST2*, *ZmVGT1*, *ZmVGT2* and *ZmSUT2* showed up-regulation during embryo development
301 and down-regulation during endosperm development. Conversely, *ZmSTP7* were down-regulated
302 during embryo development but up-regulated during endosperm development. Some genes like
303 *ZmINT4*, *ZmPMT4*, *ZmPMT8*, *ZmSFP10*, *ZmSTP15* and *ZmSUT1* were down-regulated, while
304 *ZmSFP7* was up-regulated, during both embryo and endosperm development (Fig. 8). In
305 particular, the expression levels of most tested *ZmST* genes, such as *ZmINT4*, *ZmpGlcT4*,
306 *ZmPMT4*, *ZmPMT5*, *ZmPMT8*, *ZmPMT9*, *ZmPMT13*, *ZmSFP5*, *ZmSFP7*, *ZmSFP9*, *ZmSFP10*,
307 *ZmSTP7*, *ZmSTP15*, *ZmVGT1* and *ZmSUT2*, increased gradually in the early stage of grain
308 filling, reaching a peak at 8-12 days after pollination, and then declined gradually (Fig. 9A). As
309 supported by previous studies emphasizing the significance of the grain filling stage for seed
310 quality and yield (Ji et al., 2022), we conducted an investigation into the grain-filling rate of B73

over a period of four days from 4 DAP to 28 DAP. Our findings revealed a substantial increase in the grain-filling rate during the developmental stages of 8-12 DAP and 16-20 DAP, aligning with the observed expression pattern of seed maturation (Fig. 9B). These results underlined the crucial role of *ZmST* genes in the embryonic, endospermic, and seed development stages.

## 4. Discussion

The structures, phylogenetic relationship, and functional evolutions of sugar transporters have been extensively studied in various plant species (Julius et al., 2017; Zhu et al., 2021). However, knowledge on their possible roles and regulation processes among different classes of sugar transporters in maize is still limited. In this study, we identified 68 *ZmST* genes in the maize genome and analyzed their physicochemical properties (Table 1). As we know, there were sixty-two *ST* genes in *Arabidopsis* (Büttner, 2007), sixty-five *ST* genes in grape (Afoufa-Bastien et al., 2010), sixty-six *ST* genes in strawberry (Liu et al., 2020), sixty-nine *ST* genes in rice (Deng et al., 2019), seventy-three *ST* genes in apple (Wei et al., 2014), seventy-five *ST* genes in pear (Li et al., 2015). The number of *ST* genes in maize was similar to those found in other plant species, suggesting that the functions of *ST* genes were relatively conserved in functions and crucial for plant growth and development throughout different species. Therefore, *ST* gene families in plants didn't participate in gene expansion, maintaining the number of genes basically unchanged. The subcellular localization of sugar transporters is crucial to explore their potential functions in various biological processes. It is known that sugar transporters are typically localized in membrane system of cells, facilitating the transport of sugar from source leaves to sink tissues, such as developing seeds, providing essential carbon and energy sources for plant growth and development. The sugar content is higher in sink tissues, which requires the proton pumps on the membrane to counteract sugar concentration gradient. The AtSTs in *Arabidopsis* were primarily distributed in the cell membrane and tonoplast (Hedrich et al., 2015). Consistent with this, ZmST proteins were also mainly located on cell membrane (Table 1). Based on the phylogenetic relationships in maize, *Arabidopsis* and rice, ZmSTs were clustered into 8 groups (Fig. 1),

consistent with previous evolutionary classification of STs from other species. ZmSTs showed a closer relationship with ST proteins from rice, which also belonged to graminaceous crop. This result indicated that the functions of *ST* genes were relatively conserved, especially in graminaceous crops.

In this study, we identified that 68 *ZmST* genes were located on 10 chromosomes in the maize genome (Fig. 2A). In the *ZmST* gene family, we only observed 15 gene pairs with segmental duplication (Fig. 2B, Table 3). Therefore, segmental duplication was probably the predominant driver for the expansion of *ST* gene family in maize. Several duplicated gene pairs, such as *ZmpGlcT1* and *ZmpGlcT2*, *ZmPMT7* and *ZmPMT10*, *ZmSFP3* and *ZmSFP4*, *ZmSTP5* and *ZmSTP20*, *ZmSUT1* and *ZmSUT7*, exhibited similar exon-intron structures and conserved motifs, indicating a certain degree of functional redundancy (Fig. 3B-D). During the course of evolution, changes in gene structures may lead to variations in expression patterns and functions among *ZmST* genes.

Gene expression patterns can help us understand the biological functions of genes. In *Arabidopsis*, the gene *AtPLT5* was primarily expressed in sink tissues (Reinders et al., 2005), while in maize, a few members of *ZmPMT* group were expressed in seeds and vegetative organs like old leaves, roots and stems, indicating their involvement in sugar transport to specific sink tissues in plants. Neither *AtSTPs* in Arabidopsis nor *ZmSTPs* in maize were found in the female gametophyte or developing seeds (Büttner, 2010), suggesting that the *STP* family may not be involved in the seed maturation process. *AtERDL6* orthologs showed higher expression levels in fleshy fruits that accumulated a large amount of sugar, such as tomatoes (McCurdy et al., 2010), oranges (Zheng et al., 2014) and apples (Li et al., 2016). However, *ZmSFPs* in maize showed lower expression levels than fruits, indicating that the role of *SFPs* may not be prominent in crops. *AtSUC2* was expressed in source-leaf companion cells of phloem (Stadler and Sauer, 1996), while *OsSUT1* in rice played a role in sucrose transport during grain filling (Scofield et al., 2002). Similarly, certain *ZmSUTs* showed high expression levels in leaves and developing

seeds, implying conserved functions of *SUTs* across *Arabidopsis*, rice and maize. *AtTMT1* gene was strongly expressed in young developing tissues (Wormit et al., 2006), and *ZmTSTs* in maize exhibited higher expression levels in the young leaves, suggesting their involvement in sugar transport during rapid tissue expansion of cells in young tissues. The expression levels of *OsTMT1* and *OsTMT2* in rice were high in various organs excluding the endosperm (Cho et al., 2010), and similarly, *ZmTSTs* in maize were highly expressed in leaves, roots, stems, tassels, embryos and seeds, with lower expression in endosperm. Moreover, *ZmTSTs* showed higher expression levels in the early stage of grain filling compared to the late stage of grain-filling, indicating their importance in early seed development. *AtVGT1* was detected in all developmental stages and organs except roots in *Arabidopsis* (Aluri and Büttner, 2007), while *ZmVGT* genes were expressed in roots. Overall, most *ZmPMT*, *ZmSFP* and *ZmSTP* genes were predominantly expressed in vegetative organs and barely expressed in developing kernels, whereas, most *ZmpGlcT*, *ZmVGT* and *ZmTST* genes were highly expressed in seeds (Fig. 7, 8, 9). Notably, most tested *ZmST* genes exhibited an increase in expression during the early stage of grain filling and a subsequent decline during seed development in maize, suggesting their potential role in grain filling and seed maturation.

## 5. Conclusions

In summary, sixty-eight *ZmST* genes were identified and systematically analyzed in maize. Gene family analyses were conducted to investigate their physicochemical properties, chromosomal localizations, gene structures and biological functions in maize. The expression pattern analyses suggested that these *ZmST* genes may play a vital role in maize kernel development and could be potential candidates for improving maize yield. These significant findings will serve as valuable references for further research on *ZmSTs* and provide new genetic resources for the high-yield maize breed

## Acknowledgements

All the authors thank the raw data download from the public database. We thank all the

389 colleagues in our laboratory for providing useful discussions and technical assistance.

