

Major Facilitator Superfamily transporters balance sugar metabolism in peach

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Abstract

Sugar content is a key determinant of peach (*Prunus persica*) fruit quality, influencing taste, consumer preferences, and market value. However, the roles of Major Facilitator Superfamily (MFS) transporters in sugar metabolism and regulation remain largely unexplored. This study employed a combination of spatial metabolomics, quantitative genetics, transcriptomics, comparative genomics, and functional genomics to investigate the role of 67 MFS members in balancing sugar metabolism during peach fruit development. Spatial metabolomics revealed dynamic sugar distribution patterns, with ERD6-like transporters (*PpERDL16-1*) and tonoplasmic sugar transporters 1 (*PpTST1*) promoting sucrose accumulation and polyol/monosaccharide transporters 5 (*PpPMT5-1*) and sucrose transporters 4 (*PpSUT4*) reducing sucrose transport during fruit ripening. Functional studies confirmed these roles: *PpERDL16-1* overexpression enhanced sucrose transport, and *PpPMT5-1* or *PpSUT4* silencing reduced sugar levels in peach fruit. Quantitative trait locus (QTL) mapping identified a major locus on chromosome 5, upstream of *PpTST1*, forming distinct haplotypes (Hap1 and Hap2). Hap1 was associated with lower *PpTST1* expression and higher sugar and soluble solids content (SSC), while Hap2 was linked to higher *PpTST1* expression and lower sugar content. This inverse relationship suggests that upstream genetic variants fine-tune *PpTST1* expression in a context-dependent manner, potentially through interactions with transcription factors or epigenetic modifiers. Notably, *PpTST1* overexpression increased sugar content but did not alter SSC, indicating compensatory mechanisms such as changes in organic acid metabolism or water content. These results illuminate the molecular mechanisms regulating sugar homeostasis in peach fruits, providing valuable targets for the genetic improvement of fruit quality through breeding programs.

Introduction

Fruit flavor in peaches (*Prunus persica*), a complex trait influenced by the balance of sugars and acids, is a critical determinant of fruit quality and consumer preference (Predieri et al. 2006). Sweetness, often quantified by soluble sugar content (SSC), primarily reflects the accumulation of sugars such as sucrose, glucose, and fructose, as well as their relative balance (Kroger et al. 2006; Cirilli et al. 2016). Previous studies have linked SSC and sugar metabolism to multiple chromosomes in peaches, underscoring its polygenic nature (Quilot et al. 2004; Salazar et al. 2014; Zanon et al. 2015; Cirilli et al. 2016; Tian et al. 2017; Shi et al. 2020; Peng et al. 2020b; Yu et al. 2021). Despite substantial advances, the molecular mechanisms driving sugar transport and accumulation during fruit development remain poorly understood.

Peach fruit development follows a double-sigmoid growth curve, with distinct stages marked by dynamic shifts in sugar composition, where sucrose becomes the dominant sugar during maturation (Falchi et al. 2013). In peaches, as in other Rosaceae fruit trees such as apples and pears, sorbitol is the dominant sugar transported, unlike other plants where sucrose is primarily used for transport (Zhang and Turgeon 2018). Several key sugar transporter families mediate sugar movement across membranes, including Sugar Will Eventually be Exported Transporters (SWEET) proteins (Ren et al. 2020; Singh et al. 2023), and members of the Major Facilitator Superfamily (MFS), such as sugar porters (SP/MST) and sucrose transporters (SUT/SUC) (Niño-González et al. 2019). These transporters regulate sugar partitioning within cells and between tissues, playing crucial roles in plant development

Received January 13, 2025. Accepted April 8, 2025.

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and fruit ripening (Zhu et al. 2022). Specifically, MFS transporters facilitate sugar loading into the phloem, transport through the sieve element-companion cell complex, and subsequent unloading into sink tissues (Andersen et al. 2023).

In sink tissues such as fruits, sugar unloading occurs via 2 distinct pathways: the symplastic pathway, where sugars move passively through plasmodesmata, and the apoplastic pathway, which involves active sugar transport across membranes (Paniagua et al. 2021). Unlike pome fruits such as apples, which predominantly rely on apoplastic unloading throughout development, stone fruits like peaches exhibit remarkable developmental plasticity in sugar unloading, switching between symplastic and apoplastic pathways depending on developmental stage, metabolic demands, and environmental conditions (Zanon et al. 2015). For instance, in the Japanese plum, both pathways coexist and alternate based on time of day and environmental cues, allowing flexible regulation of osmotic potential and sugar accumulation in sink tissues (Corelli Grappadelli et al. 2019). This unique plasticity highlights the need for a deeper understanding of sugar transport mechanisms in peach fruit, as these processes are tightly regulated by MFS transporters that mediate sugar allocation during development (Tan et al. 2022).

Sugar transporter proteins (STPs), or hexose transporters (HTs), function as proton/sugar symporters for various monosaccharides, including glucose and fructose (Büttner 2007; Johnson and Thomas 2007; Wang et al. 2020). Polyol/monosaccharide transporters (PMTs/PLTs) and sugar facilitator proteins (SFPs) include plasma membrane symporters for sugar alcohols and vacuolar hexose exporters contributing to osmotic regulation (Noiraud et al. 2001; Juchaux-Cachau et al. 2007; Klepek et al. 2010; Wang et al. 2016; Tian et al. 2017). Tonoplastic sugar transporters (TSTs/TMTs) (Wormit et al. 2006; Cheng et al. 2018a, 2018b; Braun 2022; Li et al. 2022), and vacuolar glucose transporters (VGTs) (Aluri and Büttner 2007) mediate vacuolar sugar transport. Inositol transporters (INTs/ITRs) import inositol, a regulatory molecule precursor (Schneider et al. 2006, 2008; Sambe et al. 2015; Schneider 2015; Strobl et al. 2018). ERD6-like transporters are vacuolar membrane transporters (Yu et al. 2021; Zhu et al. 2021), while plastidic glucose translocators (pGlcTs) export starch degradation products (Cho et al. 2011; Valifard et al. 2023). Sucrose transporters (SUTs/SUCs) enable proton/sucrose symport across the vacuolar membrane, playing a crucial role in phloem transport (Peng et al. 2020a; Braun 2022; Zhang et al. 2023).

Leveraging Matrix-Assisted Laser Desorption/Ionization Mass Spectrometry Imaging (MALDI-MSI), which allows high-resolution visualization of metabolite distribution during fruit ripening (Lu et al. 2023; Mao et al. 2024), this study aims to investigate the roles of MFS transporters in regulating sugar metabolism and distribution. Spatial and temporal regulation of these genes is essential for sugar partitioning and accumulation, as observed in other fruit crops like melons and strawberries, where specific MFS transporters enhance sugar content (Cheng et al. 2018a; Liu et al. 2020).

Despite advances in identifying MFS in fruit crops, the mechanisms regulating sugar accumulation in peaches remain poorly understood. While studies on apples (Zhu et al. 2021, 2023), grapes (Liu et al. 2024), strawberries (Liu et al. 2020; Milosavljević et al. 2023), and melons (Cheng et al. 2018a; Lu et al. 2020) have provided valuable insights, the genetic and molecular basis of sugar metabolism in peaches is less explored. By integrating spatial metabolomics, transcriptomics, quantitative genetics, and functional genomics, we seek to uncover the genetic and molecular basis of sugar accumulation. Understanding these processes will not only provide insights into the regulatory networks governing

sugar transport but also offer valuable targets for genetic improvement of peach fruit sweetness in breeding programs.

Results

To explore the roles of MFS members in sugar metabolism and transport during peach fruit development, we employed a combination of multiomics approaches, as outlined in [Supplementary Fig. S1](#).

Comparative genomic studies of 67 MFS members in peach

We used the Rosaceae Genome Database (GDR) and the diploid peach genome Lovell 2.0 (Verde et al. 2017) to identify and characterize 67 MFS genes involved in sugar transport. Bioinformatics analysis, supported by functional annotation from SwissProt, TrEMBL, NCBI, and TAIR databases, revealed 8 distinct MFS subfamilies: STP/HT, PMT/PLT/SFP, TST/TMT, INT/ITR, ERD6-like, pGlcT, VGT, and SUT. Phylogenetic analysis demonstrated that the SUT/SUC subfamily formed a separate branch, while the SP subfamily clustered into a coherent group ([Fig. 1A](#), [Supplementary Table S1](#)), consistent with previous findings (Niño-González et al. 2019).

