frontiers in Ecology and Evolution

ORIGINAL RESEARCH
published: 15 February 2021
doi: 10.3389/fevo.2021.537398

Comprehensive Analysis of Evolutionary Characterization and Expression for Monosaccharide Transporter Family Genes in *Nelumbo nucifera*

Peng Wu[1†], Yongyan Zhang[1†], Shuping Zhao[1†] and Liangjun Li[1,2]*

[1] School of Horticulture and Plant Protection, Yangzhou University, Yangzhou, China, [2] Joint International Research Laboratory of Agriculture and Agri-Product Safety of Ministry of Education of China, Yangzhou University, Yangzhou, China

Sugar transporters, an important class of transporters for sugar function, regulate many processes associated with growth, maturation, and senescence processes in plants. In this study, a total of 35 NuMSTs were identified in the *Nelumbo nucifera* genome and grouped by conserved domains and phylogenetic analysis. Additionally, we identified 316 MST genes in 10 other representative plants and performed a comparative analysis with *Nelumbo nucifera* genes, including evolutionary trajectory, gene duplication, and expression pattern. A large number of analyses across plants and algae indicated that the MST family could have originated from *STP* and *Glct*, expanding to form *STP* and *SFP* by dispersed duplication. Finally, a quantitative real-time polymerase chain reaction and cis-element analysis showed that some of them may be regulated by plant hormones (e.g., abscisic acid), biotic stress factors, and abiotic factors (e.g., drought, excessive cold, and light). We found that under the four abiotic stress conditions, only *NuSTP5* expression was upregulated, generating a stress response, and ARBE and LTR were present in *NuSTP5*. In summary, our findings are significant for understanding and exploring the molecular evolution and mechanisms of NuMSTs in plants.

Keywords: gene rentain, conserved synteny, evolutionary model, gene expression pattern analysis, abiotic stresses

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***Correspondence:**
Liangjun Li
ljli@yzu.edu.cn

[†] These authors have contributed equally to this work

Specialty section:
This article was submitted to Phylogenetics, Phylogenomics, and Systematics, a section of the journal Frontiers in Ecology and Evolution

Received: *02 November 2020*
Accepted: *18 January 2021*
Published: *15 February 2021*

Citation:
Wu P, Zhang Y, Zhao S and Li L (2021) Comprehensive Analysis of Evolutionary Characterization and Expression for Monosaccharide Transporter Family Genes in Nelumbo nucifera. Front. Ecol. Evol. 9:537398. doi: 10.3389/fevo.2021.537398

INTRODUCTION

Sugars (sucrose, monosaccharides, and polyols), as the main product of photosynthesis, occupy an important place in plant growth and development. Sugars constitute not only osmotic and signaling molecules but also metabolites and nutrients. The movement of sugars on the whole plant occurs in two different ways (Aje, 2003). One way is the loading and unloading of transport tissues (Aje, 2003). In long-distance carbon partitioning, sugar alcohols can be transported on sucrose (Zimmermann and Ziegler, 1975). The other form of movement is the sugar transporter,

Abbreviations: DAF, days after flowering; WGT, whole genome triplication; MST, monosaccharide transporters; ST, sugar transporter; SUT, sucrose transporter; FPKM, Fragments per kilobase of exon per million fragments mapped; PCC, Pearson Correlation Coefficient.

which controls the distribution of sugars in the sink and source, such as mediating the transport of sucrose (Kühn, 2003, 2010), monosaccharides (Büttner, 2007) or polyols (Noiraud et al., 2001; Juchauxcachau et al., 2007). Sugars exist in different organelles of source and sink cells or more biochemically for the transport of hexoses into the vacuoles (Martinoia et al., 2000), chloroplasts (Weber and Flügge, 2000), and Golgi apparatus (Wang et al., 2006). Thus, they have constructed sink and source organs (Chaffey, 2015). At present, the most studied sugar transporter belongs to the main promoter superfamily (MFS), which has a classic structure with 12 putative transmembrane helices and 11 loops (Chaudhry et al., 2007). Sugar transporters (STs) are responsible for the transmembrane transport of sucrose and play a key role in the sour-sink transport mediated by phloem and the sucrose supply of the sink tissues (Lemoine, 2000). In a number of fauna and flora studies, some ST genes have been identified, for instance, hexose transporters in *Juglans regia* (Decourteix et al., 2008), *Vitis* (Fillion et al., 1999; Hayes and Dry, 2007) and some polyol transporters in *Prunus cerasus* (Maurousset et al., 2003), *Malus domestica* (Watari et al., 2004), and *Olea europea* (Conde et al., 2007). In many plants, the sugar transporters, principally monosaccharide transporters (MSTs) and sucrose transporters (SUTs), have been identified. These species with sugar transporters include rice (*Oryza sativa*) (Aoki et al., 2003), *Arabidopsis thaliana* (Wormit et al., 2006), wheat (*Triticum aestivum*) (Aoki et al., 2002), populus (Payyavula et al., 2011), sorghum (*Sorghum bicolor*) (Milne et al., 2013), *Medicago truncatula* (Doidy et al., 2012), tomato (Reuscher et al., 2014a), and *Solanum lycopersicum* (Moore and Purugganan, 2003; Hackel et al., 2006), as well as woody plants such as *Rosa hybrida* (Henry et al., 2011). In recent years, SWEETs (Sugars Will Eventually Be Exported Transporters) have been identified as newly discovered sugar transporter proteins from *Arabidopsis* and *Oryza sativa* (Chen et al., 2010).

The MST gene family has important functions. The monosaccharide transporter encoded by the MST gene mediates the transport of multiple monosaccharides (such as glucose, fructose, mannose, galactose, and xylose). According to the location of monosaccharide transporters and the transport substances, MSTs contain seven distinct subfamilies (STP, VGT, PLT, INT, SFP, TMT, and Glct) (Büttner, 2007). In previous studies, 53 and 69 MST members were identified in *Arabidopsis* and pear, respectively (Li J.M. et al., 2015). Afterward, 58, 52, 64, and 76 monosaccharide transporters were identified from sweet orange (*Citrus sinensis*), tomato (*Solanum lycopersicum*), rice, and peanut (*Arachis hypogaea* L.) sugar transporter genes, respectively (Reuscher et al., 2014a; Zheng et al., 2014; Deng et al., 2019; Wan et al., 2020). In plants, STPs that play important roles in sugar accumulation have been proven by related studies. The STP protein belongs to the main facilitator superfamily, usually has 12 transmembrane domains and is considered a H+/sugar transporter (Ming et al., 2013). For example, the three hexose transporters were RNAi-mediated knockdown in the tomato, resulting in a significant decrease in hexose content in fruits, which proved that they could control hexoses in tomato fruits (Mccurdy et al., 2010). *MdSTP13a* takes up both hexose and sucrose for sorbitol-modulated pollen tube growth in apple, revealing a situation where the acquisition of sugars for pollen tube growth is regulated by a sugar alcohol (Li et al., 2020). *TaSTP6*, a sugar transporter protein in wheat (*Triticum aestivum*), was previously shown to exhibit enhanced expression in leaves upon infection by *Puccinia striiformis f.* sp. *tritici* (Pst), the causal agent of wheat stripe rust (Huai et al., 2019). Overexpression of *TaSTP13* promoted *Arabidopsis* susceptibility to powdery mildew and led to increased glucose accumulation in the leaves (Huai et al., 2020). Furthermore, other MST subfamilies have been identified as located in the tonoplast. In *Arabidopsis* and rice, AtTMTs (tonoplast monosaccharide transporter), AtVGTs (vacuolar glucose transporter) and OsTMTs, which play key roles in vacuole sugar partitioning, were proven to import hexoses into the vacuole (Michael, 2007). Studies have also shown that BvTST2.1 is a sucrose-specific transporter. Current evidence shows that it also functions as a proton reversal transporter, combining sucrose input into the vacuole with proton output. Because BvTST2.1 has a high degree of amino acid sequence similarity with members of the *Arabidopsis* tonoplast monosaccharide transporter family, this group of proteins has been renamed vacuole sugar transporters (TSTs) (Jung et al., 2015). *PpTST1* out of the TST gene family in the peachis likely responsible for sucrose accumulation in fruit. It is worth noting that silencing of *PpTST1* in peach fruit also inhibited hexose accumulation suggesting that it may also have influence on hexose accumulation (Peng et al., 2020). Previous studies have indicated that *AtERDL6* is a tonoplast-localized glucose exporter that releases glucose from vacuoles into the cytosol (Poschet and Büttner, 2011). In addition, sugar transporters respond to abiotic stress in plants. After cold stress treatment, the expression of *AtTMT1* was upregulated, and more glucose and fructose accumulated in the vacuole membrane of *Arabidopsis* leaf cells to increase the stress resistance of the plant (Wormit et al., 2006; Wingenter et al., 2010). In rice, the expression of the Golgi monosaccharide transporter *OsGMST1* is positively correlated to salt treatment (Cao et al., 2011). The overexpression of *OsMST6* gene can confer greater tolerance to drought and salinity stress in *A. thaliana* (Monfared et al., 2020). In cucumber, *CsTST2* is responsive to various abiotic stresses, inducing salt, drought and cold (Hu et al., 2019). Although the expression patterns and functional analysis of the sugar transporter gene family have been previously studied in a variety of plants, knowledge of the sugar transporter gene family is lacking in lotus roots.