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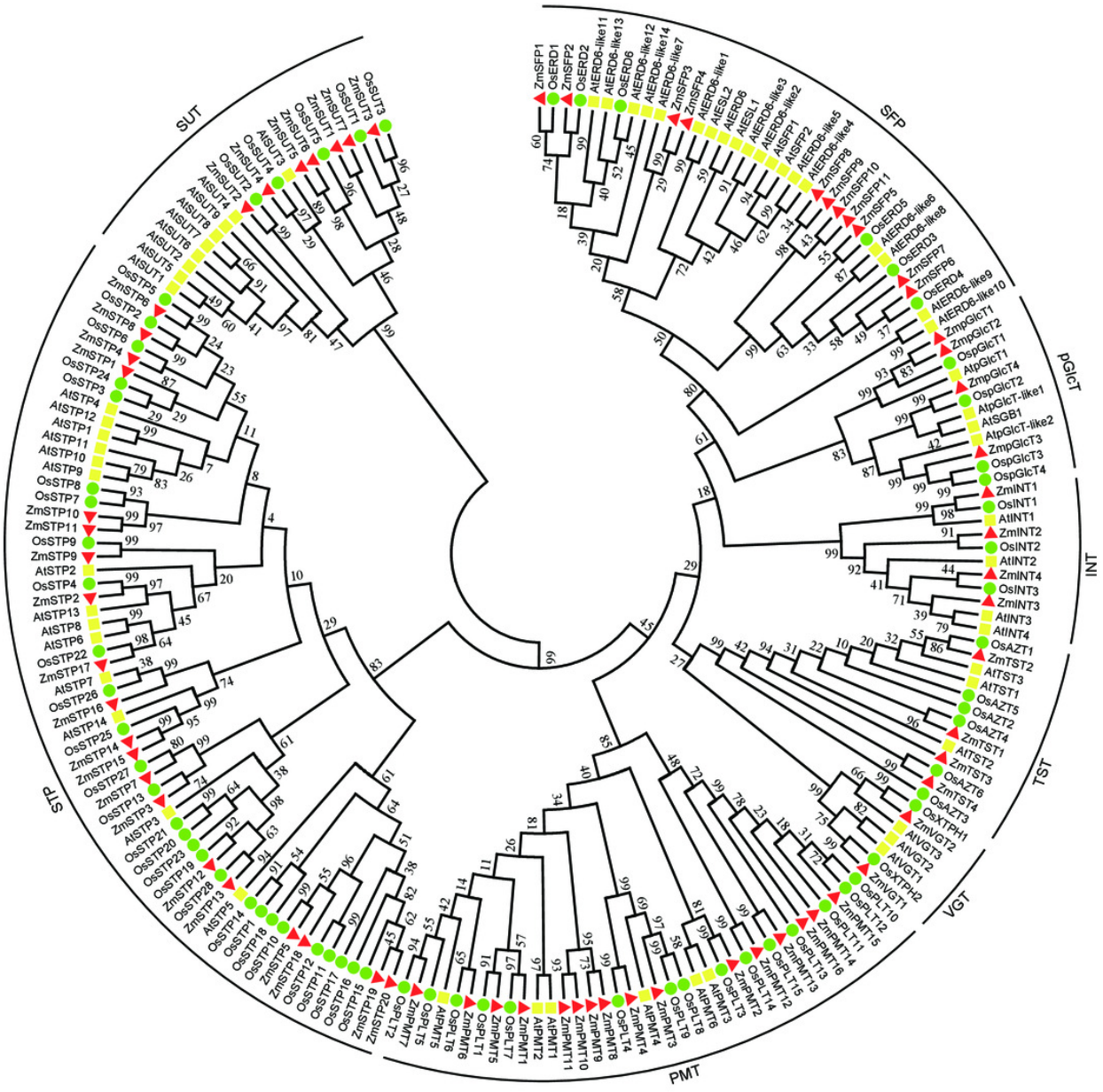
# Figure 1

Phylogenetic analysis of sugar transporters from *Z. mays*, *A. thaliana* and *O. sativa*.

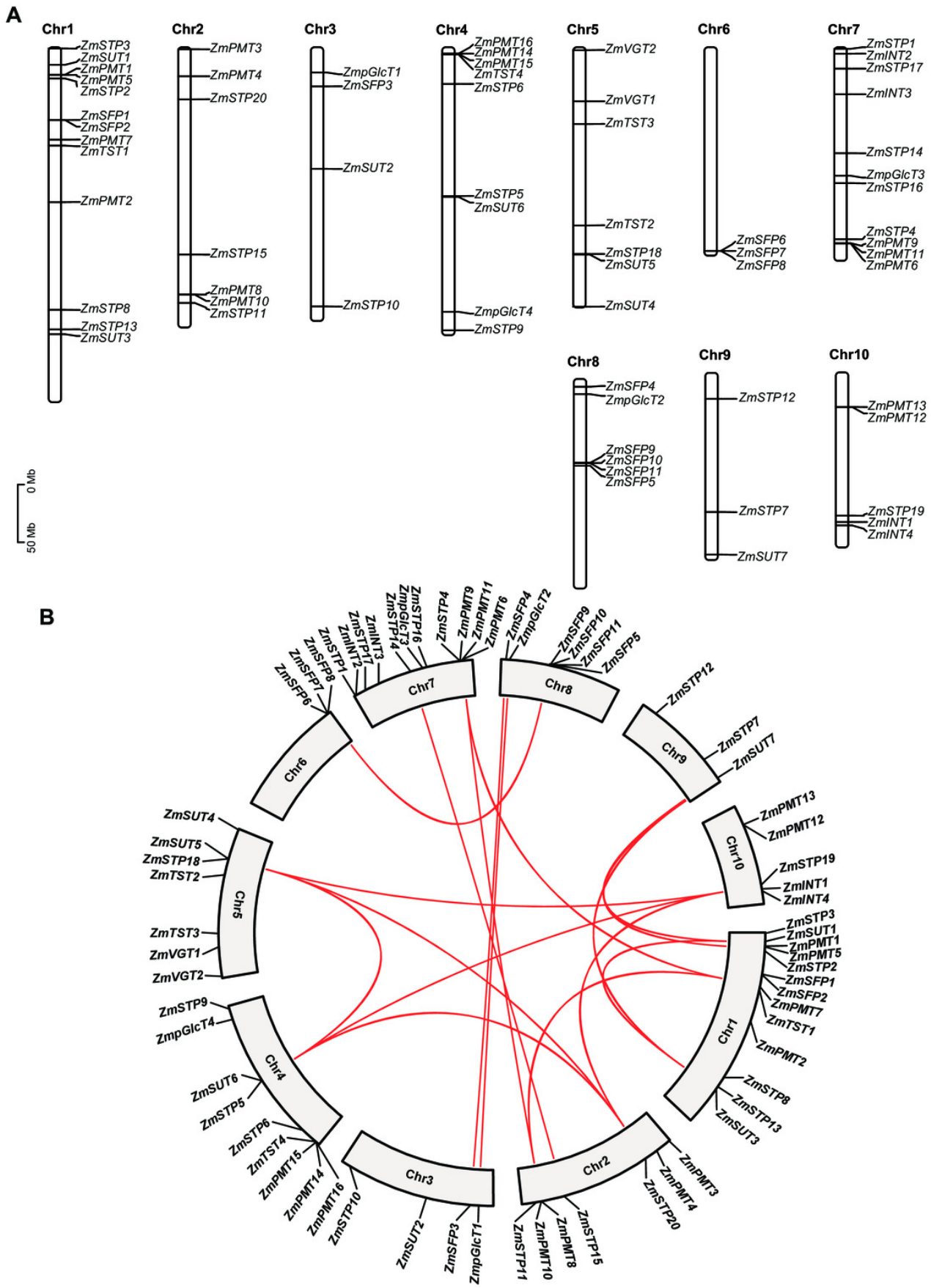
A total number of 68 ZmSTs from maize, 62 AtSTs from *Arabidopsis* and 69 OsSTs from rice were used to construct the phylogenetic tree by MEGA 7.0 using the neighbor-joining (NJ) method with 1000 bootstrap replications. All sugar transporter members were classified into eight groups (STP, PMT, SFP, SUT, TST, pGlcT, INT, VGT). The red triangle, yellow square and green circle signs represented *Z. mays*, *A. thaliana* and *O. sativa*, respectively.


SUT
SFP
pGlcT
INT
TST
VGT
PMT
STP

# Figure 2

Chromosomal distribution and collinearity analysis of *ZmST* genes.