Expression analysis of MFS genes in the fruit flesh of 3 peach varieties—"Daguo Heitao" (DG), "Zheng Bai 5-2" (ZB), and "Babygold 5" (BG5)—revealed a set of highly expressed genes during fruit maturation. Genes with expression levels exceeding >2 fragments per kilobase per million mapped reads (FPKM) in at least 2 developmental stages were classified as "highly expressed," based on established thresholds for peach fruit transcriptomics. Genes such as *Prupe.8G101200* (PpPMT5-2), *Prupe.1G144800* (PpERDL16-1), *Prupe.5G006300* (PpTST1), *Prupe.3G066300* (suppressor of G protein beta1, PpSGB1) (Wang et al. 2006), *Prupe.8G101500* (PpPMT5-1), *Prupe.8G180600* (PpSTS2), *Prupe.1G378500* (PpERDL6), *Prupe.7G234100* (suppressor of G protein beta2, PpSGB2), *Prupe.3G144600* (PppGlcT), and *Prupe.1G542000* (PpSUT4) were clustered together and exhibited differential expression across fruit developmental stages ([Fig. 1B](#), [Supplementary Table S2](#)).

To elucidate the relationship between MFS genes and sugar metabolism, we analyzed sugar content dynamics during fruit development in the variety "Babygold 5" (BG5), which is not classified as a high-sugar variety by modern standards. Fructose content remained relatively stable throughout development, while glucose content showed a slight increase during the second rapid growth phase (BG5_3—BG5_4). In contrast, sucrose accumulation was substantially more pronounced, peaking during the same period, which highlights the second rapid growth phase as a critical stage for sugar accumulation ([Fig. 2A](#), [Supplementary Table S3](#)). Statistically validated correlation analysis of candidate MFS genes with sucrose, glucose, and fructose levels throughout the developmental stages (BG5_1—BG5_4) revealed significant positive correlations ($P \leq 0.05$) between sucrose content and genes such as PpERDL6, PpSGB1, PpSGB2, and PpTST1, while PpPMT5-2 and PppGlcT exhibited significant negative correlations with sucrose content ([Supplementary Fig. S2](#)). Further differential expression analysis during the premature (BG5_3) and mature (BG5_4) stages revealed highly significant positive correlations ($P \leq 0.01$) for PpERDL16-1, PpERDL6, PpSGB1, PpSTS2, and PpTST1 with sucrose content, while PpPMT5-2, PpPMT5-1, PpSUT4, and PppGlcT exhibited highly significant negative correlations during these stages ([Fig. 2B](#), [Supplementary Table S4](#)).

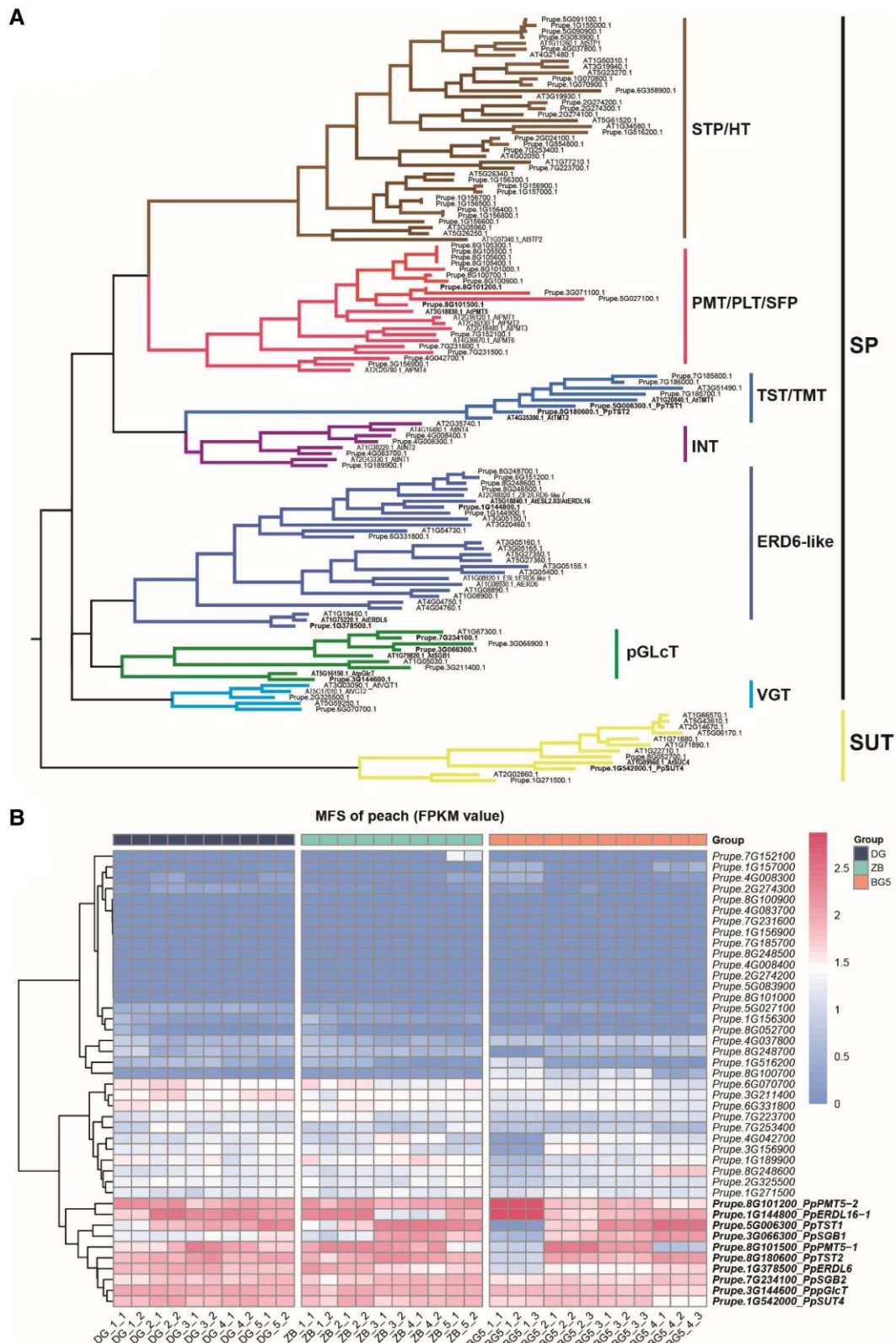


Figure 1. Phylogenetic and expression analysis of MFS transporters in peach (*P. persica*). **A**) The phylogenetic tree of MFS members highlights the sucrose transporter (SUT) subfamily in yellow and SP subfamilies (colored segments): STP/HT, PMT/PLT/SFP, TST/TMT, INT/ITR, ERD6-like, pGLcT, and VGT). Branch lengths are not proportional to evolutionary distance. **B**) Heatmap of normalized expression (FPKM) for SUT and SP genes across fruit developmental stages in 3 cultivars: DG (10, 60, 80, 100, and 120 days after full bloom [DAFB]), ZB (10, 30, 50, 70, and 90 DAFB), and BG5 (60, 75, 90, and 105 DAFB). Color scale shows $\log_2(\text{FPKM} + 1)$ values.