Lotus root (*Nelumbo nucifera* Gaertn) is an ancient perennial aquatic herb plant that originated from India and China (Xue et al., 2012). Archeological research has estimated that the history of lotus cultivation in China is more than 2,000 years old (Shen-Miller, 2002; Na et al., 2009). In the long-term evolution of lotus, various types of mutations have appeared. After long-term artificial selection, the plants were grouped into three major types: ornament, seed, and rhizome lotus. Lotus roots, powder and seeds are recognized as nourishing foods that are rich in starch, protein, vitamins and mineral substances, have high edible value and medicinal value and are popular among many people (Pagels, 2005; Liu et al., 2010). Starch is the main storage material of lotus roots, accounting for more than 70% of the dry matter weight.

Starch is the main factor that affects the quality of lotus root products (Libao et al., 2013).

In plants, sugar transporters in sugar transport, absorption and utilization affect the growth and development of plants (Wu and Zhu, 2004). Sucrose synthesized in the source organ enters the phloem under the action of sugar transporters, and after long-distance transportation, it enters the sink organ through the apoplast or extracellular pathway (Carpaneto et al., 2005). In the storage organ of lotus roots, synthetic starch from sucrose is produced by the synthesis or degradation of starch in photosynthetic tissue. It enters the storage organ via sugar transporters and synthesizes starch with the action of a series of enzymes.

However, little information is currently available on the expression of the sugar transporter gene family in different tissues and developmental stages of lotus roots. Due to the significant functions of sugar transporters in lotus roots, it is of great significance to study the gene families of sugar transporters in lotus roots. In this study, we comprehensively analyzed the influences of drought, exogenous hormones, extreme temperatures, and salinity on NuMST. In addition, based on cis-regulatory elements and qRT-PCR, we further analyzed the roles of NuMST in *N. nucifera*. Finally, we constructed NuMST gene interaction networks and analyzed NuMST gene expression patterns through comparative genomics. This is the first study to report on MST genes in *N. nucifera*, extending our comprehension of the MST gene family in evolution stress responses and providing basic information for improving the edible quality of lotus.

MATERIALS AND METHODS

Retrieval of Genome Sequences

The *N. nucifera* and 10 representative genome sequences Were used for comparative analyses in this study. the genome sequences of *N. nucifera* Were downloaded From the NCBI genome database Under the link https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=4432.
the *Arabidopsis* sequences Were downloaded From the TAIR database Under the link http://www.Arabidopsis.org/. the sequences of the other 9 species Were downloaded From Phytozome (Goodstein et al., 2012).

Identification and Characterization of MST Gene Family

The Pfam database Was used to identify MST genes From all protein sequences of the examined species, With a threshold of $e < 1e^{-5}$. ST genes Have the typical MFS domain (PF07690). Using SMART to further verify the retrieved ST candidates (Song et al., 2018). MEME Was used to search for conserved motifs (Bailey et al., 2009).

Sequence alignment and phylogenetic analyses uses MUSCLE with default parameters for Multiple sequence alignment (Tong et al., 2013). Based on alignment, we generated phylogeny using a previously reported method (Li Q. et al., 2015). Phylogenetic analyses were conducted using Maximum Likelihood (Kühn, 2010).

Syntenic Analysis

The NuMST genes were mapped to *N. nucifera* chromosomes using Circos. The Multiple Collinearity Scan toolkit (MCScanX) exhibited the collinear relationship of the NuMST genes and other selected species, and uses the Dual Systeny Plotter software[1] was used to construct the syntenic analysis maps (Chen et al., 2020). Non-synonymous and synonymous (ks) were calculated using KaKs_Calculator 2.0 (Reuscher et al., 2014a).

Conditions and Treatments and qRT-PCR

Experimental samples were used cultivar *N. nucifera* seedlings "MeiRenHong," which grew in plastic containers containing water in a controlled-environment growth chamber. Under the same condition, seedlings at the leaf stage were transferred to the following treatment. Including (1) control; (2) ABA 18.9 mM; (3) polyethylene glycol (PEG) 300 mM; (4) NaCl 102.7 mM; (5) 4°C. The samples were collected seedling leaves at the leaf stage at 0, 8, 16, 24 h after treatment and frozen in liquid nitrogen, and stored at −70°C and used for bioassays. The material sample is repeated three times.

Total RNA was isolated from treated leaves using TaKaRa MiniBEST Plant RNA Extraction Kit (Takara, Dalian, China). Total RNA was reverse transcribed into cDNA for RT-PCR (Takara, Dalian, China). To verify the primer specificity, we used the program BLAST against the Euryale ferox Salisb genome. qRT-PCR was performed in 20 μl reactions consisting of 10 μl 2 × ChamQ SYBR qPCR Master Mix (Vazyme, NanJin, China), 0.8 μl of a mixture of the forward and reverse primers, 1.0 μl cDNA template, and 8.2 μl ddH_2O. The reactions were performed on a CFX-96 real-time PCR system (Bio-Rad). According to the instructions of ChamQ SYBR qPCR Master Mix (there is no need to add 50 × ROX Reference Dye when using Bio-Rad CFX96) (Liu et al., 2018; Hong et al., 2019). The qRT-PCR assays were carried out with three biological and technical replicates. According to the $2^{-\Delta\Delta CT}$ method, gene expression levels were calculated (Pfaffl, 2001).