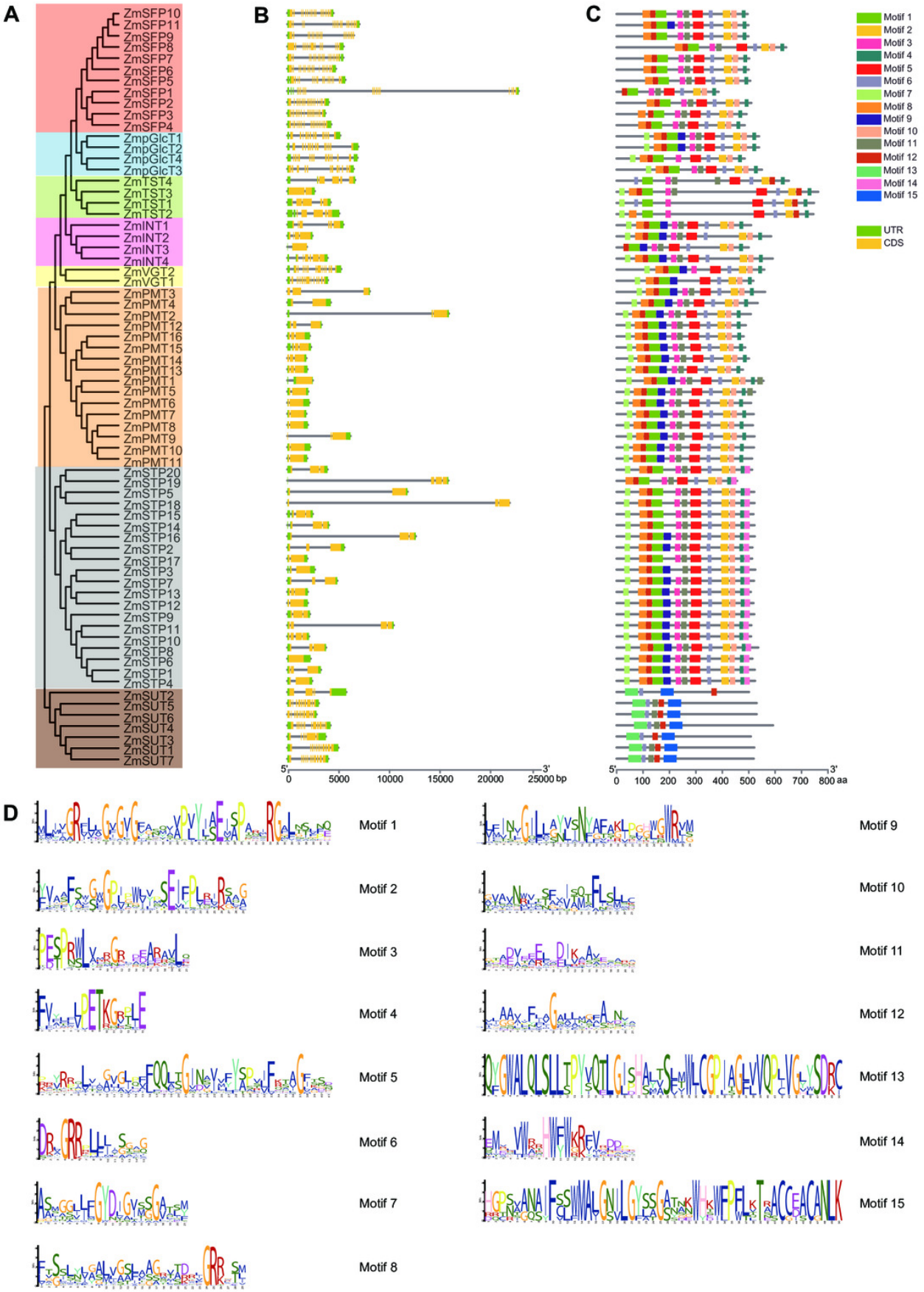
(**A**) Chromosome distribution of *ZmSTs* in maize genome using TBtools software. The chromosomal location of each *ZmST* gene was mapped according to the maize genome. The chromosome number is indicated at the top of each chromosome. (**B**) *ZmST* gene duplications analysis with TBtools. The syntenic *ZmST* gene pairs are connected by red lines.


A
Chr1
ZmSTP3
ZmSUT1
ZmPMT1
ZmPMT5
ZmSTP2
ZmSFP1
ZmSFP2
ZmPMT7
ZmTST1
ZmPMT2
ZmSTP8
ZmSTP13
ZmSUT3
Chr2
ZmPMT3
ZmPMT4
ZmSTP20
ZmSTP15
ZmPMT8
ZmPMT10
ZmSTP11
Chr3
ZmpGlcT1
ZmSFP3
ZmSUT2
ZmSTP10
Chr4
ZmPMT16
ZmPMT14
ZmPMT15
ZmTST4
ZmSTP6
ZmSTP5
ZmSUT6
ZmpGlcT4
ZmSTP9
Chr5
ZmVGT2
ZmVGT1
ZmTST3
ZmTST2
ZmSTP18
ZmSUT5
ZmSUT4
Chr6
ZmSFP6
ZmSFP7
ZmSFP8
Chr7
ZmSTP1
ZmINT2
ZmSTP17
ZmINT3
ZmSTP14
ZmpGlcT3
ZmSTP16
ZmSTP4
ZmPMT9
ZmPMT11
ZmPMT6
Chr8
ZmSFP4
ZmpGlcT2
ZmSFP9
ZmSFP10
ZmSFP11
ZmSFP5
Chr9
ZmSTP12
ZmSTP7
ZmSUT7
Chr10
ZmPMT13
ZmPMT12
ZmSTP19
ZmINT1
ZmINT4
0 Mb
50 Mb
B
Chr1
Chr2
Chr3
Chr4
Chr5
Chr6
Chr7
Chr8
Chr9
Chr10

# Figure 3

Exon-intron structure and conserved motifs of *ZmST* gene family.

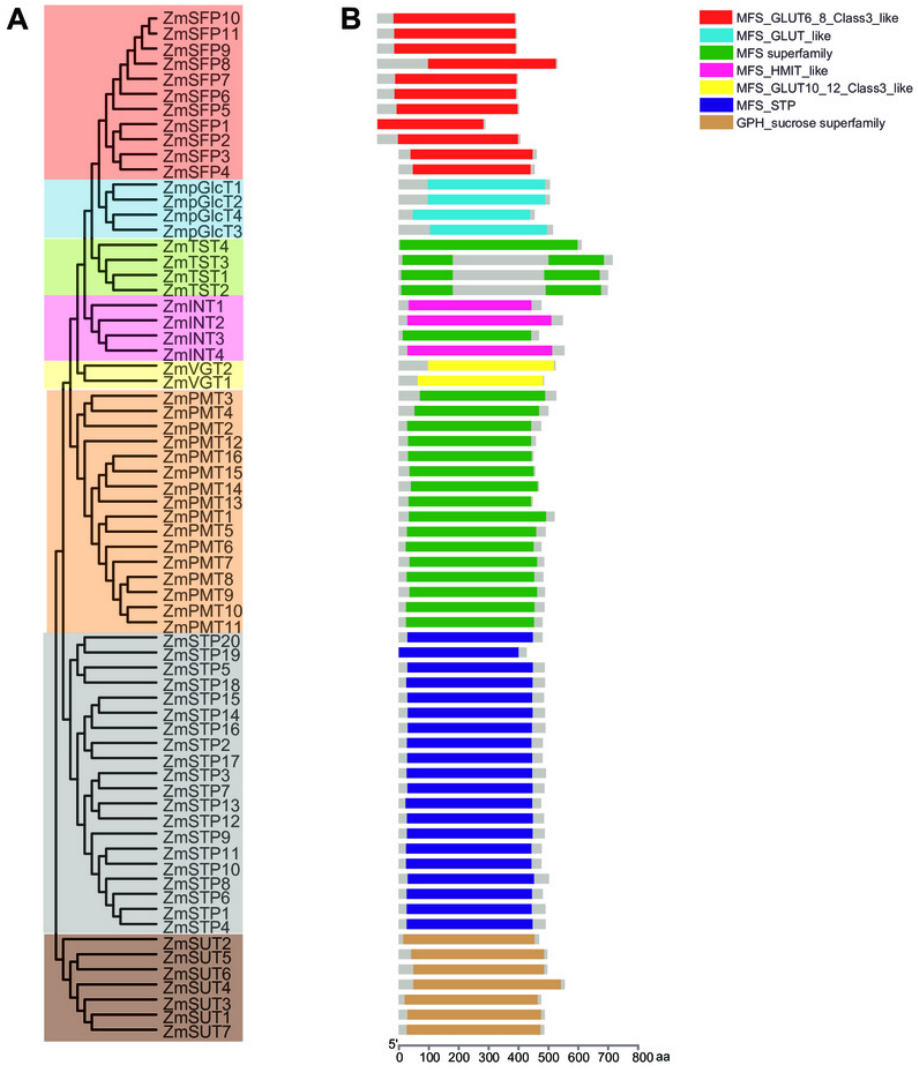
(**A**) Phylogenetic tree of 68 sugar transporters in maize. The phylogenetic tree was constructed by MEGA 7.0 with 1000 bootstrap replications. (**B**) Gene structure analysis of *ZmST* genes. Yellow blocks, black lines and green blocks represented exons, introns and untranslated regions, respectively. (**C**) The conserved motifs in ZmSTs. The different colored boxes represented different motifs. (**D**) Sequence logos for 15 conserved motifs were performed using MEME online tool. The x-axis represented the width of the motif and the y-axis represented the bits of each letter.