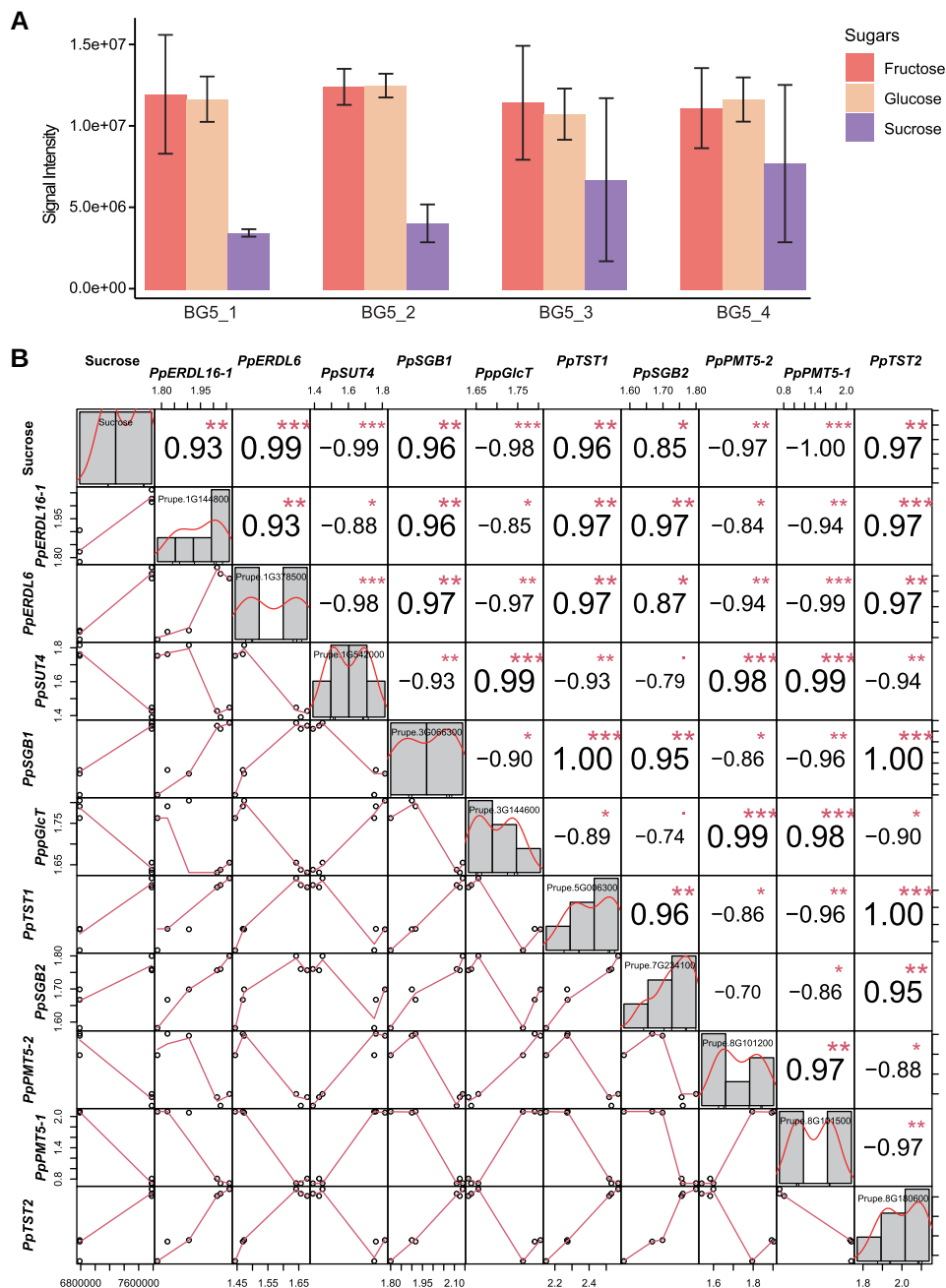
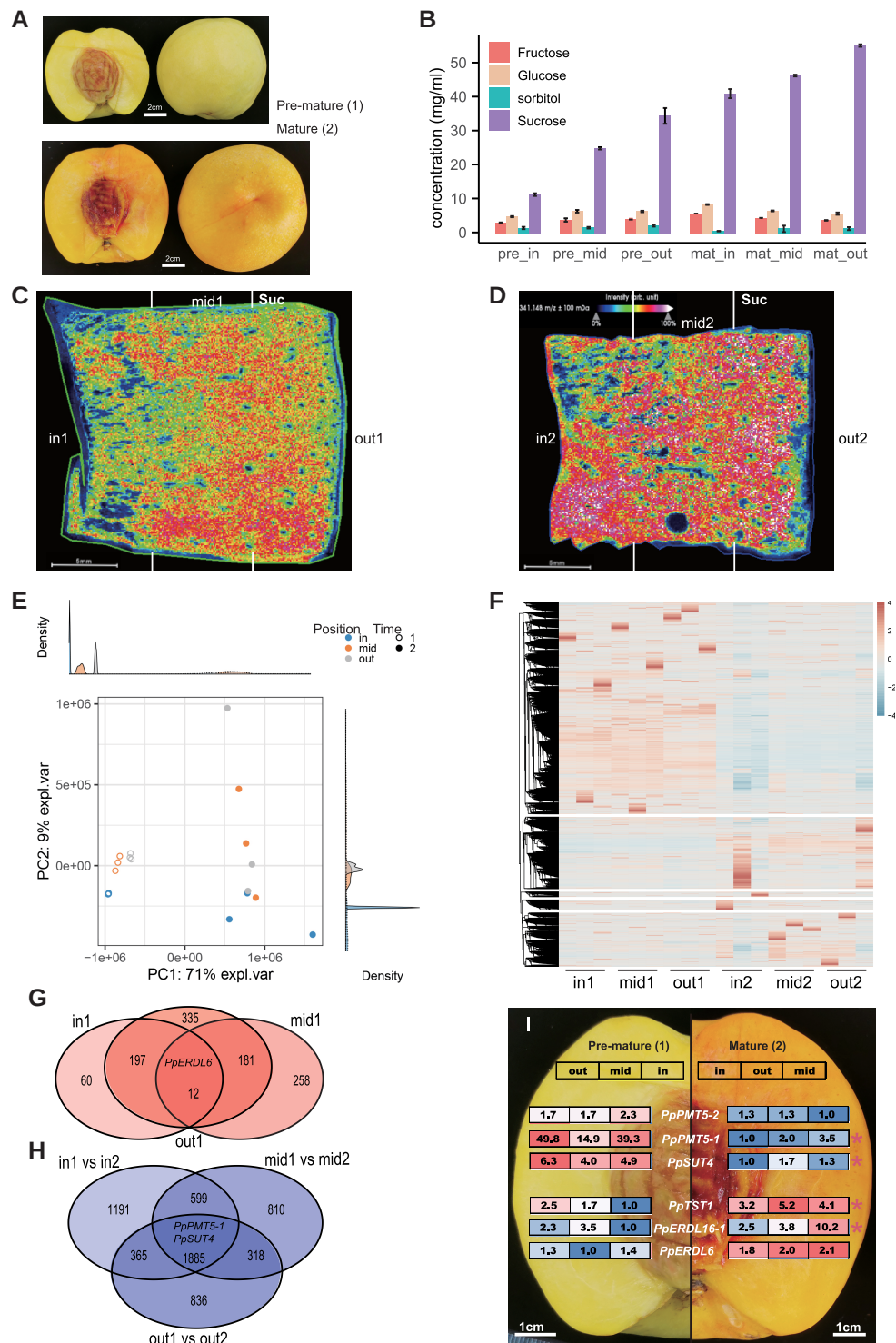


Figure 2. Sugar accumulation patterns and gene-phenotype correlations in “BG5” peach. **A**) Sugar content at 4 developmental stages (BG5_1—BG5_4). **B**) Correlation network between MFS gene expression (middle: top 10 expressed genes) and sucrose content (right: Pearson correlation coefficients) at BG5_3 (90 DAFB) and BG5_4 (105 DAFB). Significance: *** $P \leq 0.001$, ** $P \leq 0.01$, * $P \leq 0.05$, and “.” ≤ 0.1 , t-test. Error bars represent SD ($n = 3$).

Targeted spatial metabolomics and transcriptomics of peach fruit

To further analyze metabolite accumulation patterns and tissue-specific variability during peach ripening, we employed MALDI-MSI and HPLC to visualize the spatial distribution of 4 key metabolites across the premature and mature stages (Fig. 3, A to D). MALDI-MSI offers high spatial resolution, enabling the visualization of metabolite distribution across different tissue regions (e.g. inner, middle, and outer flesh), while HPLC provides precise quantification of metabolite concentrations. By integrating these approaches, we were able to both map the spatial dynamics of key metabolites and validate their quantitative changes during ripening (Skoczowsky et al. 2025).

We visualized the spatial distribution of 4 key metabolites—sucrose (m/z 341.148), glucose/fructose (m/z 179.05), glucose-6-phosphate/fructose-6-phosphate (G6P, m/z 259.064), and sorbitol (m/z 181.076)—across premature and mature stages (Fig. 3, C and D, Supplementary Fig. S3). The MALDI-MSI results were consistent with high-performance HPLC data. Sucrose showed a marked increase in intensity from the premature to mature stages, indicating progressive accumulation during ripening. Meanwhile, sorbitol exhibited a decreasing trend. The distribution of these metabolites was spatially variable, with distinct patterns observed between the inner, middle, and outer tissue regions of the fruit (Fig. 3B, Supplementary Table S5). Notably, sucrose concentrations increased substantially during ripening, forming clear



spatial gradients across the tissue zones, with the highest accumulation observed in the outer flesh (Fig. 3, C and D). The HPLC data corroborated the MALDI-MSI findings, validating the tissue-specific metabolic shifts observed during ripening. The integration of MALDI-MSI and HPLC data highlighted the importance

of sucrose accumulation and its spatial dynamics in the ripening process (Fig. 3, A to D, Supplementary Fig. S3).

To further support the spatial metabolomic findings, transcriptome sequencing (RNA-seq) was conducted, revealing dynamic changes in gene expression between premature and mature

stages. Principal component analysis demonstrated clear distinctions in gene expression between these developmental stages along PC1. Additionally, spatial proximity to the fruit core (“in,” “mid,” and “out”) substantially influenced expression patterns, particularly in the premature stage. During this stage, gene expression exhibited pronounced variability across tissues, whereas in the mature stage, this variability diminished slightly, as reflected along PC2 (Fig. 3, E and F). This pattern of gene expression aligns with the ripening-associated metabolic shifts observed in the MALDI-MSI data.