RESULTS

Identification and Phylogenetic Relationship of the MST Gene Family in *N. nucifera*

To identify all putative MST proteins in the *N. nucifera* genome, we used the HMM profile of the MFS domain (PF07690) and Blastp to search against the database. A total of 35 MSTs were identified and subjected to Pfam and SMART analyses, which are shown in **Supplementary Table S1**. The MST genes were clustered into seven groups: STP, VGT, PLT, INT, SFP, TMT, and Glct. The SUT subfamily was not found in *N. nucifera*. To study the classification and phylogenetic relationship of the MST gene

[1]https://github.com/CJ-Chen/TBtools

family in *N. nucifera*, we constructed a phylogenetic tree using the MST protein. According to the phylogenetic tree (**Figure 1**), all the MST proteins were consistent with *Arabidopsis* groups.

To explore the diversity in each group, we used the MEME program to identify motifs. As shown in **Figure 1**, the MST proteins that share common motifs 1–10 were conversed in MST. Motifs 1, 4, 7, 8, and 10 were representative of MFS domains. Each subgroup that shared similar motifs and motif compositions was clustered into the same group. The results showed that the MST gene family has highly conserved domains and motifs (**Figure 1** and **Supplementary Table S1**).

Evolution and Expansion of MST in Different Plant Species

To study the evolution of the MST gene family in the kingdom of plants, we chose the 9 most representative Angiospermaes from low to high (6 eudicots and 1 basal angiosperm, 1 Pteridophyta and 1 Bryophyta species) for comparative analysis (**Figure 2**). According to the whole-genome level, the number of MSTs in each species was counted (**Figure 2A**). Land plants have a relatively large number of MST genes. Moreover, species that have a larger genome seem to contain more MST genes except for *Am. Trichopoda* and *P. patens*. In all species, the densities of MST proteins in *A. thaliana* (0.3926 number/Mb) were the highest, followed by *B. rapa* (0.2403 number/Mb) and *C. papaya* (0.1704 number/Mb), which were higher than those in lower plants. The reason is that *C. papaya*, *Vitis vinifera*, and *P. trichocarpa* do not have α and β duplications; *Am. trichopoda*, a basal angiosperm, is missing the γ duplication event. Furthermore, some species have more members of the MST gene family due to specific WGT events (Tuskan et al., 2006; Wang et al., 2011). We found that no MSTs were detected in *V. carteri* and *C. reinhardtii*. Then, a phylogenetic tree of the MST genes was constructed to analyze the evolutionary relationships of these species (**Figure 2B**). The phylogenetic tree indicated that the

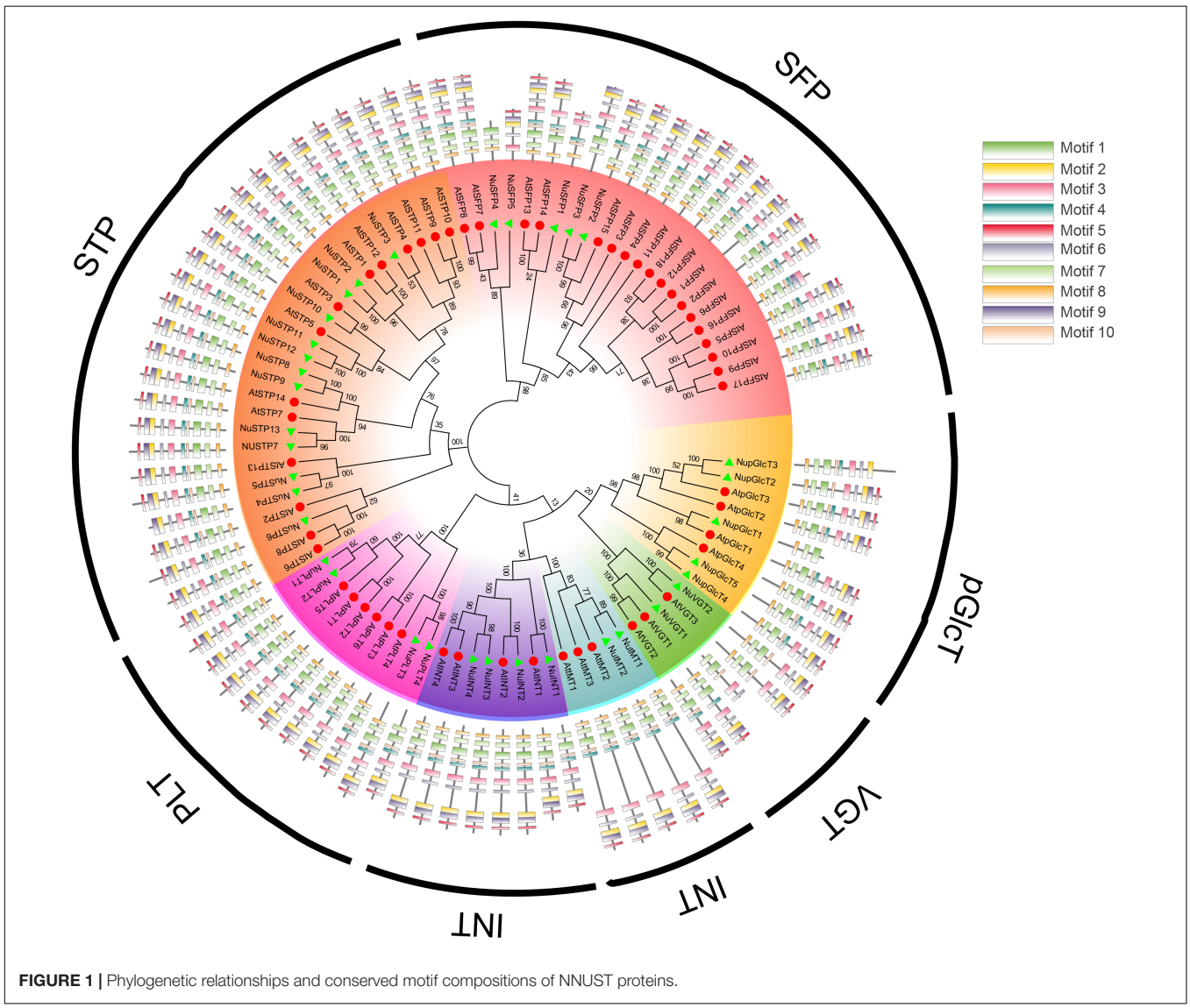

FIGURE 1 | Phylogenetic relationships and conserved motif compositions of NNUST proteins.