A
ZmSFP10
ZmSFP11
ZmSFP9
ZmSFP8
ZmSFP7
ZmSFP6
ZmSFP5
ZmSFP1
ZmSFP2
ZmSFP3
ZmSFP4
ZmpGlcT1
ZmpGlcT2
ZmpGlcT4
ZmpGlcT3
ZmTST4
ZmTST3
ZmTST1
ZmTST2
ZmINT1
ZmINT2
ZmINT3
ZmINT4
ZmVGT2
ZmVGT1
ZmPMT3
ZmPMT4
ZmPMT2
ZmPMT12
ZmPMT16
ZmPMT15
ZmPMT14
ZmPMT13
ZmPMT1
ZmPMT5
ZmPMT6
ZmPMT7
ZmPMT8
ZmPMT9
ZmPMT10
ZmPMT11
ZmSTP20
ZmSTP19
ZmSTP5
ZmSTP18
ZmSTP15
ZmSTP14
ZmSTP16
ZmSTP2
ZmSTP17
ZmSTP3
ZmSTP7
ZmSTP13
ZmSTP12
ZmSTP9
ZmSTP11
ZmSTP10
ZmSTP8
ZmSTP6
ZmSTP1
ZmSTP4
ZmSUT2
ZmSUT5
ZmSUT6
ZmSUT4
ZmSUT3
ZmSUT1
ZmSUT7
B
5'
0
5000
10000
15000
20000
25000 bp
3'
C
Motif 1
Motif 2
Motif 3
Motif 4
Motif 5
Motif 6
Motif 7
Motif 8
Motif 9
Motif 10
Motif 11
Motif 12
Motif 13
Motif 14
Motif 15
UTR
CDS
5'
0
100
200
300
400
500
600
700
800 aa
3'
D
Motif 1
Motif 2
Motif 3
Motif 4
Motif 5
Motif 6
Motif 7
Motif 8
Motif 9
Motif 10
Motif 11
Motif 12
Motif 13
Motif 14
Motif 15

# Figure 4

Analysis of the conserved domains in ZmST proteins.

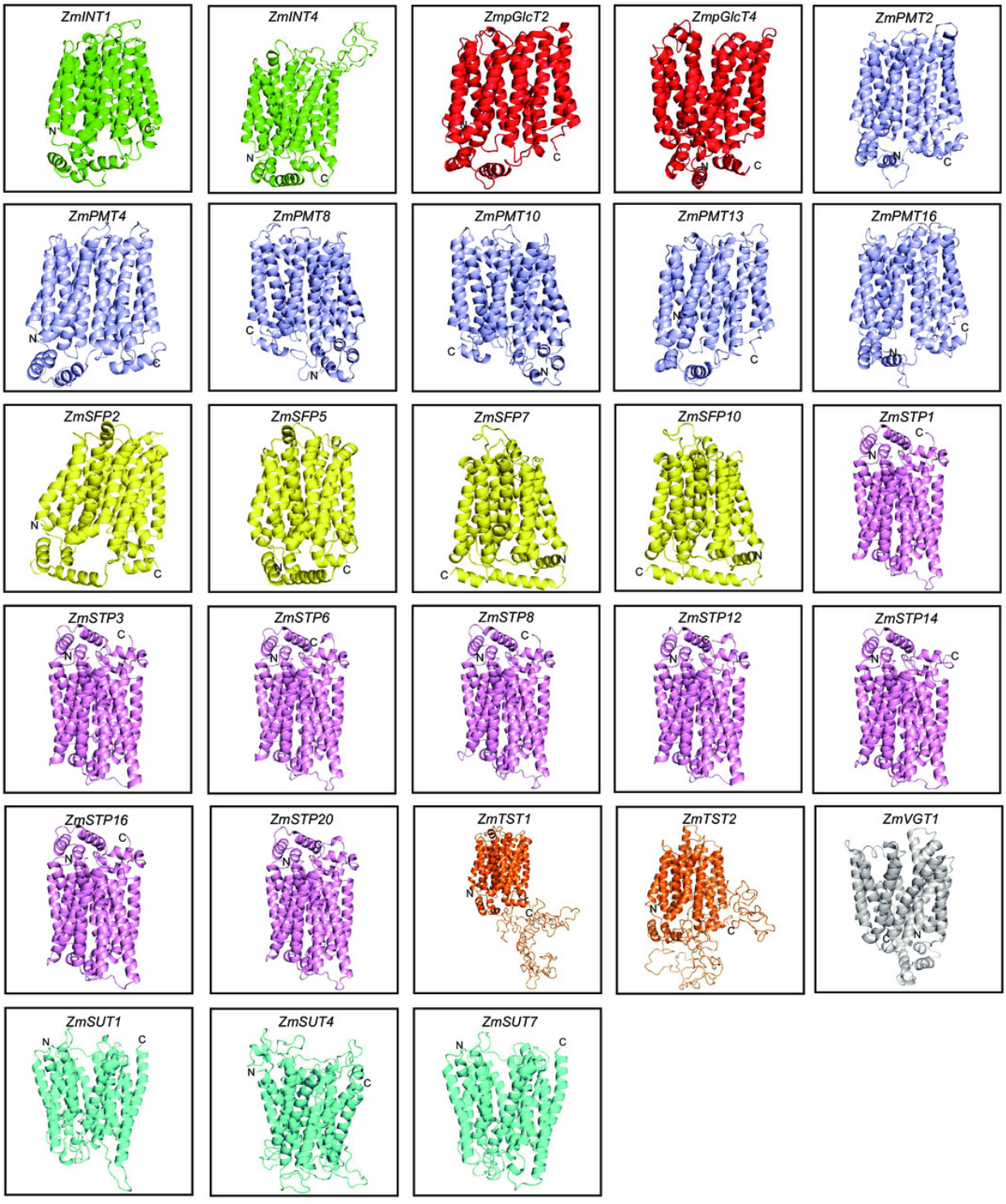
(**A**) Phylogenetic tree of 68 sugar transporters in maize. (**B**) The conserved domains in ZmSTs were identified with NCBI-CDD. The conserved domains were presented with different colors.


A
ZmSFP10
ZmSFP11
ZmSFP9
ZmSFP8
ZmSFP7
ZmSFP6
ZmSFP5
ZmSFP1
ZmSFP2
ZmSFP3
ZmSFP4
ZmpGlcT1
ZmpGlcT2
ZmpGlcT4
ZmpGlcT3
ZmTST4
ZmTST3
ZmTST1
ZmTST2
ZmINT1
ZmINT2
ZmINT3
ZmINT4
ZmVGT2
ZmVGT1
ZmPMT3
ZmPMT4
ZmPMT2
ZmPMT12
ZmPMT16
ZmPMT15
ZmPMT14
ZmPMT13
ZmPMT1
ZmPMT5
ZmPMT6
ZmPMT7
ZmPMT8
ZmPMT9
ZmPMT10
ZmPMT11
ZmSTP20
ZmSTP19
ZmSTP5
ZmSTP18
ZmSTP15
ZmSTP14
ZmSTP16
ZmSTP2
ZmSTP17
ZmSTP3
ZmSTP7
ZmSTP13
ZmSTP12
ZmSTP9
ZmSTP11
ZmSTP10
ZmSTP8
ZmSTP6
ZmSTP1
ZmSTP4
ZmSUT2
ZmSUT5
ZmSUT6
ZmSUT4
ZmSUT3
ZmSUT1
ZmSUT7
B
MFS_GLUT6_8_Class3_like
MFS_GLUT_like
MFS superfamily
MFS_HMIT_like
MFS_GLUT10_12_Class3_like
MFS_STP
GPH_sucrose superfamily
5'
0 100 200 300 400 500 600 700 800 aa

# Figure 5

Predicted 3D structures of ZmST proteins.