Differentially expressed genes (DEGs) analysis provided key insights into the molecular dynamics of peach ripening, highlighting significant transcriptional regulation influenced by both spatial tissue variability and developmental stage (Fig. 3, G and H, Supplementary Fig. S4, and Supplementary Table S6). In the premature stage, 12 genes, including *PpERDL6*, were commonly upregulated across the inner (“in1”), middle (“mid1”), and outer (“out1”) regions (Fig. 3G), while 37 genes were downregulated (Supplementary Fig. S4D). During the mature stage, the number of commonly upregulated genes across these tissue regions (“in2,” “mid2,” “out2”) decreased to 4, with 24 genes being downregulated, including *PpSUT4* (Supplementary Fig. S4, E and F). When both tissue region and developmental time were considered, the number of DEGs increased significantly, with 1,106 upregulated genes, including *PpTST1* (Supplementary Fig. S4A), and 1,885 downregulated genes, including *PpPMT5-1* and *PpSUT4* (Fig. 3H). These findings illustrate a clear pattern of dynamic transcriptional regulation driven by both spatial and temporal factors during ripening. When only developmental time was considered (without spatial variability), genes such as *PpERDL16-1* and *PpTST1* were significantly upregulated, while *PpPMT5-1* and *PpSUT4* were significantly downregulated (Fig. 3I, Supplementary Fig. S5, Supplementary Tables S7 and S8).

Impacts of MFS transporters on SSC and sugar in peach fruits

Our previous research demonstrated that structural variation in *PpTST1* affects SSC and sugar accumulation in peach fruits (Peng et al. 2020b; Wang et al. 2022). Building on this, we identified several DEGs for functional validation, including *PpERDL16-1*, *PpERDL6*, *PpPMT5-1*, and *PpSUT4*. Notably, *PpPMT5-2* and *PpPMT5-1* belong to the same gene family and are located adjacently on chromosome 8. To investigate the functional roles of MFS transporters in sugar metabolism, we performed transient overexpression (OE) and virus-induced gene silencing (VIGS) experiments in peach fruits using *Agrobacterium*-mediated injection (Supplementary Tables S9 and S10). Peach fruit at the premature stage was transiently transformed via injection, and samples were collected 10 days post-transformation. Browning at the injection site, referred to as “inject,” distinguished these tissues from surrounding “uninject” regions that showed no visible color change (Fig. 4, A and B). RT-qPCR analysis revealed significantly higher expression levels of MFS genes in “inject” regions compared with “uninject” and control samples, demonstrating the effectiveness of transient transformation (Supplementary Fig. S6 and Supplementary Table S11).

HPLC analysis quantified fructose, glucose, sorbitol, and sucrose in OE, silencing, and empty vector transformation samples, and “uninject” regions (Supplementary Fig. S7). Sucrose levels in “inject” regions were generally depleted, although slight variations were observed in certain genes. Notably, significant differences in sucrose levels for the same gene across different regions

underscore the complexity of sugar transport evaluation in peach flesh (Fig. 4C, Supplementary Table S12). The analysis demonstrated that MFS genes play a significant role in transporting fructose, glucose, sorbitol, and sucrose between the “inject” and “uninject” regions (Fig. 4D, Supplementary Table S13). The transport direction was determined by sugar level differences: lower sugar levels in the “Experimental” group indicated transport from “inject” to “uninject,” while higher levels indicated transport from “uninject” to “inject.” For (OE/VIGS) *PpPMT5-2*, all sugars were transported from “inject” to “uninject.” Conversely, (OE) *PpPMT5-1* transported all sugars from “uninject” to “inject,” while (VIGS) *PpPMT5-1* showed mixed transport, with sucrose moving from “uninject” to “inject” and other sugars in the opposite direction. Similarly, (OE) *PpSUT4* transported all sugars from “inject” to “uninject,” but (VIGS) *PpSUT4* reversed the direction for sucrose and sorbitol while maintaining “inject” to “uninject” transport for fructose and glucose. For (OE/VIGS) *PpERDL16-1*, all sugars were transported from “uninject” to “inject,” while (OE) *PpERDL6* moved all sugars from “inject” to “uninject.” Interestingly, in (VIGS) *PpERDL6*, sucrose transport reversed to “uninject” to “inject,” while other sugars moved in the opposite direction. Overall, *PpPMT5-1* and *PpERDL16-1* promoted sucrose accumulation in the “inject” region, while *PpPMT5-2*, *PpSUT4*, and *PpERDL6* reduced it. These findings highlight the specialized roles of MFS transporters in sugar transport pathways and their complex regulatory mechanisms.

SSC reflects total dissolved solids, including sugars, organic acids, and minerals. Here, we focused on sugar components (sucrose, glucose, fructose, and sorbitol) quantified by HPLC, while SSC was measured as a complementary trait for breeding relevance. In the control group, SSC and sugar levels were lower in the “inject” regions, likely due to sucrose consumption by injected *Agrobacterium* (Figs. 4C and 5H, Supplementary Fig. S8A, Supplementary Table S14). Among the tested genes, only (OE) *PpPMT5-1* significantly increased SSC levels ($P < 0.05$), while no significant changes were observed for others (Supplementary Fig. S8B and Supplementary Table S15). These results suggest that MFS transporters not only regulate sugar accumulation but also influence overall fruit quality through their effects on SSC.

Genome-wide quantitative trait locus mapping and functional studies of *PpTST1* in balancing sugar contents and SSC in peach

PpTST1 has been identified as a key gene in a quantitative trait locus (QTL) hotspot region (Salazar et al. 2014; Cirilli et al. 2016; Shi et al. 2020; Yu et al. 2021), with previous studies demonstrating that its structural variation affects SSC and sugar accumulation (Peng et al. 2020b; Wang et al. 2022). As shown in Fig. 1B, *PpTST1* expression increases significantly during different developmental stages across 3 peach varieties, highlighting the need to explore the mechanisms regulating its expression. To investigate the genetic basis of sugar content in peaches, we analyzed a hybrid population (“wen_07-2-30” × “wen_07-2-47”) consisting of 160 lines. Under mature conditions, substantial phenotypic variation in sugar content was observed (Fig. 5A). Using QTL mapping and genome-wide significance thresholds ($P \leq 0.05$) established through permutation tests, significant loci were identified using Lep-MAP3 software (Rastas 2017). Notably, hotspots of marker density were observed on chromosomes 2, 4, and 5, which aligns with prior findings (Verde et al. 2013). A particularly strong association was detected on chromosome 5, with a peak SNP located at position 5:1,093,132 ($-\log_{10}(p) = 5.20$) (Fig. 5A). Among the candidate genes in this region, several were linked to sugar