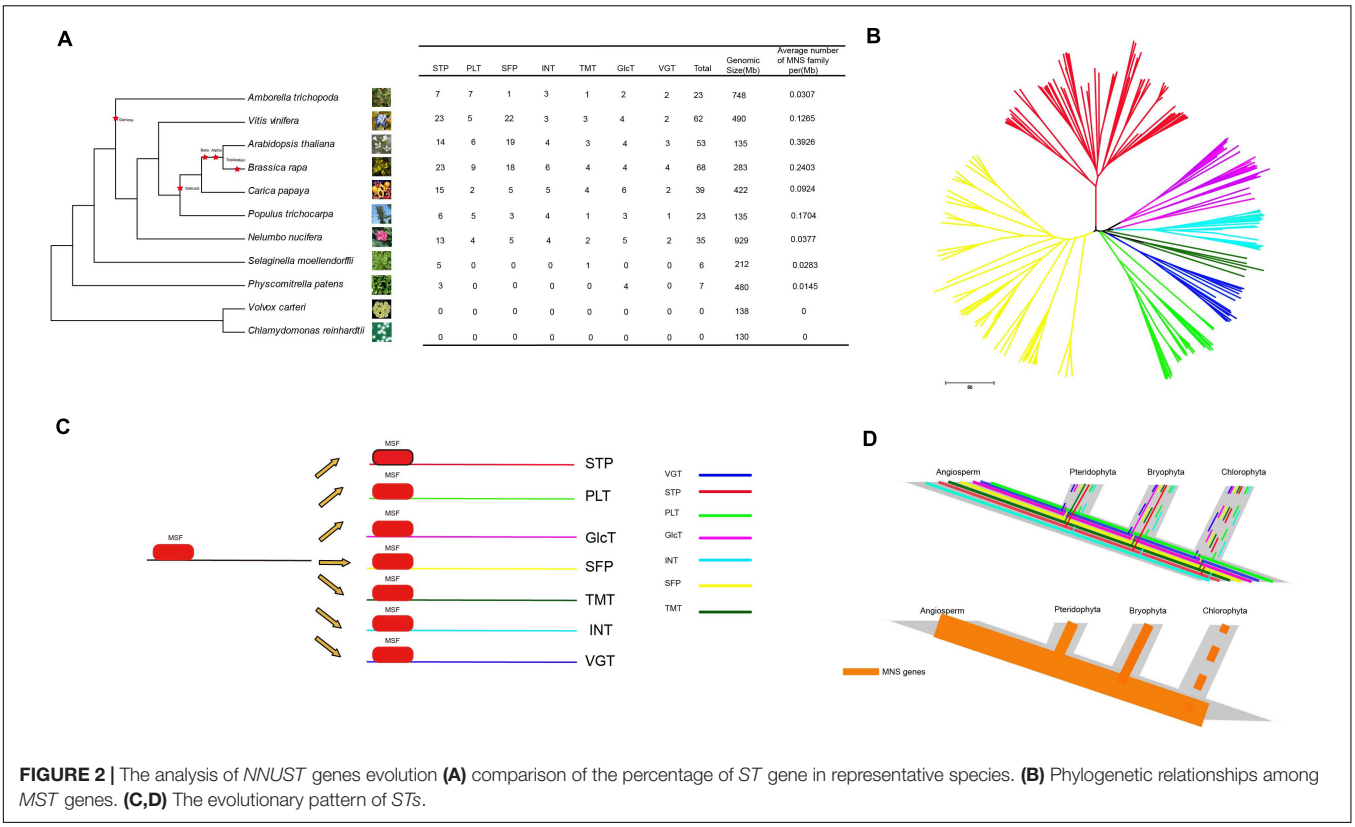

FIGURE 2 | The analysis of *NNUST* genes evolution **(A)** comparison of the percentage of *ST* gene in representative species. **(B)** Phylogenetic relationships among *MST* genes. **(C,D)** The evolutionary pattern of *STs*.

MST gene family can be divided into 7 distinct groups (STP, VGT, PLT, INT, SFP, TMT, and Glct), which is consistent with the result for *A. thaliana* (**Figure 2C**). The expansion occurred in the process of evolution from lower to higher plants, and the density of MST proteins increased as the plants evolved. From algae to angiosperms, we found that the MST gene family has highly conserved domains and motifs (**Figure 2D**). According to these findings, the evolutionary history of MST in the plant kingdom was constructed.

Chromosomal Distribution and Synteny Analysis of NuMST Genes

The NuMST genes were unevenly mapped on the 11 scaffolds (**Figure 3**). Some scaffolds have more genes, whereas others have few. The scaffold contained the largest number of NuMST genes (11). In other scaffolds, the highest numbers of MST genes were found in scaffolds6 (4), followed by scaffolds2 and scaffolds3 (3), and scaffolds10, scaffolds14, scaffolds5, and scaffolds8 had only one gene. The NuMST duplicate genes were identified with PlantDGD. The duplicate genes were derived from four modes of gene duplication, including 7 whole-genome duplications (WGD), 2 tandem duplications (TD), 7 transposed duplications (TRD), and 17 dispersed duplications (DSD). These results indicate that dispersed duplication may be a major driving force for NuMST gene evolution.

To investigate the evolution of the NuMST family, three dicots (*Arabidopsis*, *S. tuberosum* and grape) and one monocot (maize) were constructed from four comparative microsynteny maps with *N. nucifera* (**Figure 4**). The collinear gene pairs showed syntenic relationships in maize (135), followed by grape (107), *Arabidopsis* (89), *S.* tuberosum (22) (**Supplementary Table S2**). In these syntenic gene pairs, we found that some genes correspond to at least 4 collinear genes, especially in maize and grape, such as *NuSTP7* and *NuSFP5*. Some NuMST collinear gene pairs (corresponding to at least 4 collinear genes) were identified in *N. nucifera*/*Arabidopsis*, *N. nucifera*/grape and *N. nucifera*/maize, indicating that these genes may have already existed before ancestral divergence and played a vital role in the NuMST gene family during evolution. In contrast, subgroups of INT collinear gene pairs were not identified between *N. nucifera* and any of the other four species, indicating that they may have occurred after the divergence of dicotyledonous and monocotyledonous plants.

To further investigate the evolution footprint of the NuMST family, the Ka/Ks ratios of the NuMST gene pairs were calculated between *N. nucifera* and *Arabidopsis* (**Supplementary Table S3**). All collinear gene pairs NuMST gene pairs had Ka/Ks < 1. Thus, these findings suggest that the NuMST gene family might have had a purifying selective pressure in the process of evolution (**Supplementary Figure S1**).

Comparative Expression Pattern Analysis of the MST Genes in Different Tissues From *N. nucifera*

Comparing the expression of different sugar transporters in different tissues, we investigated divergence expression patterns