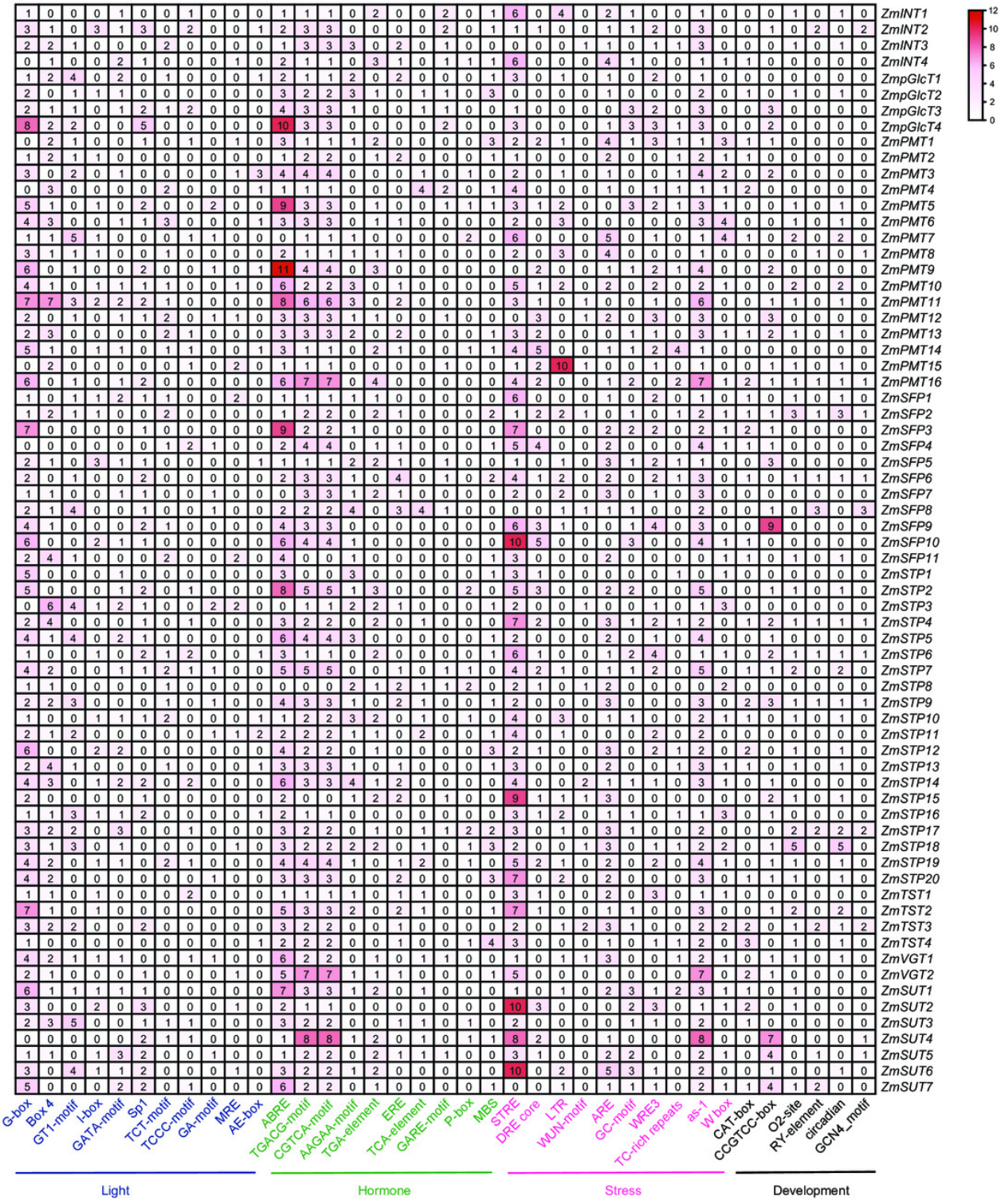
Different subfamilies were represented by different colors. All ZmSTs were folded into 8-13 transmembrane domains to form a compact helix bundle.


ZmINT1
ZmINT4
ZmpGlcT2
ZmpGlcT4
ZmPMT2
ZmPMT4
ZmPMT8
ZmPMT10
ZmPMT13
ZmPMT16
ZmSFP2
ZmSFP5
ZmSFP7
ZmSFP10
ZmSTP1
ZmSTP3
ZmSTP6
ZmSTP8
ZmSTP12
ZmSTP14
ZmSTP16
ZmSTP20
ZmTST1
ZmTST2
ZmVGT1
ZmSUT1
ZmSUT4
ZmSUT7

# Figure 6

The *cis*-acting elements in the promoters of *ZmST* gene family.

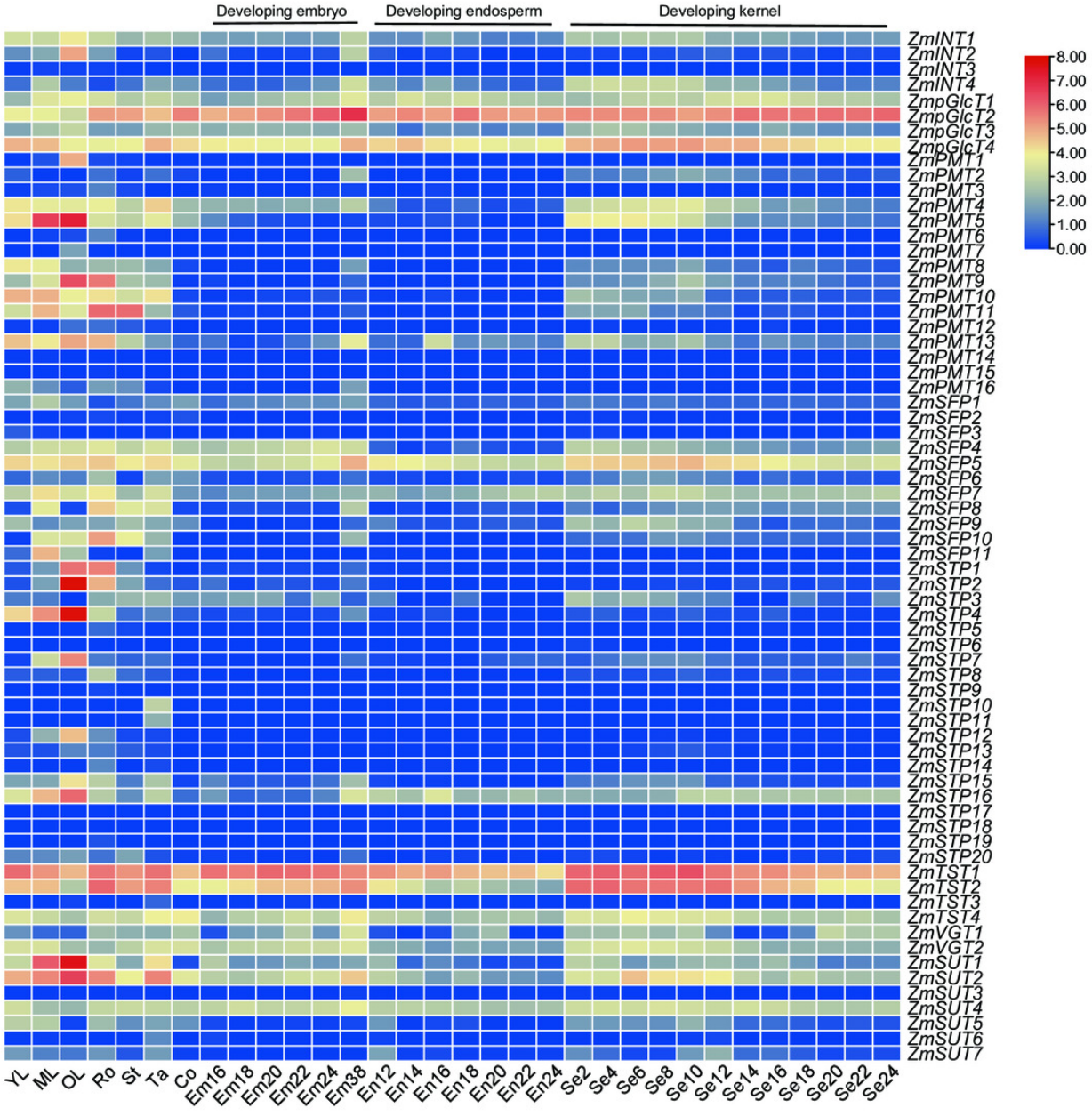
The names of 66 *cis*-elements were labeled at the bottom of the figure.


ZmINT1
ZmINT2
ZmINT3
ZmINT4
ZmpGlcT1
ZmpGlcT2
ZmpGlcT3
ZmpGlcT4
ZmPMT1
ZmPMT2
ZmPMT3
ZmPMT4
ZmPMT5
ZmPMT6
ZmPMT7
ZmPMT8
ZmPMT9
ZmPMT10
ZmPMT11
ZmPMT12
ZmPMT13
ZmPMT14
ZmPMT15
ZmPMT16
ZmSFP1
ZmSFP2
ZmSFP3
ZmSFP4
ZmSFP5
ZmSFP6
ZmSFP7
ZmSFP8
ZmSFP9
ZmSFP10
ZmSFP11
ZmSTP1
ZmSTP2
ZmSTP3
ZmSTP4
ZmSTP5
ZmSTP6
ZmSTP7
ZmSTP8
ZmSTP9
ZmSTP10
ZmSTP11
ZmSTP12
ZmSTP13
ZmSTP14
ZmSTP15
ZmSTP16
ZmSTP17
ZmSTP18
ZmSTP19
ZmSTP20
ZmTST1
ZmTST2
ZmTST3
ZmTST4
ZmVGT1
ZmVGT2
ZmSUT1
ZmSUT2
ZmSUT3
ZmSUT4
ZmSUT5
ZmSUT6
ZmSUT7
12
10
8
6
4
2
0
G-box
Box 4
GT1-motif
I-box
GATA-motif
Sp1
TCT-motif
TCCC-motif
GA-motif
MRE
AE-box
ABRE
TGACG-motif
CGTCA-motif
AAGAA-motif
TGA-element
ERE
TCA-element
GARE-motif
P-box
MBS
STRE
DRE core
LTR
WUN-motif
ARE
GC-motif
WRE3
TC-rich repeats
as-1
W box
CAT-box
CCGTCC-box
O2-site
RY-element
circadian
GCN4_motif
Light
Hormone
Stress
Development

# Figure 7

Expression patterns of *ZmSTs* in 10 tissues.