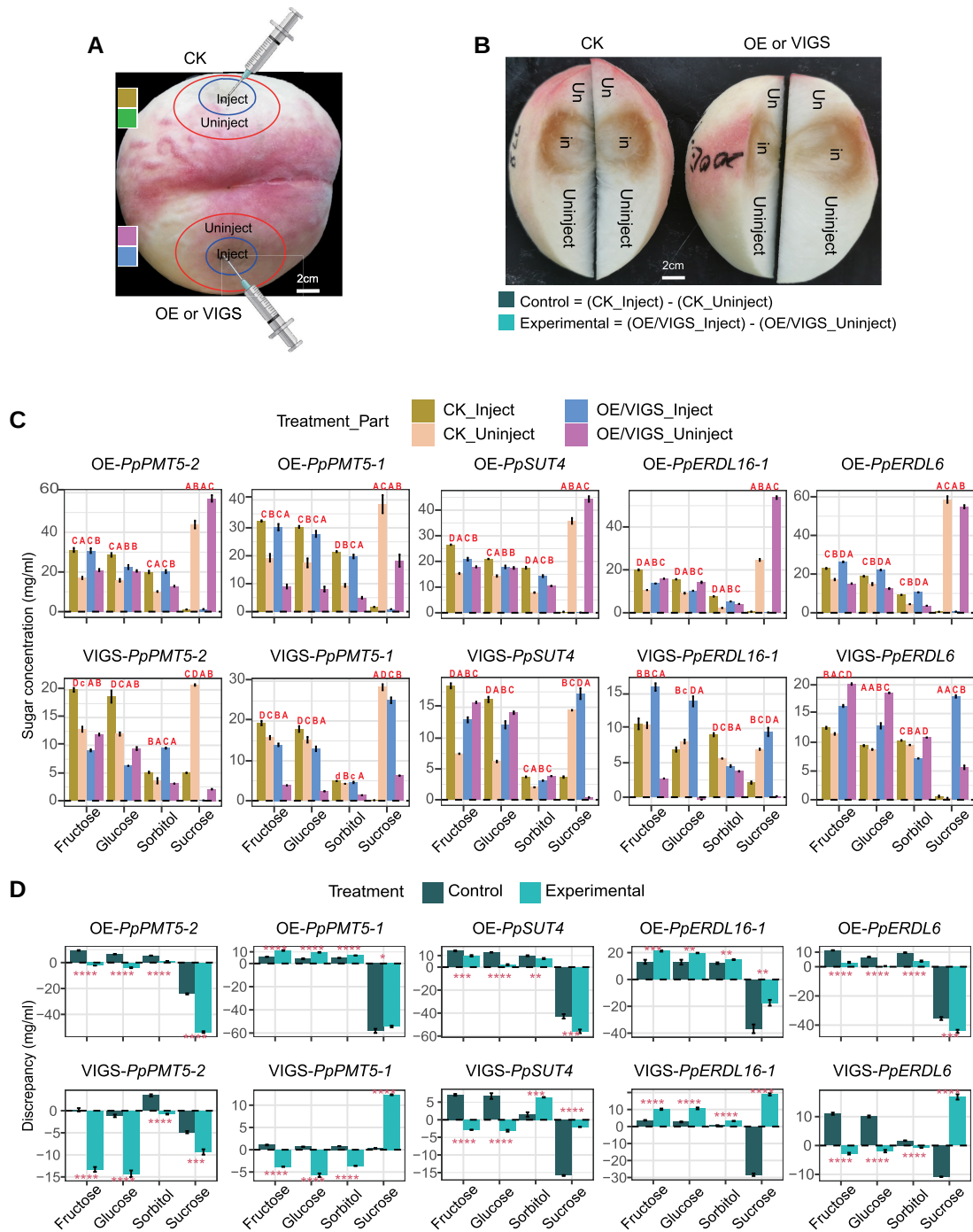


Figure 4. Schematic of transiently injected transformed peach flesh and gene expression analysis. **A)** Schematic of *Agrobacterium*-mediated injection (Inner circle: transformed region; Outer ring: control uninjected area). CK, empty vector control; OE/VIGS, OE or silencing constructs. **B)** Calculation of concentration differences between transformed and non-transformed regions. $\Delta\text{Concentration} = \text{Inject} - \text{Uninject}$. Control: $\Delta\text{Concentration} = (\text{CK_Inject}) - (\text{CK_Uninject})$; Experimental: $\Delta\text{Concentration} = (\text{OE/VIGS_Inject}) - (\text{OE/VIGS_Uninject})$. **C)** Sugar concentration (mg/mL) in "inject" and "uninject" regions. Lower sugar levels in the CK-inject regions are likely due to sucrose consumption by *Agrobacterium*. Significant differences are indicated by letters: uppercase A to D for $P \leq 0.01$ and lowercase c, d for $P \leq 0.05$ (t-test; $n \geq 3$). **D)** Differences in sugar levels between "Control" and "Experimental," calculated as "inject" minus "uninject." Significance: **** $P \leq 0.0001$, *** $P \leq 0.001$, ** $P \leq 0.01$, and * $P \leq 0.05$, t-test. Error bars represent SD ($n \geq 3$).

metabolism. Whole-genome alignment confirmed 100% similarity between *PrupeTJSM.5G000540* and *Prupe.5G006300.1* (Supplementary Table S16), demonstrating that *PrupeTJSM.5G000540* is identical to the *PpTST1* gene reported in Wang et al. (Wang et al. 2022). However, we identified a previously unreported SNP locus (Chr5:1,093,132) upstream of *PpTST1*, distinct from the exonic SNP

described previously. This upstream SNP resides within a putative regulatory region, suggesting it may modulate *PpTST1* expression levels rather than altering its protein function. This distinction highlights the potential for genetic variation in regulatory regions to fine-tune gene expression and contribute to phenotypic diversity in sugar accumulation among peach varieties.

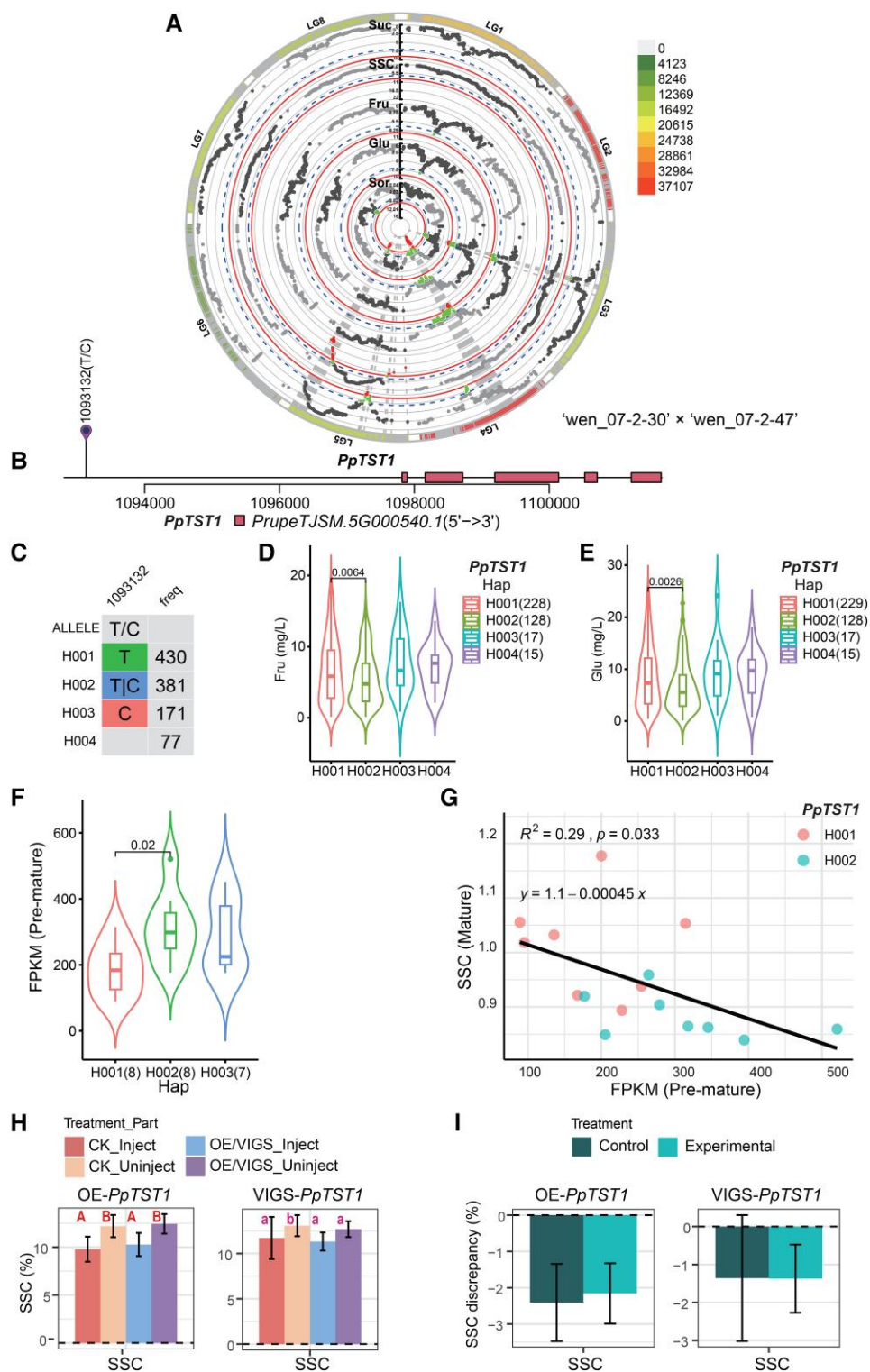


Figure 5. QTL mapping and functional analysis of sugar-related traits associated with *PpTST1* variants in peach. **A**) QTL mapping of 5 sugar traits in "wen_07-2-30" × "wen_07-2-47" hybrid population. Color scale (LOD score): dashed circles: $P \leq 0.05$; solid circles: $P \leq 0.01$. **B**) The *PpTST1* gene model (black line: genomic DNA; rectangles: exons) with upstream SNPs within 5 kb of the ATG start site (Chr5:1,093,132) across natural and hybrid populations ($n = 1,059$ accessions). **C**) Haplotype analysis of the *PpTST1* upstream region upstream region reveals 4 distinct SNP haplotypes (H001-H004) in natural and hybrid populations ($n = 1,059$ accessions), with H004 containing a T/C deletion. **D** and **E**) Significant differences in sugar-related phenotypes between haplotypes across 4 hybrid populations ($n = 495$ lines), facilitating identification of markers associated with sugar accumulation. **F** and **G**) Haplotype-based QTL mapping identifies upstream variant sites of *PpTST1* associated with gene expression and SSC in 23 natural populations. **H**) SSC from OE and VIGS experiments in "inject" and "uninject" peach fruit for *PpTST1*. **I**) SSC discrepancy between injected and uninjected sites in peach fruit for *PpTST1*. Boxplot elements: center line, median; box limits, 25th to 75th percentiles; whiskers, $1.5 \times IQR$; points, outliers. Significant differences are indicated by letters: uppercase A to D for $P \leq 0.01$ and lowercase a to d for $P \leq 0.05$, t-test; Error bars represent SD ($n \geq 3$).