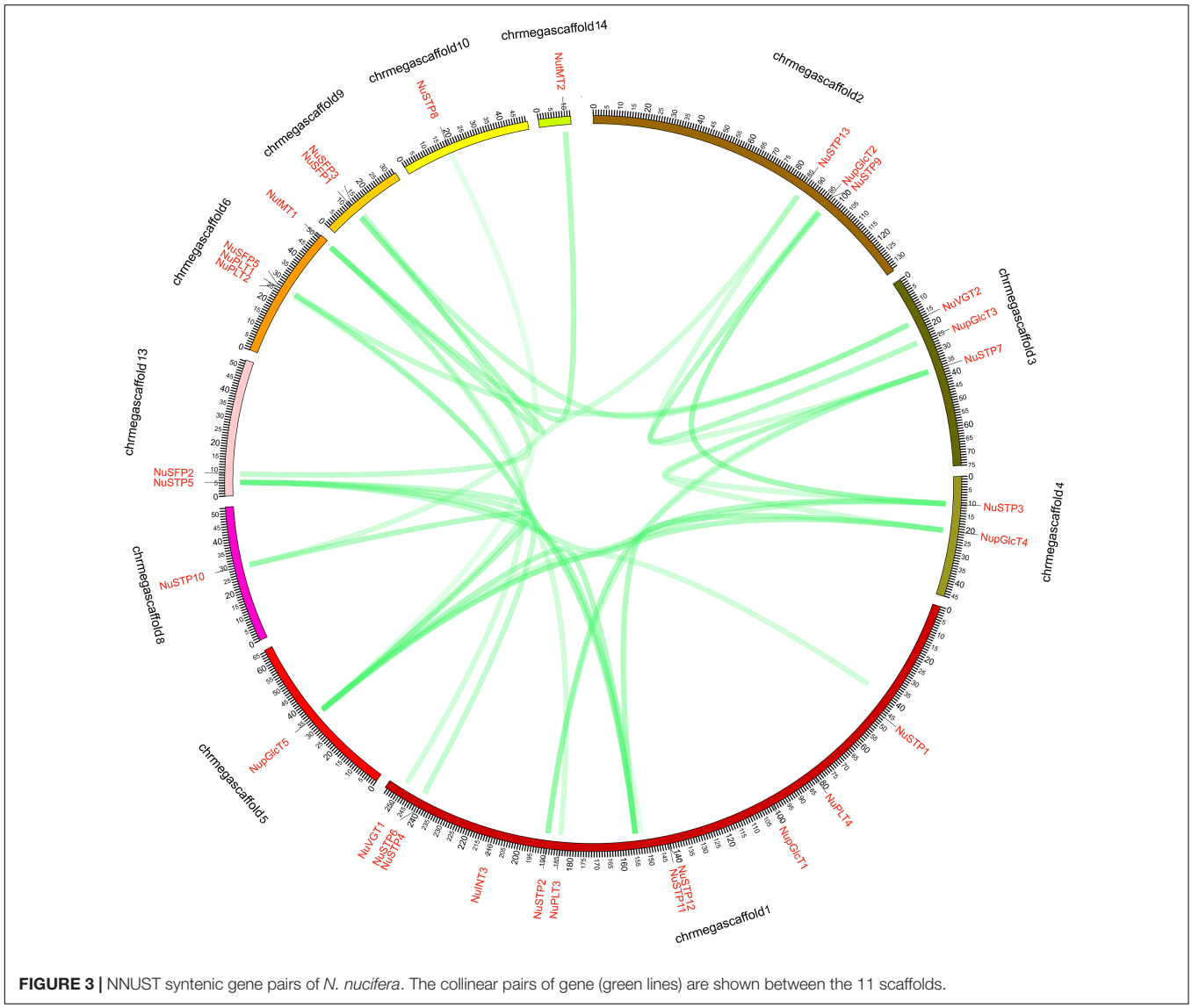

FIGURE 3 | NNUST syntenic gene pairs of *N. nucifera*. The collinear pairs of gene (green lines) are shown between the 11 scaffolds.

(**Figure 5** and **Supplementary Tables S4**, **S6**). We detected different MST gene expression levels, including the leaves, petioles, flowers, and rhizome. In the SFP subfamily, all genes were downregulated in leaves and petioles, of which the expression of the *NuSFP3* gene was upregulated. The expression of the *NuSFP3* gene was upregulated in the rhizome, followed by *NuSFP4*, *NuSFP5,* and *NuSFP2*, which remained almost unchanged. In the pGlcT subfamily, all genes were upregulated in flowers, of which the upregulated expression of *NupGlcT2* and *NupGlcT4* was most significant. *NupGlcT4* was the predominantly expressed member in this gene family and was expressed in all four tissues, followed by *NupGlcT5*. In the STP subfamily, the *NuSTP6* gene is a member with low activity because it is not detectable. *NuSTP3* and *NuSTP9* were the main members of the subfamily, showing relative levels in all four tissues, of which the upregulated expression of the *STP9* gene was most significant, especially in flowers. *NuSTP1*, *NuSTP4*, *NuSTP5*, *NuSTP7*, and *NuSTP8* expression was detected in leaves, but these genes were not expressed or expressed at lower levels in other tissues. In the INT and PLT subfamilies, some genes (including *NuINT2*, *NuINT3*, *NuPLT1*, and *NuPLT2*) were detected in only leaves. Both the *NuINT4* and *NuPLT4* genes were upregulated in only flowers. The *NuINT1* and *NuPLT3* genes were not expressed or downregulated in any of the four tissues. In the tMT subfamily, the expression of all genes was detected in all four tissues, and *NutMT2* was the most active. In the VGT subfamily, both *NuVGT1* and *NuVGT2* were upregulated in leaves and flowers but expressed at lower levels in other tissues.

Cis-Element and Interaction Network Analysis Among MST Proteins in *N. nucifera*

The MST gene of cis-elements was identified at the 1.5 kb promoter region. Then, we analyzed the cis-elements by the Plant Cis-acting Regulatory DNA Elements (PLACE) website. We

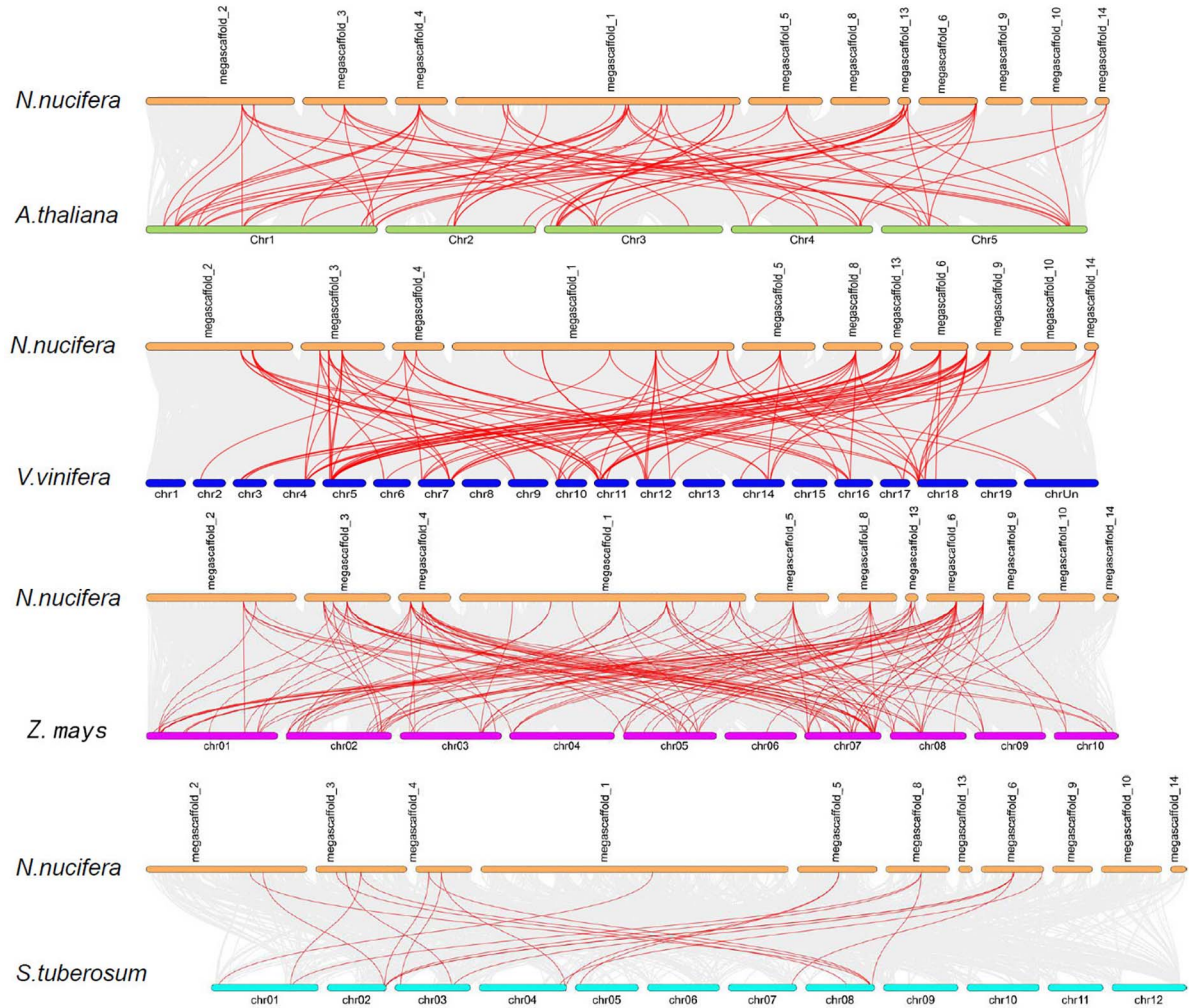

FIGURE 4 | Synteny of *NNUSTs* in maize, grape, *S. tuberosum*and and *Arabidopsis*. Gray lines in the background indicate the collinear blocks within *N. nucifera* and other plant genomes, while the red lines highlight the syntenic NNUST gene pairs.