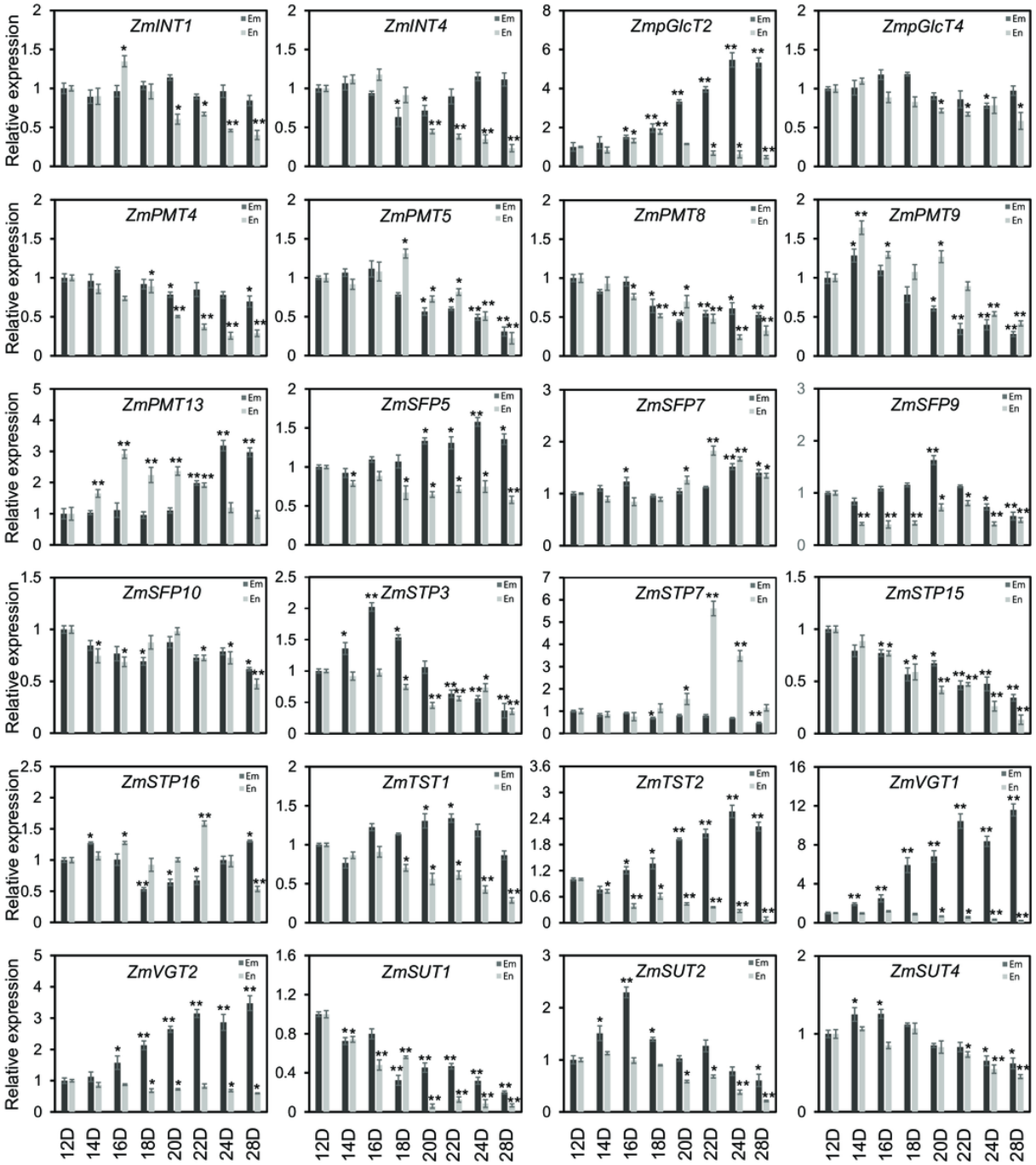
The genes were labeled on the right and the tissues were displayed at the bottom of each column. YL: young leaf when the ninth leaf is fully unfolded ; ML: mature leaf when the ninth leaf is fully unfolded ; OL: old leaf in blister stage; Ro: crown roots node5 when the seventh leaf is fully unfolded; St: stem when the third leaf is fully unfolded; Tassel: miotic tassel when the eighteenth leaf is fully unfolded ; Co: immature cob when the eighteenth leaf is fully unfolded; Em16, Em18, Em20, Em22, Em24, Em38: embryo of 16 DAP (days after pollination), 18 DAP, 20 DAP, 22 DAP, 24 DAP, 38 DAP, respectively; En12, En14, En16, En18, En20, En22, En 24: endosperm of 12 DAP, 14 DAP, 16 DAP,18 DAP, 20 DAP, 22 DAP, 24 DAP, respectively; Se2, Se4, Se6, Se8, Se10, Se12, Se14, Se16, Se18, Se20, Se22, Se24: whole seed of 2 DAP, 4 DAP, 6 DAP, 8 DAP, 10 DAP, 12 DAP, 14 DAP, 16 DAP, 18 DAP, 20 DAP, 22 DAP, 24 DAP, respectively.


Developing embryo
Developing endosperm
Developing kernel
ZmINT1
ZmINT2
ZmINT3
ZmINT4
ZmpGlcT1
ZmpGlcT2
ZmpGlcT3
ZmpGlcT4
ZmPMT1
ZmPMT2
ZmPMT3
ZmPMT4
ZmPMT5
ZmPMT6
ZmPMT7
ZmPMT8
ZmPMT9
ZmPMT10
ZmPMT11
ZmPMT12
ZmPMT13
ZmPMT14
ZmPMT15
ZmPMT16
ZmSFP1
ZmSFP2
ZmSFP3
ZmSFP4
ZmSFP5
ZmSFP6
ZmSFP7
ZmSFP8
ZmSFP9
ZmSFP10
ZmSFP11
ZmSTP1
ZmSTP2
ZmSTP3
ZmSTP4
ZmSTP5
ZmSTP6
ZmSTP7
ZmSTP8
ZmSTP9
ZmSTP10
ZmSTP11
ZmSTP12
ZmSTP13
ZmSTP14
ZmSTP15
ZmSTP16
ZmSTP17
ZmSTP18
ZmSTP19
ZmSTP20
ZmTST1
ZmTST2
ZmTST3
ZmTST4
ZmVGT1
ZmVGT2
ZmSUT1
ZmSUT2
ZmSUT3
ZmSUT4
ZmSUT5
ZmSUT6
ZmSUT7
YL ML OL Ro St Ta Co Em16 Em18 Em20 Em22 Em24 Em38 En12 En14 En16 En18 En20 En22 En24 Se2 Se4 Se6 Se8 Se10 Se12 Se14 Se16 Se18 Se20 Se22 Se24
8.00
7.00
6.00
5.00
4.00
3.00
2.00
1.00
0.00

# Figure 8

The expression profiles of the *ZmST* genes in embryo and endosperm.