Analysis of 1,059 accessions (Supplementary Table S17) revealed SNP variations in the 5000 bp upstream region of the *PpTST1* start codon (ATG), resulting in 4 distinct haplotype groups (Fig. 5, B and C). Statistical analysis of the offspring from 4 hybrid populations, including the “wen_07-2-30” × “wen_07-2-47” population (Supplementary Table S17), revealed that Hap1 was significantly associated with higher fructose ($P=0.0064$) and glucose ($P=0.0026$) levels, while Hap2 was linked to lower levels of these sugars (Fig. 5, D and E, Supplementary Fig. S9, A to J, and Supplementary Tables S18 and S19). Functional characterization of *PpTST1* demonstrated that it encodes a tonoplast-localized MFS transporter responsible for the inward transport of Fru, Glu, hexoses, and sucrose via H^+ antiport (Peng et al. 2020b; Wang et al. 2022). These results suggest that SNP variations in the upstream region of *PpTST1* influence its expression and its role in sugar accumulation.

To verify this hypothesis, genetic variants in the upstream region of *PpTST1*, *PpTST1* expression, and SSC were analyzed in 23 natural population lines (Supplementary Fig. S9, K to L, and Supplementary Table S20). The analysis revealed a significant ($P=0.02$) association between upstream genetic variants (Hap1 and Hap2) and *PpTST1* expression ($r^2=0.5123$, $P=0.0002$), indicating that haplotypes explain approximately 51.23% of the variation in *PpTST1* FPKM levels. Furthermore, under *PpTST1* expression conditions, Hap1 and Hap2 exhibited significant differences ($P=0.02$) (Fig. 5F), highlighting the substantial influence of haplotypes on *PpTST1* expression. Additionally, a negative correlation between *PpTST1* expression and SSC ($r^2=0.29$, $P=0.033$) (Fig. 5G). At the premature stage, *PpTST1* expression was significantly lower in Hap1 compared with Hap2 (Fig. 5F). Notably, by contrast, the expression response of *PpTST1* under premature was insensitive in low SSC lines in mature, *PpTST1* exhibited a negative correlation between the expression response to SSC under premature (Fig. 5G), suggesting the expression of *PpTST1* in premature is likely the causal gene leading to the natural variation in peach. In the “Control” group, SSC levels were generally lower in the “inject” regions compared with the “uninject” regions (Fig. 5H, Supplementary Table S21), consistent with Supplementary Fig. S8A. Similarly, OE of *PpTST1* resulted in a slight reduction in SSC levels, further supporting its role as a negative regulator of sugar accumulation (Fig. 5I, Supplementary Table S22). These findings demonstrate the critical role of *PpTST1* in balancing sugar content and SSC during peach fruit development, with its expression dynamics and upstream genetic variants contributing to variations in fruit quality.

Discussion

Fruit sweetness in peaches is regulated by a complex network of sugar transport and metabolism, mediated by the coordinated activity of multiple transporter families (Cirilli et al. 2016). This study highlights the critical role of MFS transporters in sugar transport and storage, offering insights into their function during peach fruit development, as previously studied in *Arabidopsis* (Niño-González et al. 2019). By integrating spatial metabolomics, transcriptomics, quantitative genetics, and functional genomics, we identified and characterized 67 MFS genes in the peach genome (Fig. 1A). Among these, *PpERDL16-1* and *PpTST1* were positively correlated with sucrose accumulation during ripening, while *PpPMT5-1*, *PpPMT5-2*, and *PpSUT4* exhibited negative correlations, suggesting their involvement in sugar efflux or catabolism during early fruit development (Figs. 1B and 3). Functional studies confirmed these roles, with OE of *PpERDL16-1* enhancing sucrose

transport and storage, while silencing *PpPMT5-1* and *PpERDL6* reduced sugar levels, underscoring their roles in sugar partitioning and compartmentalization (Fig. 4). Notably, sucrose transport pathways differed from those of glucose and fructose, indicating independent regulatory mechanisms.

Spatiotemporal regulation of sugar partitioning by MFS transporters

Our multiomics approach uncovered a spatiotemporal regulatory network in which MFS transporters dynamically coordinate sugar partitioning. For instance, *PpERDL16-1* and *PpTST1* dominate sucrose accumulation in the outer flesh during ripening (Fig. 3, D and I), while *PpPMT5-1* and *PpSUT4* may limit sugar influx into vacuoles at early stages (Zanon et al. 2015). This stage-specific specialization aligns with findings in apples, where *MdTST1/2* expression peaks during fruit maturation (Zhu et al. 2021), but contrasts with grapes, where *VvERDL6L13* is stress-inducible (Breia et al. 2020). These parallels suggest that MFS transporters play conserved yet adaptable roles in sugar homeostasis across species. By integrating spatial metabolomics and transcriptomics, we revealed how different MFS transporters contribute to sugar accumulation at distinct developmental stages and tissue regions, providing a more comprehensive understanding of the regulatory networks governing fruit sweetness.

PpTST1 as a key regulator of sugar accumulation

Genome-wide QTL mapping identified *PpTST1* as a major regulator of sugar content, located on chromosome 5 (Salazar et al. 2014; Cirilli et al. 2016; Shi et al. 2020; Yu et al. 2021) within the *D* locus, a critical region where structural variations in *Prunus* species influence traits like titratable acidity, pH, and sugar content (Yang et al. 2024). The increase in *PpTST1* expression from the pre-ripening to the ripening stage aligns with the fruit's need for enhanced sugar transport and storage as it matures (Wang et al. 2022). However, the negative correlation between high pre-ripening *PpTST1* expression and low SSC at ripening in some varieties suggests that the timing and level of *PpTST1* expression are crucial for optimal sugar accumulation (Fig. 5, F and G). This developmental regulation may involve interactions with other metabolic pathways or transcription factors.

Interestingly, while transient OE of *PpTST1* increased sugar content and reduced organic acid levels in peach fruits (Wang et al. 2022), it did not significantly alter SSC in tree-based assays (Fig. 5, H and I). This discrepancy highlights the complexity of SSC as a composite trait influenced by multiple factors, including sugars, organic acids, and other soluble compounds (Kroger et al. 2006; Cirilli et al. 2016). The lack of SSC change despite increased sucrose levels suggests that *PpTST1* OE may trigger compensatory mechanisms, such as altered organic acid metabolism or changes in water content, to maintain SSC homeostasis. This finding underscores the importance of considering broader metabolic networks when interpreting the effects of gene manipulation on fruit quality traits.

Upstream genetic variants fine-tune *PpTST1* expression

We identified an upstream genetic variant associated with distinct haplotypes (Hap1 and Hap2) providing insights into the regulatory mechanisms controlling *PpTST1* expression (Fig. 5, D and E). Hap1, linked to lower *PpTST1* expression during the pre-ripening stage and higher fructose and glucose levels, likely contains a repressive regulatory element. In contrast, Hap2,

associated with higher *PpTST1* expression, may enhance transcriptional activity. These findings suggest that natural variation in the *PpTST1* promoter region contributes to phenotypic diversity in sugar accumulation among peach varieties.

Haplotype analysis revealed that Hap1, associated with lower *PpTST1* expression, correlates with higher SSC, while Hap2, linked to higher *PpTST1* expression, results in lower SSC (Fig. 5, F and G). This inverse relationship highlights the regulatory role of upstream genetic variants in modulating *PpTST1* expression and sugar accumulation. The apparent contradiction between our findings and those of previous studies (Peng et al. 2020b; Wang et al. 2022) may arise from the differential effects of upstream genetic variants (Hap1 and Hap2) on *PpTST1* expression. We hypothesize that the upstream SNP locus (Chr5:1,093,132) may act as a regulatory element (e.g. an enhancer or repressor) that fine-tunes *PpTST1* expression in a context-dependent manner. For instance, Hap1 may enhance *PpTST1* expression during early fruit development, promoting sugar import into vacuoles, while Hap2 may suppress *PpTST1* expression during ripening, facilitating sugar export. This regulatory mechanism could explain the stage-specific roles of *PpTST1* in sugar homeostasis.

Implications for fruit quality and breeding

The functional characterization of *PpTST1* (Peng et al. 2020b; Wang et al. 2022) and its upstream regulatory variants provides valuable targets for genetic improvement of peach fruit quality (Fig. 5, D and E). By manipulating *PpTST1* expression or selecting favorable haplotypes, breeders can optimize sugar accumulation and metabolic balance in peach fruits. Additionally, our findings highlight the importance of considering developmental timing and genetic background when designing strategies to enhance fruit quality.

Some limitations should be acknowledged (Wang et al. 2022). For instance, the transient nature of our OE and silencing assays may not fully capture the long-term effects of *PpTST1* manipulation (Fig. 5, H and I). Future studies should employ stable transgenic approaches to validate these findings. Additionally, the integration of multi-omics data (e.g. transcriptomics, metabolomics, and epigenomics) will be essential to unravel the complex regulatory networks governing *PpTST1* expression and function.