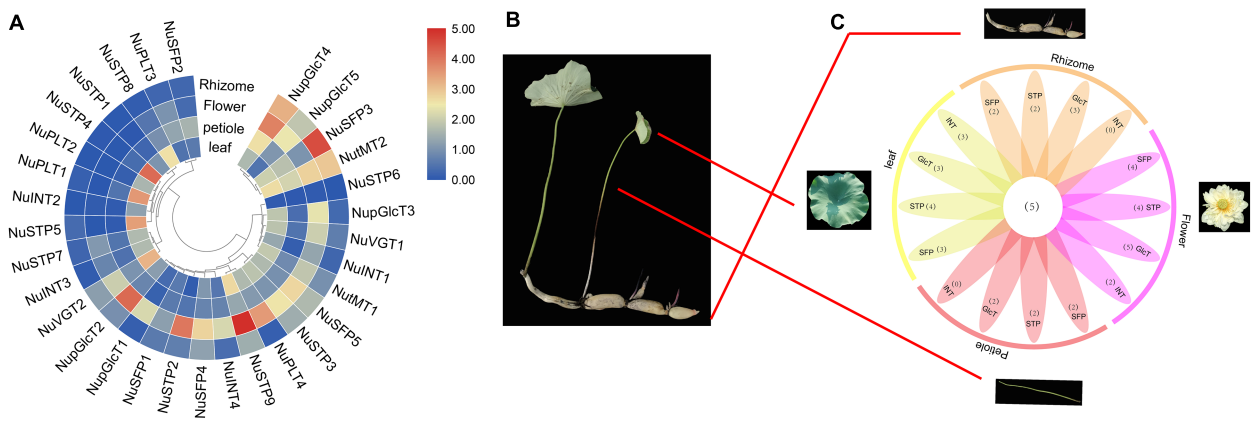

FIGURE 5 | Phylogenetic relationships, expression among four *N. nucifera* tissues of ST proteins. **(A)** ST genes among four *N. nucifera* tissues. **(B)** *N. nucifera* tissues. **(C)** Venn diagram depicting the distribution of shared expression of the ST.

identified the 10 most common cis-elements in MST genes in *N. nucifera* (**Supplementary Figure S2**).

In the promoter regions of the MST gene, we found a total of 10 common cis-regulatory elements, which were highly conserved among all the studied MST genes in *N. nucifera* (**Supplementary Figure S2**). We found that ARBE, TGACG, and GARE motifs responded to plant hormones, such as GA, ABA, and JA. A number of common cis-regulatory elements were responsive to both abiotic and biotic stresses, including a drought-responsive element (MBS), defense and stress responsiveness (TC-rich repeats), a light-responsive element pathogen (G-Box), low-temperature responsiveness (LTR), and one fungal elicitor-responsive element (W-box), indicating the importance of MST genes in stress tolerance. The MST genes were responsive to drought, cold, salinity and other stresses, which may be due to upstream gene specificity and the combination of corresponding cis-elements regulating the expression of MST genes. This speculation needs further verification in future work.

Expression Profiles of MST Genes Under Abiotic Stresses in *N. nucifera*

In plants, the MST gene family plays particularly important roles in development and stress responses. Abiotic stress conditions such as drought, extreme temperatures, and salinity, adversely affects plant growth and crop productivity. Therefore, we chose NaCl, PEG, ABA, and cold treatments to identify the stress-responsive MST genes (**Figure 6** and **Supplementary Tables S5**, **S6**). With ABA treatment, we found that 23 MST genes were upregulated, and two MST genes were downregulated. Among the 31 MST genes, *NuSFP5*, *NuSTP2*, *NuSTP4*, and *NuSTP5*, their expression was over 6 times that of the control at 8 h. With NaCl treatment, 25 MST genes were upregulated. We found that *NuSFP2*, *NuSFP3*, *NuSTP3*, *NuSTP4*, *NuSTP6*, *NuSTP7*, and *NuVGT2* expression was upregulated at 8 h or 16 h but downregulated at 24 h. By contrast, when PEG treatment was applied, we found that upregulated *NuSTP5* and *NuSTP8* expression reached the highest levels at 24 h and was more than 6 times that of the control. Including the 12 MST genes that were upregulated at 8 h. Under Cold treatment, 7 MST genes were upregulated at 8 h and reached their highest levels. Among the 31 MST genes, especially *NuINT3*, *NuSTP2,* and *NuSTP5*, their upregulated expression was over 5 times that of the control at 8 h; the upregulated expression of *NuINT1, NuINT2, NuSTP3, NuSTP5,* and *NuSTP8* was over 4 times that of the control at 24 h. Specifically, we observed that *NuVGTs* did not respond or responded less to the four stress treatments. Under the four abiotic stress conditions, only *NuSTP5* expression was upregulated at 8 h and 24 h, generating a stress response. Combined with cis-element analysis, ARBE and LTR were present in *NuSTP5*.

To further investigate the link between these MST genes, PCCs based on the relative expression of these genes established a related and co-regulatory network (**Figure 6B**). We collected NuMST gene pairs evaluated at the $p \geq 0.05$ significance level and visualized them to build a hormone and abiotic stress co-regulatory network (**Supplementary Figure S3**). In the co-regulatory network, a total of 35 nodes were positively significantly correlated with the gene pairs. The NuMST gene interaction network shows a very complex correlation with other genes of *N. nucifera*, which may reveal that NuMST genes take part in a number of basic mechanisms and are regulated by many upstream genes and/or regulate many downstream factors. In this described network, increasing cooperation and obtaining new functions help plants adapt to abiotic stresses and changing environments.

DISCUSSION

The production of sugar by photosynthesis in plants is a very important process. Sugar transporters are distributed in various tissues and organs and expressed in different tissues. Sugar transporters play different roles in the growth and development of plants. In the current study, we found that the MST gene family was likely to be involved in growth, development, and responses to stress.

If gene products are involved in complex regulatory networks, these genes may be retained (Birchler and Veitia, 2007; Aad et al., 2012). Previous research has shown that gene duplicates generate new functions or expression through the processes of neo- and subfunctionalization, and they are retained more frequently than the classical model permits. In our study, we found 35 NuMST genes encoding putative sugar transporters in the *N. nucifera* genome. The NuSTP (13) genes were preferably retained in *N. nucifera*. The duplication genes went through three fates: subfunctionalization, neofunctionalization, or nonfunctionalization (deletion or pseudogenization). In subfunctionalization, the expression or function of the progenitor gene is divided into daughter genes by complementary mutations in regulatory or coding regions (Hughes and Friedman, 2005). In neofunctionalization, through preliminary relaxation of purification choices and accumulation of mutations, under either neutral or positive selection, related or new functions may appear in one of the duplicates, endowing it with a new function (Gu et al., 2002). The ST genes provided opportunities to gain functional diversification.