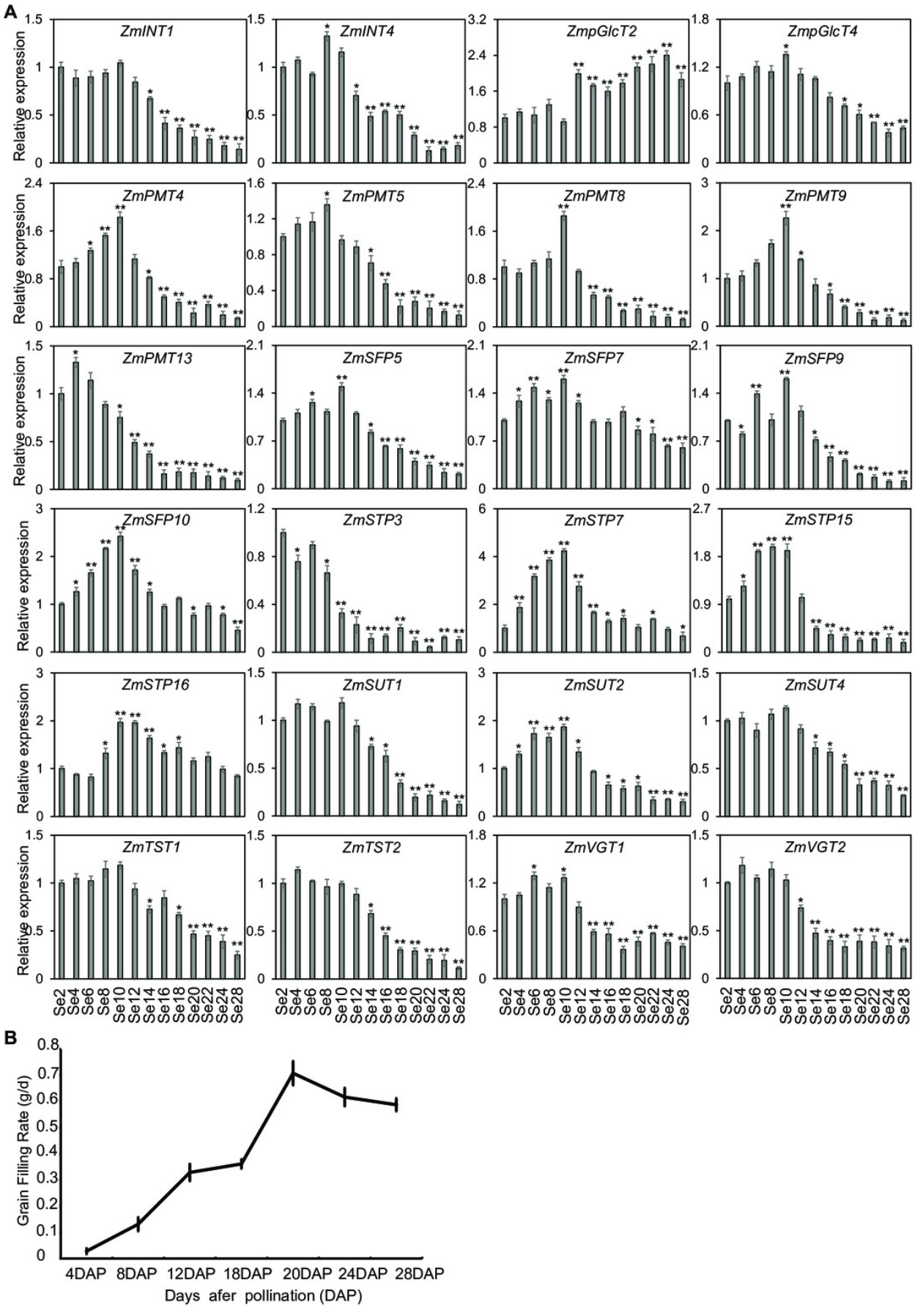
The qRT-PCR analysis was used to analyze the expression of selected *ZmST* genes in embryo (Em, shown in dark gray) and endosperm (En, shown in light gray). The names of the genes were labeled at the top of each diagram. 12D, 14D, 16D, 18D, 20D, 22D, 24D, 28D: embryo and endosperm of 12 DAP, 14 DAP, 16 DAP, 18 DAP, 20 DAP, 22 DAP, 24 DAP, 28 DAP, respectively. DAP: days after pollination. Columns were the mean of three independent replicates, and error bars represented SD. * and ** indicated significant differences with $P<0.05$ and $P<0.01$, respectively.


ZmINT1
ZmINT4
ZmpGlcT2
ZmpGlcT4
ZmPMT4
ZmPMT5
ZmPMT8
ZmPMT9
ZmPMT13
ZmSFP5
ZmSFP7
ZmSFP9
ZmSFP10
ZmSTP3
ZmSTP7
ZmSTP15
ZmSTP16
ZmTST1
ZmTST2
ZmVGT1
ZmVGT2
ZmSUT1
ZmSUT2
ZmSUT4
Em
En
Relative expression
12D
14D
16D
18D
20D
22D
24D
28D

# Figure 9

Expression profiles and grain-filling rate of the *ZmST* genes in seed.

(A) The qRT-PCR was used to analyze the expression levels of selected *ZmST* genes in seed (Se). The names of the genes were labeled at the top of each diagram. Se2, Se4, Se6, Se8, Se10, Se12, Se14, Se16, Se18, Se20, Se22, Se24, Se28: seed of 2 DAP, 4 DAP, 6 DAP, 8 DAP, 10 DAP, 12 DAP, 14 DAP, 16 DAP, 18 DAP, 20 DAP, 22 DAP, 24 DAP, 28 DAP, respectively. DAP: days after pollination. Columns were the mean of three independent replicates, and error bars represented SD. * and ** indicated significant differences with $P<0.05$ and $P<0.01$, respectively. (B) The grain-filling rate calculated by the weight increase of hundred-grain weight was measured from 4 to 28 DAP every 4 days.


A
ZmINT1
ZmINT4
ZmpGlcT2
ZmpGlcT4
ZmPMT4
ZmPMT5
ZmPMT8
ZmPMT9
ZmPMT13
ZmSFP5
ZmSFP7
ZmSFP9
ZmSFP10
ZmSTP3
ZmSTP7
ZmSTP15
ZmSTP16
ZmSUT1
ZmSUT2
ZmSUT4
ZmTST1
ZmTST2
ZmVGT1
ZmVGT2
Relative expression
Se2 Se4 Se6 Se8 Se10 Se12 Se14 Se16 Se18 Se20 Se22 Se24 Se28
B
Grain Filling Rate (g/d)
4DAP 8DAP 12DAP 18DAP 20DAP 24DAP 28DAP
Days afer pollination (DAP)

## Table 1(on next page)

Physicochemical characteristics of 68 ST protein

| Gene name | Gene ID | Protein Size (aa) | (KDa) | | GR | Localization Prediction |
|---|---|---|---|---|---|---|
| *ZmINT1* | Zm00001eb425560 | 509 | 53.92 | 5.31 | 0.609 | Cell membrane |
| *ZmINT2* | Zm00001eb300060 | 585 | 62.44 | 8.82 | 0.361 | Cell membrane |
| *ZmINT3* | Zm00001eb306230 | 500 | 53.18 | 9.06 | 0.482 | Cell membrane |
| *ZmINT4* | Zm00001eb426370 | 591 | 63.84 | 8.67 | 0.364 | Cell membrane |
| *ZmpGlcT1* | Zm00001eb125210 | 539 | 56.81 | 9.22 | 0.567 | Cell membrane |
| *ZmpGlcT2* | Zm00001eb335350 | 539 | 56.59 | 9.08 | 0.585 | Cell membrane |
| *ZmpGlcT3* | Zm00001eb311910 | 550 | 58.08 | 6.27 | 0.466 | Cell membrane |
| *ZmpGlcT4* | Zm00001eb203690 | 485 | 52.35 | 8.59 | 0.535 | Cell membrane |
| *ZmPMT1* | Zm00001eb008070 | 556 | 59.39 | 8.13 | 0.335 | Cell membrane |
| *ZmPMT2* | Zm00001eb027550 | 508 | 54.03 | 9.22 | 0.541 | Cell membrane |
| *ZmPMT3* | Zm00001eb066030 | 562 | 58.31 | 7.14 | 0.579 | Cell membrane |
| *ZmPMT4* | Zm00001eb075840 | 534 | 58.04 | 6.01 | 0.431 | Cell membrane |
| *ZmPMT5* | Zm00001eb008080 | 524 | 56.15 | 9.08 | 0.444 | Cell membrane |
| *ZmPMT6* | Zm00001eb325680 | 509 | 54 | 8.88 | 0.617 | Cell membrane |
| *ZmPMT7* | Zm00001eb021140 | 519 | 55.3 | 9.53 | 0.558 | Cell membrane |
| *ZmPMT8* | Zm00001eb107810 | 516 | 54.37 | 9.16 | 0.621 | Cell membrane |
| *ZmPMT9* | Zm00001eb325640 | 522 | 55.59 | 8.9 | 0.61 | Cell membrane |
| *ZmPMT10* | Zm00001eb107870 | 520 | 55.08 | 9.16 | 0.589 | Cell membrane |
| *ZmPMT11* | Zm00001eb325650 | 513 | 54.26 | 9 | 0.612 | Cell membrane |
| *ZmPMT12* | Zm00001eb411020 | 489 | 50.58 | 8.7 | 0.599 | Cell membrane |
| *ZmPMT13* | Zm00001eb411000 | 478 | 50.16 | 8.75 | 0.742 | Cell membrane |
| *ZmPMT14* | Zm00001eb166230 | 501 | 52.14 | 9.25 | 0.633 | Cell membrane |
| *ZmPMT15* | Zm00001eb166250 | 487 | 50.94 | 8.89 | 0.658 | Cell membrane |
| *ZmPMT16* | Zm00001eb166210 | 481 | 50.2 | 8.79 | 0.73 | Cell membrane |
| *ZmSFP1* | Zm00001eb017730 | 386 | 41.83 | 9.03 | 0.69 | Cell membrane |