The upstream regulatory region may interact with transcription factors or epigenetic modifiers to modulate *PpTST1* expression in response to developmental or environmental cues. For example, ABA signaling, which is known to influence sugar accumulation (Falchi et al. 2013; Zhu et al. 2021, 2023), may interact with the upstream regulatory region to fine-tune *PpTST1* expression during fruit ripening. Future studies should explore the molecular mechanisms underlying these regulatory interactions and their impact on sugar metabolism.

Conclusion

This study underscores the critical roles of MFS transporters, particularly *PpTST1*, in regulating sugar transport and accumulation during peach fruit development. By leveraging multi-omics approaches, we have provided a robust framework for understanding the molecular mechanisms underlying sugar accumulation. These findings not only deepen our understanding of fruit sweetness regulation but also offer practical targets for breeding programs aimed at enhancing peach fruit sweetness and quality. Future studies should explore the mechanisms underlying *PpTST1*'s stage-specific functions, particularly its interactions with hormonal signals (e.g. ABA) and environmental factors, as well as the potential regulatory roles of transcription factors and

epigenetic modifications in mediating the effects of Hap1 and Hap2 on *PpTST1* expression.

Materials and methods

Plant materials and sample collection

This study analyzed 3 peach varieties: ZB, DG, and BG5, all 14 years old, to investigate changes in sugar composition at different fruit developmental stages. These cultivars were selected based on their contrasting sugar accumulation patterns and unique metabolic profiles, providing a representative range of phenotypes for investigating sugar metabolism in peach fruit. Specifically, DG is characterized by moderate sugar content (12% soluble solids) and a distinct acidic flavor, making it a valuable model for studying the balance between sugar and acid accumulation. ZB exhibits high sugar content (13% soluble solids) and a sweet flavor, ideal for exploring mechanisms of sugar accumulation and partitioning in high-sugar peaches. BG5, with a soluble solids content of 10%, is not classified as a high-sugar variety by modern standards but exhibits unique sugar metabolism traits, particularly in sucrose accumulation, making it useful for comparative studies.

Fruit development was divided into 4 stages: S1 (rapid growth), S2 (pit hardening), S3 (second rapid growth), and S4 (fruit maturity). Stage S4 was further categorized into S4-1 (fruit reaches final size) and S4-2 (ethylene-dependent ripening) (Borsani et al. 2009). Samples from each stage were taken from at least 9 fruits, with 3 fruits per biological replicate. Additionally, tissue samples from the cultivar "KX_1729_93" were collected at premature and mature stages, categorized as "in", "mid", and "out" based on proximity to the fruit core. "KX_1729_93" has an average SSC of 9.29% at maturity, providing a contrasting phenotype to the other cultivars studied. For MALDI-MSI analysis, 1 mm thick tissue slices were prepared, covering developmental stages from premature to mature fruit (Fig. 3, C and D, Supplementary Fig. S3). For transcriptome analysis, the 6 sub-tissue samples were pooled, immediately frozen in liquid nitrogen, and used for RNA extraction (Fig. 3, E to I). Five to 6 fruits from each of the 23 natural population peach accessions were harvested 15 days before maturity, in 2016 (Cao et al. 2021), and their mesocarps were sampled to perform SSC and RNA-seq analysis (Fig. 5, F and G). The accessions exhibited an average SSC of 10.997% at maturity in 2016. To account for environmental variation, multi-year normalized SSC values were used as phenotypic data, calculated by adjusting for year-specific effects.

Evolutionary analysis

To explore the evolutionary relationships of MFS genes, the diploid peach genome (Lovell 2.0) (Verde et al. 2017) was referenced from the GDR to comprehensively identify MFS genes. Functional characterization and bioinformatics analysis of these genes were conducted using databases including SwissProt, TrEMBL, NCBI, and TAIR. We performed sequence alignment of 129 MFS amino acid sequences from *Arabidopsis thaliana* and peach using CLUSTAL W. A phylogenetic tree was constructed with MEGA 7 software using the neighbor-joining method, applying the p-distance model, 1,000 bootstrap replicates for statistical confidence, and pairwise deletion to handle missing data (Fig. 1A).

Transcriptome sequencing and expression analysis

Total RNA was extracted using a kit (Waryong, Beijing, China), treated with RNase-free DNase, and reverse-transcribed using

the Supremo III RT kit (BioTeKe, Beijing, China). RNA concentration and purity were assessed using the Agilent Bioanalyzer 2,100 and NanoDrop 2000. mRNA was enriched using Oligo(dT) magnetic beads, randomly fragmented, and first-strand cDNA was synthesized with random hexamers, followed by second-strand cDNA synthesis. The constructed library was sequenced on the HiSeq 2,500 system (Illumina) with 3 biological replicates for all peach flesh samples. Quality control of RNA sequence data included the removal of adapters, low-quality reads, and reads containing N. Clean reads were aligned to the peach reference genome (Lovell 2.0) (Verde et al. 2017) using TopHat2 (Kim et al. 2013), and gene expression levels were quantified both as raw counts and as fragments per kilobase of transcript per million mapped reads (FPKM) for correlation studies. Raw counts were normalized using VST or rlog for correlation analysis with sugar content, while FPKM values were also used for similar analyses. Pearson correlation coefficients were computed, and their statistical significance was evaluated using t-tests ($df=n-2$), with results visualized as a correlation matrix using the *corrplot* package in R (Fig. 2B). DEGs were identified from raw counts using DESeq2 ($\log_2FC \geq 1$ or ≤ -1 , $p_{adj} < 0.05$) (Fig. 3, G and H).

Phenotypic analysis for fruit flavor

Phenotypic data for fruit flavor were collected from hybrid peach populations in 2021. A single plant was sampled multiple times, each time collecting 8 to 10 commercially mature fruits. Fruit juice was obtained, filtered, and stored for subsequent analysis of sugar components. SSC was measured with a hand refractometer (Frett et al. 2012), and sugar contents (sucrose, fructose, glucose, and sorbitol) were quantified using HPLC, following methods from previous studies (Figs. 4 and 5).

Targeted spatial metabolomics

Spatial metabolomics analysis was conducted using tissue samples from the “KX_1729_93” cultivar. Frozen samples were equilibrated at -20°C for 1 h to ensure temperature uniformity before slicing. Using a Leica CM1950 cryostat, the tissues were sectioned to a thickness of $14\text{ }\mu\text{m}$ and mounted onto indium tin oxide-coated conductive glass slides. The mounted sections were dried in a vacuum desiccator for 30 min to remove residual moisture. MSI was performed on a rapifleX MALDI-TOF/TOF MS system in positive reflectron mode. Data were acquired over a mass range of m/z 200 to 600 Da with a spatial resolution of $50\text{ }\mu\text{m}$, ensuring detailed mapping of metabolites. Each spectrum was averaged over 100 laser shots, and the resulting signal intensities were normalized using total ion current for consistent data analysis. To confirm metabolite identities, MS/MS fragmentation was conducted in LIFT mode, enabling precise structural validation of the targeted metabolites. This approach provided detailed spatial localization and profiling of key metabolites within the tissue samples (Fig. 3, C and D).

Transient OE and VIGS functional validation

Transient OE and VIGS were used to validate the target gene's function. The target gene was amplified using specific primers and cDNA from ZB and “Tianjin Shuimi.” PCR products were purified and cloned into the pSAK778 vector for OE or the pTRV2 vector for VIGS. The plasmids were transformed into *E. coli* DH5 α , and positive clones were confirmed by colony PCR and sequencing (Supplementary Table S9). Recombinant plasmids were introduced into *Agrobacterium* GV3101. For OE, *Agrobacterium* carrying pSAK778_MFS was used, and for VIGS, pTRV2_MFS was used.

Cultures ($\text{OD}_{600}=0.8$) were infiltrated into peach fruits on the tree. After 7 to 10 days, peach flesh was sampled for sugar measurement and RNA extraction for RT-qPCR analysis (Supplementary Fig. S6, and Tables S10 and S11).

Peach flesh was homogenized into juice, and SSC, fructose, glucose, sorbitol, and sucrose concentrations were quantified in both OE and virus-induced gene silencing (VIGS) experiments (Supplementary Tables S12 and S14). Concentration discrepancies between transformed and non-transformed regions were calculated as: $\Delta\text{Concentration} = \text{Inject} - \text{Uninject}$ (Supplementary Tables S13 and S15).

RT-qPCR expression analysis

Total RNA was reverse-transcribed to cDNA, and real-time qPCR was performed using the SYBR Premix ExTaq Green PCR Kit on a QuantStudio 7 Flex system. *PpActin* was used as the reference gene, and relative gene expression levels were calculated using the $2^{-\Delta\Delta\text{CT}}$ method. Expression levels were normalized against the control, and technical replicates were used for each gene (Supplementary Fig. S6C, and Table S12).