Expression analysis of the MST gene family of *N. nucifera* in different tissues was performed to identify the most important MSTs. In our study, *NuINT2* and *NuINT3* were expressed at relatively low levels in all tested tissues but responded to abiotic stresses and sugar treatments very rapidly, which indicated that *NuINT2* and *NuINT3* responded to environmental changes. The *NuINT4* gene was expressed only in flowers of *N. nucifera*. In the tomato, *SlINT4* was expressed in all tested tissues, apart from fruits (days after flowering) (Reuscher et al., 2014b). pGlcT has a significant contribution to the output of chloroplast starch degradation products in *Arabidopsis* leaves and the starch-mediated export of pGlcT photoassimilates (Cho et al., 2010). A pGlcT protein was first discovered in olive trees during fruit development (Rafal et al., 2003). *CspGlcT2* and *CspGlcT4* were upregulated as fruit ripened in the orange (*Citrus sinensis* Osbeck) (Zheng et al., 2014). In our study, all pGlcT genes were expressed

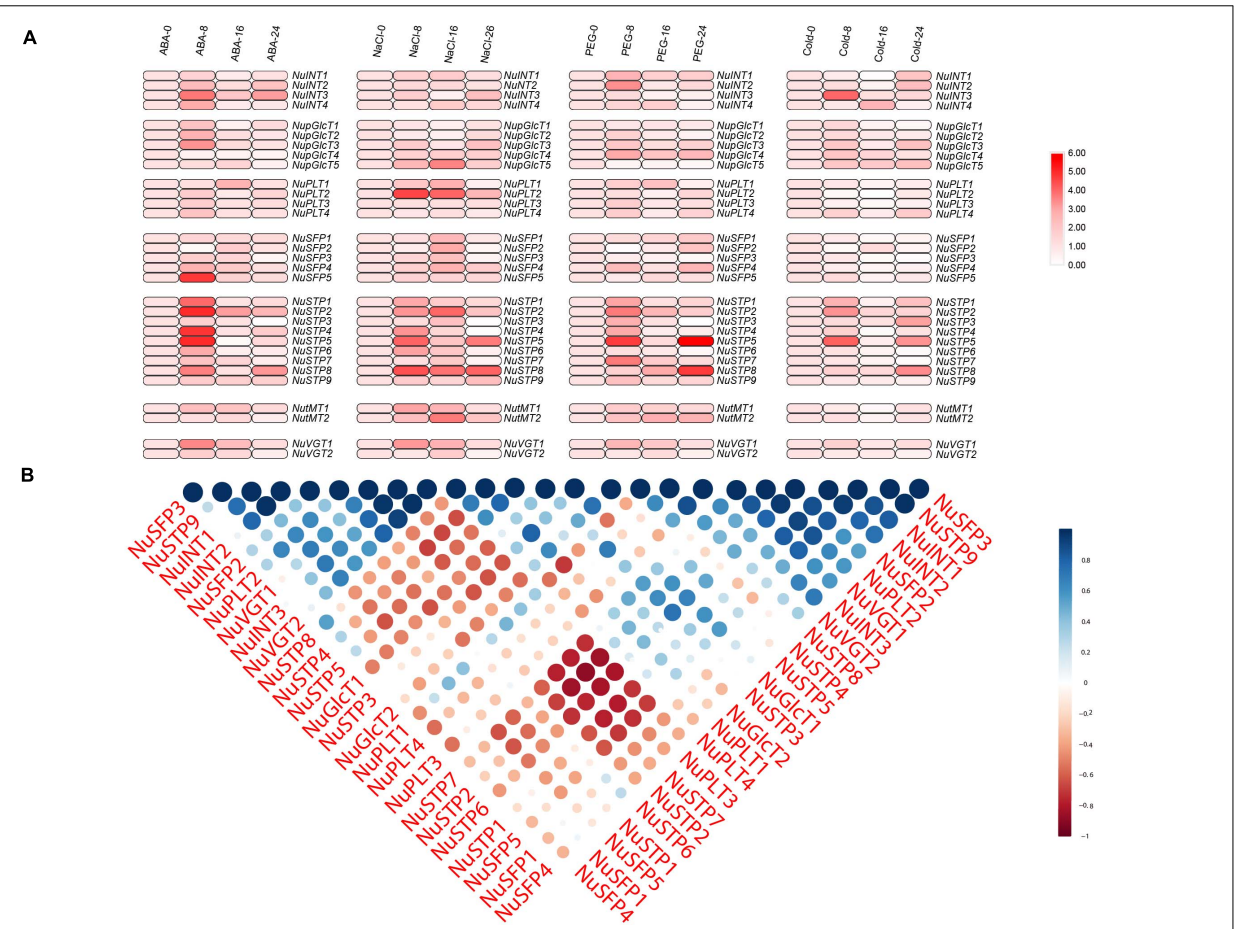

FIGURE 6 | Expression analysis of *NNUSTs* under abiotic stresses. **(A)** Heat map representation and hierarchical clustering of *NNUSTs* during a ABA stress, Nacl stress, PEG stress, cold stress. **(B)** Correlation analysis by using the R package program.

in flowers, of which the expression of *NupGlcT2* and *NupGlcT4* was most significant, where *NupGlcT4* was a ubiquitously expressed gene with high expression, especially at ripening rhizomes, consistent with the results in oranges. *NupGlcT5* showed a strong response to salt treatment, which supported its role in tolerance to salt stress (Cao et al., 2011). *NuPLT1*, *NuPLT2*, and *NuPLT4* were expressed in leaves, among which *NuPLT4* was the most active. However, NuPLTs were not detected in rhizomes, indicating that these genes may not play an important role in the accumulation of sugar during the development of rhizomes, which is consistent with the results of pear research (Li J.M. et al., 2015). *NuPLT2* was strongly induced by NaCl treatment, which suggests an important role of *NuPLT2* in tolerance to salt stress. We found that *NuSTP3* and *NuSTP9* were ubiquitously expressed genes with high expression in flowers. In particular, *NuSTP9* was not sensitive to the abiotic stress response, which is consistent with the research results of sweet orange (Zheng et al., 2014). *NuSTP1* and *NuSTP6* were expressed at relatively low levels in all tested tissues but responded to ABA treatment. This has the same result as the wheat study. In wheat research, it was found that Pst infection caused increased accumulation of abscisic acid (ABA) and that application of exogenous ABA significantly enhanced *TaSTP6* expression (Huai et al., 2019). *NuSTP4*, *NuSTP5*, and *NuSTP8* were only active in leaves, but they were all induced by ABA, NaCl, and PEG, suggesting that they might be important emergency response genes (**Figure 7**). *NuSTP8* is an important abiotic stress gene, which is consistent with the results of rice (Mamun et al., 2006). In previous studies, TMTs of *Arabidopsis* and rice have been identified. In *Arabidopsis*, *AtTMT1* and *AtTMT2* are located in the VM and considered to be antiporters of glucose or fructose/H^+, which introduce sugar into vacuoles (Wormit et al., 2006; Wingenter et al., 2010). In sweet orange, *CsTMT2* showed relatively high expression levels in fruits (Zheng et al., 2014). In our study, *NutMT1* and *NutMT2* were ubiquitously expressed, but *NutMT2* showed more redundancy in the petiole and rhizome sheath than *NutMT1*. *NutMT2* plays a role in vacuolar sugar accumulation during fruit ripening, which is consistent with the results of sweet orange studies. Moreover, *NutMT2* was induced by salt treatment, which suggests an important role of *NutMT2* in tolerance to salt stress. Both *NuVGT1* and *NuVGT2* are expressed in leaves and flowers, with *NuVGT1* responding to ABA and salt. There are few studies on sugar transporters in lotus roots, which provides a good reference for future research on lotus root sugar transporters.