| | | | | | | |
|---|---|---|---|---|---|---|
| *ZmSFP2* | Zm00001eb017760 | 510 | 54.33 | 6.91 | 0.547 | Cell membrane |
| *ZmSFP3* | Zm00001eb127290 | 492 | 52.06 | 8.33 | 0.644 | Cell membrane |
| *ZmSFP4* | Zm00001eb333940 | 485 | 51.43 | 5.67 | 0.634 | Cell membrane |
| *ZmSFP5* | Zm00001eb344570 | 506 | 54.09 | 9.23 | 0.635 | Cell membrane |
| *ZmSFP6* | Zm00001eb296190 | 500 | 53.74 | 8.54 | 0.552 | Cell membrane |
| *ZmSFP7* | Zm00001eb296200 | 502 | 54.16 | 8.31 | 0.58 | Cell membrane |
| *ZmSFP8* | Zm00001eb296220 | 642 | 68.5 | 9.25 | 0.476 | Cell membrane |
| *ZmSFP9* | Zm00001eb344010 | 499 | 53.28 | 8.49 | 0.58 | Cell membrane |
| *ZmSFP10* | Zm00001eb344020 | 496 | 52.62 | 9.08 | 0.632 | Cell membrane |
| *ZmSFP11* | Zm00001eb344040 | 499 | 52.92 | 9.24 | 0.613 | Cell membrane |
| *ZmSTP1* | Zm00001eb298310 | 523 | 57.04 | 9.38 | 0.467 | Cell membrane |
| *ZmSTP2* | Zm00001eb008810 | 514 | 56.69 | 9.05 | 0.507 | Cell membrane |
| *ZmSTP3* | Zm00001eb000240 | 525 | 57.5 | 9.21 | 0.515 | Cell membrane |
| *ZmSTP4* | Zm00001eb324180 | 524 | 56.91 | 9.13 | 0.606 | Cell membrane |
| *ZmSTP5* | Zm00001eb182870 | 521 | 56.35 | 9.21 | 0.609 | Cell membrane |
| *ZmSTP6* | Zm00001eb171880 | 514 | 55.43 | 7.54 | 0.647 | Cell membrane |
| *ZmSTP7* | Zm00001eb391140 | 520 | 56.83 | 9.37 | 0.503 | Cell membrane |
| *ZmSTP8* | Zm00001eb043150 | 536 | 56.85 | 9.35 | 0.601 | Cell membrane |
| *ZmSTP9* | Zm00001eb207680 | 521 | 56.91 | 9.1 | 0.603 | Cell membrane |
| *ZmSTP10* | Zm00001eb159990 | 509 | 55.65 | 9.54 | 0.672 | Cell membrane |
| *ZmSTP11* | Zm00001eb110690 | 510 | 55.64 | 9.43 | 0.686 | Cell membrane |
| *ZmSTP12* | Zm00001eb377440 | 518 | 54.52 | 9.82 | 0.581 | Cell membrane |
| *ZmSTP13* | Zm00001eb047630 | 508 | 54.32 | 9.14 | 0.554 | Cell membrane |
| *ZmSTP14* | Zm00001eb309780 | 522 | 57.27 | 9.41 | 0.47 | Cell membrane |
| *ZmSTP15* | Zm00001eb098100 | 518 | 56.63 | 9.16 | 0.527 | Cell membrane |
| *ZmSTP16* | Zm00001eb312640 | 523 | 56.2 | 9.34 | 0.557 | Cell membrane |
| *ZmSTP17* | Zm00001eb303000 | 513 | 56.14 | 9.04 | 0.483 | Cell membrane |

| | | | | | | |
|---|---|---|---|---|---|---|
| *ZmSTP18* | Zm00001eb244790 | 522 | 55.82 | 9.58 | 0.58 | Cell membrane |
| *ZmSTP19* | Zm00001eb423910 | 456 | 49.86 | 9.71 | 0.627 | Cell membrane |
| *ZmSTP20* | Zm00001eb081130 | 513 | 54.77 | 9.12 | 0.609 | Cell membrane |
| *ZmTST1* | Zm00001eb022230 | 747 | 79.55 | 4.85 | 0.401 | Cell membrane |
| *ZmTST2* | Zm00001eb239520 | 745 | 79.82 | 5.26 | 0.397 | Cell membrane |
| *ZmTST3* | Zm00001eb228740 | 763 | 80.96 | 4.72 | 0.316 | Cell membrane |
| *ZmTST4* | Zm00001eb166700 | 652 | 71.7 | 5.64 | 0.354 | Cell membrane |
| *ZmVGT1* | Zm00001eb225000 | 518 | 55.46 | 5.72 | 0.598 | Cell membrane |
| *ZmVGT2* | Zm00001eb211520 | 559 | 58.71 | 9.6 | 0.624 | Cell membrane |
| *ZmSUT1* | Zm00001eb005460 | 521 | 55.17 | 8.58 | 0.608 | Cell membrane |
| *ZmSUT2* | Zm00001eb133930 | 501 | 53.37 | 8.84 | 0.486 | Cell membrane |
| *ZmSUT3* | Zm00001eb048470 | 508 | 53.52 | 7.46 | 0.584 | Cell membrane |
| *ZmSUT4* | Zm00001eb259340 | 592 | 63.14 | 6.63 | 0.323 | Cell membrane |
| *ZmSUT5* | Zm00001eb244930 | 530 | 56.2 | 8.63 | 0.494 | Cell membrane |
| *ZmSUT6* | Zm00001eb183000 | 530 | 55.94 | 8.7 | 0.554 | Cell membrane |
| *ZmSUT7* | Zm00001eb402200 | 519 | 55.09 | 8.68 | 0.609 | Cell membrane |

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## Table 2(on next page)

Comparative analysis the gene numbers of different *ST* families in maize, *Arabidopsis*, rice, tomato, pear, strawberry, grape, longan and apple.

| | Number of Genes | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **Subfamily** | **Maize** | ***Arabidopsis*** | **Rice** | **Tomato** | **Pear** | **Strawberry** | **Grape** | **Longan** | **Apple** |
| STP | 20 | 14 | 28 | 18 | 20 | 24 | 22 | 20 | 30 |
| PMT | 16 | 6 | 15 | 8 | 23 | 7 | 5 | 6 | 10 |
| SFP | 11 | 19 | 6 | 10 | 5 | 16 | 22 | 10 | 8 |
| SUT | 7 | 9 | 5 | 3 | 6 | 8 | 4 | 6 | 9 |
| INT | 4 | 4 | 3 | 4 | 6 | 3 | 3 | 4 | 4 |
| pGlcT | 4 | 4 | 4 | 4 | 6 | 3 | 4 | 3 | 4 |
| TST | 4 | 3 | 6 | 3 | 6 | 3 | 3 | 1 | 5 |
| VGT | 2 | 3 | 2 | 2 | 3 | 2 | 2 | 2 | 3 |
| Total | 68 | 62 | 69 | 52 | 75 | 66 | 65 | 52 | 73 |

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## Table 3(on next page)

The Ka/Ks for the duplication gene pairs in *ZmST* family.

| Duplicated pair | Duplicate type | Ka | Ks | Ka/Ks | Positive selection |
|---|---|---|---|---|---|
| *ZmpGlcT1/ ZmpGlcT2* | Segmental | 0.02302462 | 0.17639281 | 0.13053037 | No |
| *ZmPMT7/ ZmPMT9* | Segmental | 0.1887097 | 0.73309884 | 0.25741372 | No |
| *ZmPMT7/ ZmPMT10* | Segmental | 0.18220538 | 0.59309584 | 0.30721068 | No |
| *ZmPMT8/ ZmPMT9* | Segmental | 0.05922607 | 0.19262997 | 0.30746033 | No |
| *ZmSFP3/ ZmSFP4* | Segmental | 0.12261777 | 0.72591107 | 0.16891569 | No |
| *ZmSFP6/ ZmSFP9* | Segmental | 0.14390645 | 0.54307605 | 0.26498398 | No |
| *ZmSTP5/ ZmSTP18* | Segmental | 0.21542769 | 0.59259227 | 0.36353442 | No |
| *ZmSTP5/ ZmSTP19* | Segmental | 0.35185515 | 0.66335247 | 0.5304196 | No |
| *ZmSTP5/ ZmSTP20* | Segmental | 0.37342149 | 0.55823017 | 0.66893821 | No |
| *ZmSTP18/ ZmSTP19* | Segmental | 0.37160006 | 0.71051129 | 0.52300373 | No |
| *ZmSTP18/ ZmSTP20* | Segmental | 0.35668412 | 0.69017173 | 0.51680489 | No |
| *ZmSTP19/ ZmSTP20* | Segmental | 0.1612672 | 0.44168081 | 0.3651216 | No |
| *ZmSUT3/ ZmSUT7* | Segmental | 0.22699235 | 0.76180805 | 0.29796528 | No |

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