Plant materials of populations and SNP calling

This study included 1,059 peach (*P. persica*) accessions, comprising 400 natural populations and 4 biparental populations. The natural populations were collected from China, the USA, Japan, South Africa, Russia, France, Italy, Canada, and Korea, consisting of wild relatives and local or breeding cultivars (Yu et al. 2018; Cao et al. 2019; Li et al. 2019; Guo et al. 2020; Guan et al. 2021). Seven high soluble solid content (SSC) parents were selected for hybridization, generating 4 biparental populations (653 plants) at the Xinxiang Comprehensive Experimental Base, Chinese Academy of Agricultural Sciences (CAAS) (Supplementary Table S17). Illumina resequencing data were processed using fastp and SNPs were identified through BWA-MEM (Jung and Han 2022) alignment and GATK (McKenna et al. 2010) filtering.

Genetic QTL mapping

SNPs from 160 individuals in the “wen_07-2-30” $\times$ “wen_07-2-47” population (160) were used for QTL mapping. The parental lines exhibited contrasting sugar accumulation: “wen_07-2-30” had an SSC of 10%, while “wen_07-2-47” had an SSC of 17%. The hybrid progeny showed a broad SSC range, averaging 12% in 2020 (7.7% to 17%) and 12.1% in 2021 (8.4% to 19.6%). Multiyear normalized SSC values, adjusted for year-specific effects, were used as phenotypic data to account for environmental variation.

SNPs were filtered using VCFtools (Danecek et al. 2011), and PLINK (Purcell et al. 2007), with common SNPs having a minor allele frequency ≥ 0.05 . Genetic maps were constructed using Lep-MAP3 (Rastas 2017), and QTLs were mapped using qtl.R and qtlPerm.R, with the female parent map containing 8 linkage groups (Fig. 5A).

Accession numbers

The genomic sequences analyzed in this study are publicly available in GenBank/EMBL databases. Key gene accessions include: *PpTST1* (Prupe.5G006300.1, and *PrupeTJSM*.5G000540). Complete accession numbers for all genes and full datasets are provided in Supplementary Tables S1 and S6.

Acknowledgments

We extend our gratitude to the members of the Cao Lab at the Zhengzhou Fruit Research Institute, CAAS, and the Zhou Lab at AGIS, CAAS, for their insightful discussions and valuable comments that contributed to this study.

Author contributions

K.C. and Y.Z. conceived and designed this project; X.Y., C.C., and H.W. collected plant material. X.Y., W.Y., S.C., and J.W. performed the data analysis. K.C. and Y.Z. provided good guidance for data analysis. W.Y., X.Y., J.L. (Jiacui), M.D., H.X., Y.L. (Yuting), J.L. (Jianzhong), and Y.W. assisted in bioinformatics analyses. X.Y. and W.Y. wrote the manuscript. Y.Z. and K.C. revised the manuscript. All authors revised and approved the final manuscript.

Supplementary data

The following materials are available in the online version of this article.

Supplementary Figure S1. Flowchart of sugar metabolism and transport-related gene identification and validation.

Supplementary Figure S2. Correlation and significance of candidate MFS genes with sucrose, glucose, and fructose during developmental stages BG5_1—BG5_4 in “Babygold 5”. BG5_1—BG5_4: 60, 75, 90, and 105 days after full bloom.

Supplementary Figure S3. Spatial metabolomic profiling of peach fruit development in cultivar “KX_1729_93”.

Supplementary Figure S4. Differential expression analysis of “KX_1729_93” across different fruit parts (inner flesh vs. middle flesh, middle flesh vs. outer flesh, inner flesh vs. outer flesh) and time points (premature and mature), highlighting significant up-regulation and downregulation in specific comparisons.

Supplementary Figure S5. Heatmap of RNA-seq counts for 6 key MFS genes in “KX_1729_93” across fruit parts (inner, middle, outer flesh) and stages (premature, mature), with “*” indicating $P \leq 0.05$, t-test.

Supplementary Figure S6. Cloning and transient transformation of candidate genes in peach fruit.

Supplementary Figure S7. HPLC validation of sugar metabolite quantification methods.

Supplementary Figure S8. Analysis of soluble solid content (SSC) following MFS gene transformation in peach fruit.

Supplementary Figure S9. Genetic analysis of haplotype associations with sugar-related traits in peach populations.

Supplementary Table S1. A total of 129 MFS genes were included in the phylogenetic tree, including 67 from peach.

Supplementary Table S2. FPKM values of selected MFS gene family members; transcriptomic data for ZB, DG, and BG5.

Supplementary Table S3. Sugar content at 4 developmental stages in BG5.

Supplementary Table S4. Statistical significance and relationships of 10 MFS genes in sucrose-increased BG5-3 and BG5-4 stages in BG5.

Supplementary Table S5. Sugar content at premature and mature stages in “KX_1729_93”, analyzed by HPLC.

Supplementary Table S6. DEGs in “KX_1729_93” across peach fruit parts (inner, middle, outer flesh) and stages (premature, mature).

Supplementary Table S7. Raw RNA-seq counts of 10 MFS genes in “KX_1729_93” across peach fruit parts (inner, middle, outer flesh) and developmental stages (premature, mature).

Supplementary Table S8. Normalized average RNA-seq raw counts of 10 MFS genes in “KX_1729_93” across fruit parts (inner, middle, outer flesh) and developmental stages (premature, mature).

Supplementary Table S9. Primer sequences for the genes *PpPMT5-2*, *PpPMT5-1*, *PpSUT4*, *PpTST1*, *PpERDL16-1*, and *PpERDL6*.

Supplementary Table S10. The RT-qPCR primer sequences for the genes *PpPMT5-2*, *PpPMT5-1*, *PpSUT4*, *PpTST1*, *PpERDL16-1*, and *PpERDL6*.

Supplementary Table S11. Relative expression levels of *PpPMT5-2*, *PpPMT5-1*, *PpSUT4*, *PpERDL16-1*, and *PpERDL6* under OE and virus-induced gene silencing (VIGS) conditions in transiently transformed peach fruit, indicating significant differences.

Supplementary Table S12. Trait data for fructose, glucose, sorbitol, and sucrose from OE and VIGS experiments in “inject” and “uninject” peach fruit parts, focusing on *PpPMT5-2*, *PpPMT5-1*, *PpSUT4*, *PpERDL16-1*, and *PpERDL6*.

Supplementary Table S13. Trait data for fructose, glucose, sorbitol, and sucrose from OE and VIGS experiments in different peach fruit parts, with discrepancies calculated as “inject” minus “uninject”, focusing on *PpPMT5-2*, *PpPMT5-1*, *PpSUT4*, *PpERDL16-1*, and *PpERDL6*.

Supplementary Table S14. SSC data from OE and VIGS experiments in “inject” and “uninject” peach fruit, focusing on *PpPMT5-2*, *PpPMT5-1*, *PpSUT4*, *PpERDL16-1*, and *PpERDL6*.

Supplementary Table S15. SSC data from OE and VIGS experiments in different peach fruit parts, with discrepancies calculated as “inject” minus “uninject”, focusing on *PpPMT5-2*, *PpPMT5-1*, *PpSUT4*, *PpERDL16-1*, and *PpERDL6*.

Supplementary Table S16. Comparative analysis of 67 MFS genes between the 2 peach cultivars “Lovell” v2.0 and “Tianjin Shuimi”.

Supplementary Table S17. Summary of 1,059 peach population samples used for analysis in this study.

Supplementary Table S18. Significantly associated loci and the genes of peak SNPs related to sugar content traits based on QTL mapping results through the hybrid populations “wen_07-2-30” × “wen_07-2-47” (QTL, $P < 0.05$).

Supplementary Table S19. Summary of haplotype analysis, including genotype and variant information for *PpTST1*.

Supplementary Table S20. The genetic variants in the upstream region of *PpTST1* and their association with *PpTST1* expression levels and SSC across 23 natural populations.

Supplementary Table S21. SSC data from OE and VIGS experiments in “inject” and “uninject” peach fruit, focusing on *PpTST1*.

Supplementary Table S22. SSC data from OE and VIGS experiments in different peach fruit parts, with discrepancies calculated as “inject” minus “uninject”, focusing on *PpTST1*.

Funding

This study was supported by grants from the National Key Research and Development Program (2023YFE0105400), the Agricultural Science and Technology Innovation Program (CAAS-ASTIP-2020-ZFRI-01), and the National Horticulture Germplasm Resources Center. Additionally, it received funding from the Science Fund Program for Distinguished Young Scholars of the National Natural Science Foundation of China (Overseas) to Y.Z., and the Open Funds of National Key Laboratory for Tropical Crop Breeding (No. NKLTCB202402).

Conflict of interest statement. None declared.

Data availability

All PacBio sequence data have been deposited in the NCBI Sequence Read Archive under project number PRJNA1054471 and the

National Genomics Data Center Genome Sequence Archive for a hybrid population with BioProject number (GSA) (<https://ngdc.cncb.ac.cn/gsa/>), with BioProject number PRJCA039584. All other relevant data supporting the findings of the study are available in this article and its Supplementary files, or from the corresponding authors upon request.

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