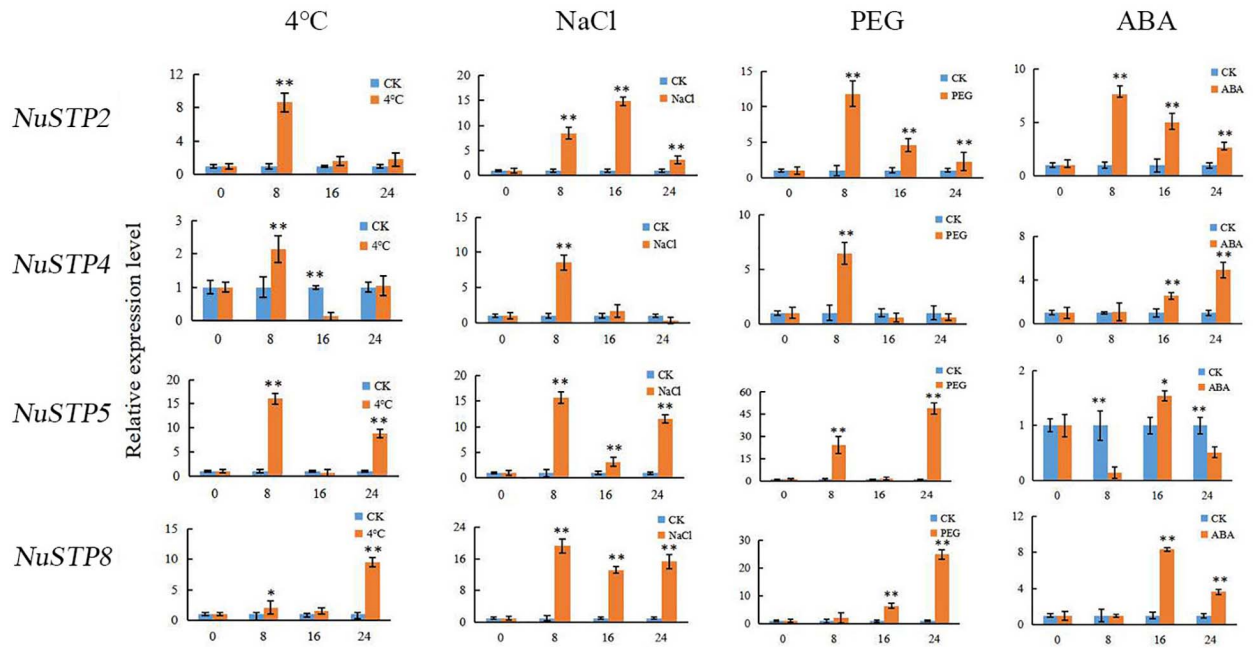

FIGURE 7 | Relative expression levels of NNUSTP2, NNUSTP4, NNUSTP5 and NNUSTP8 under four abiotic stresses. ** indicate a significant difference at $P < 0.01$.

According to the sequences of cis-elements and motifs analyzed, MST gene promoters included common cis-regulatory elements and several common motifs and domains. The MFS domain was originally thought to play a major role in sugar absorption. These observations have led to MFS being much broader in nature and more diverse in function than previously thought. The common motifs of STs, such as DOFCOREZM, suggest that DNA binding may play a significant role in regulating gene expression at the level of ST gene activity. For instance, the expression of *AtSUT2* is regulated by a putative HD-Zip transcription factor and a DOFCOREZM protein binding site in close cooperation (Schneidereit and Imlau, 2008). In previous studies, the concentration of sugar-responsive elements was regulated by some transporter genes. Transcriptional regulation via sugars, such as the transcriptional regulation of VvHT1 by glucose, was demonstrated (Atanassova and Delrot, 2003; Conde et al., 2006). Furthermore, the MYBCOREATCYCB1 promoter was found in ST members of pear, which showed that different species might have different transcriptional regulation mechanisms in the ST gene family.

Today, the great diversity of terrestrial plants on Earth provides an opportunity to study the evolution of gene families in major lineages that vary in functional complexity and life histories (Kenrick and Crane, 1997; Karol et al., 2001). In plants, gene families of evolution which prevalently combined of tandem, segmental and whole genome duplication (polyploidy) events. *N. nucifera* experienced whole genome triplication (WGT) events (Wang et al., 2011; Cheng et al., 2013). The ancient ST gene family was discovered in the moss lineage and isolated from vascular plants > 410 million years ago (Johnson et al., 2006). In our study, the MST gene family was detected in *P. patens*, which was consistent with ancient MST from the moss lineage. At the same time, the MST of the gene family shared similar evolution with MST. The expansion of gene families in plants is often due to multiple gene duplications caused by ancestral genes. In *Arabidopsis*, the large expansion of the MST gene family is attributed to tandem duplications. However, MST gene duplications in *N. nucifera* belong to dispersed duplications (**Figure 4**). Therefore, it can be concluded that the MST family experienced large expansions caused by WGDs and that most of the MST family began to expand after the differentiation of bryophytes and chlorophyta.

CONCLUSION

In summary, we comprehensive analyzed the evolutionary pattern, gene synteny, gene duplication or losses, stress treatments, and interaction network of MST genes involved in the sugar transport pathway. A total of 316 MST genes were identified among 10 representative species. The expansion of MST occurs during the evolution from low plants to high plants. The analysis of promoter sequences showed that the MST gene families of different species have different transcriptional regulatory effects. The expression analysis indicated that most MST genes respond to different stress treatments. Among these, *NuSTP2*, *NuSTP4*, *NuSTP5,* and *NuSTP8* could be used as excellent candidate genes to improve various stress tolerances of *Nelumbo nucifera*. This speculation needs further verification in future work. Our study can provide comprehensive functional characterization of MST genes genes through molecular genetics studies and reverse genetic methods.

DATA AVAILABILITY STATEMENT

The *N. nucifera* and 10 representative genome sequences were used for comparative analyses in this study. The genome sequences of *N. nucifera* were downloaded from NCBI genome database under the link https://www.ncbi.nlm.nih.gov/Taxonomy/Browser/wwwtax.cgi?mode=Info&id=4432. The *Arabidopsis* sequences were downloaded from TAIR database under the link http://www.Arabidopsis.org/. The sequences of the other 9 species were downloaded from phytozome (Goodstein et al., 2012).

AUTHOR CONTRIBUTIONS

PW conceived and designed the experimental design. LL, SZ, and YZ contributed to the experimental work. All authors read and approved the final manuscript.

FUNDING

This work was supported by the China Agriculture Research System (CARS-24). The funding bodies had no role in the design, collection and analysis, interpretation of data, or in writing the manuscript.

SUPPLEMENTARY MATERIAL

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fevo.2021.537398/full#supplementary-material

Supplementary Figure 1 | The Ka/Ks ratios of the NNUST gene pairs between *N. nucifera* and *Arabidopsis*.

Supplementary Figure 2 | The ST genes of cis-elements.

Supplementary Figure 3 | The phylogeny and motif of 6 species.

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Conflict of Interest: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.