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Identification of the *SWEET* gene family and functional characterization of PsSWEET1a and PsSWEET17b in the regulation of sugar accumulation in 'Fengtang' plum (*Prunus salicina* Lindl.)

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Abstract

Background 'Fengtang' plum is a cultivar known for its significant sugar accumulation. Although various studies have been conducted on sugar metabolism, the specific molecular mechanisms underlying the high sugar accumulation in 'Fengtang' plum remain largely unexplored. Here, we present the role of the *Sugars Will Eventually be Exported Transporters* (SWEETs) family in regulating sugar accumulation in 'Fengtang' plum fruits.

Results In this study, 18 *PsSWEET* genes were identified based on homology with *Arabidopsis* genes and the Pfam database (ID: PF03083). Alignment of multiple sequences revealed that the seven alpha-helical transmembrane regions (7-TMs) are largely conserved in the *PsSWEET* family. Phylogenetic analysis demonstrated that the 18 *SWEET* family members could be categorized into four clades. Nine predicted motifs were identified within the *PsSWEET* genes of plum. The *PsSWEET* genes were unevenly distributed across five chromosomes, and synteny analysis revealed three pairs of fragmented duplication events. *PsSWEET1a* and *PsSWEET17b* are pivotal in 'Fengtang' plum fruit development. Subcellular localization analyses indicated that *PsSWEET1a* is localized to the nucleus and cytoplasm, while *PsSWEET17b* is associated with the vacuolar membrane. Gene function was further validated through transient silencing and overexpression of the *PsSWEET1a* and *PsSWEET17b* genes in plum fruits, which significantly impacted their soluble sugar content. Heterologous expression of *PsSWEET1a* and *PsSWEET17b* in tomato resulted in an increase in soluble sugar content due to the modulation of sugar accumulation-related genes and enzyme activities.

Conclusion The genes *PsSWEET1a* and *PsSWEET17b*, which regulate the content of soluble sugar in plum fruit, were successfully identified. This study provides a comprehensive insight into the *SWEET* gene family of plum, offering novel perspectives on the regulation of sugar accumulation in fruit and laying a critical foundation for further enhancement of plum fruit quality.

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Keywords *SWEET* gene family, Sugar accumulation, Fruit, 'Fengtang' plum

Introduction

As the main product of photosynthesis, sugars serve as a carbon and energy source for plant growth and development [1], and are crucial for fruit development and quality [2]. Numerous studies have explored fruit sugar accumulation and "source-sink" sugar transport pathways, in which photosynthetic products exported from source leaves of higher plants travel through the phloem long-distance transport system before reaching fruit cells [3], providing energy for cell metabolism or accumulating in cells, and these processes are inseparable from the participation of sugar transporters [4]. The distribution of carbohydrates is critical for crop yield. Therefore, exploring the *SWEET* function in plants is of great significance. To date, three types of sugar transporters have been identified: monosaccharide transporters (MSTs), sucrose transporters (SUTs), and bidirectional sugar transporters (SWEETs), categorized based on the types of sugars they transport [5–7].

The *Sugars Will Eventually be Exported Transporters* (*SWEETs*) family consists of key transporters that play essential roles in sugar transport pathways [8]. The first member of the *SWEET* family, *MtN3*, was first identified in *Medicago truncatula* [9]. SWEET proteins possess seven transmembrane helices and are recognized as a novel group of sugar transporters that facilitate the bidirectional transport of sugars, including glucose, fructose, and sucrose. They are present in a wide range of plant genomes [10]. Biochemical and functional analyses have demonstrated that SWEET proteins are involved in various metabolic and physiological processes in plants, including phloem loading [11], nectar secretion [12], pollen development [13], bacterial infection [14], aging [15], seed filling, and transportation [7].

In plants, the *SWEET* gene family plays an important role in sugar accumulation by regulating the transmembrane transport of molecules such as sucrose, glucose, and fructose. In tomato, the gene *SlSWEET1a* was found to play a role in glucose outflow from mature leaves to young leaves [16]; similarly, *OsSWEET7* in rice has galactose transport activity [15]. In apples, molecular markers have identified *MdSWEET9b* and *MdSWEET15a* as key genes for sugar accumulation in fruits, regulating sugar content in fruits [17]. In addition, the gene *AtSWEET16* in *Arabidopsis* transfers glucose from the vacuole to the cytoplasm at low temperatures [18], and *AtSWEET17* is responsible for fructose transport in the vacuole [19]. Therefore, the *SWEET* gene family plays a decisive role in fruit sugar accumulation and the transport of sugars between source and reservoir organs. Sucrose is the main form of carbon transported from source to reservoir, which is important for sugar accumulation in fruits, and certain *SWEET* proteins can specifically transport sucrose [20]. *PuSWEET15* shows a significant positive correlation with sucrose concentration. Sucrose content increased after the overexpression of *PuSWEET15* in pear fruits, while sucrose content decreased after silencing, demonstrating its impact on sucrose accumulation in pear fruits [21]. *GmSWEET10a* and *GmSWEET10b* transport sucrose in soybeans, distributing sugars between the seed coat and embryo, thereby determining oil and protein content and seed size [22]. These studies highlight the role of the *SWEET* gene family in fruit sugar accumulation.

The 'Fengtang' plum is a regionally distinctive variety in Guizhou, known for its unique flavor, which is primarily due to its high sugar content [23]. Our team conducted a preliminary investigation into the physiological and molecular mechanisms of sugar accumulation in plum fruit [24], revealing the impact of the *SWEET* gene family on sugar accumulation. Therefore, a more comprehensive characterization of the *SWEET* gene family members was necessary to elucidate their role in sugar accumulation in plum fruit. This study identified members of the *SWEET* gene family within the entire genome of *Prunus salicina* plum and analyzed their expression patterns at different developmental stages through RNA-seq. Subsequently, the role of the *SWEET* genes in sugar accumulation in plum fruit was further clarified through transient expression in plum fruit and genetic transformation in tomato. This study enhances our understanding of the mechanisms behind plum sugar accumulation and provides a theoretical foundation for future enhancements to fruit quality, as well as potential increases in soluble sugar levels within plum fruit.

Materials and methods

Plant materials

All the plum germplasm used in this study was preserved at the 'Fengtang' plum base in Huishui County, Guiyang City, Guizhou Province, China. Material 1: The six-year-old 'Fengtang' plum tree was selected for the experiment. Samples were collected from 30 days to 110 days after flowering 'Fengtang' plum from April (average temperature 8–22℃, relative humidity 40–86%) to July (average temperature 24–33°, relative humidity 48–75%) 2023, with samples taken every 10 days. Nine fruits of uniform size were selected for each treatment, then stored at ultra-low temperatures. Various tissues and organs, including blooming flowers, functional leaves, roots, and fruit stalks, were also collected. Material 2: VIGS and transient overexpression

tests of *PsSWEET1a* and *PsSWEET17b* were performed on 90 disease-free, uniformly growing fruits from nine plum trees. A 1 mL suspension was slowly injected into the equatorial region of the live plum fruit. Simultaneously, samples for RNA extraction and sugar composition analysis were taken from the pulp 0.5 cm from the injection site. The collected plums were peeled, cored, mixed, and quickly frozen with liquid nitrogen (-196℃), then stored in an ultra-low temperature freezer (-80℃) for RNA extraction and biochemical analysis. Material 3: Tomato (*Solanum lycopersicum* L. 'Micro-Tom') was provided by Wuhan Boyuan Company. Mature leaves from T2 plants and transgenic tomato fruits at the red ripening (RR) stage were collected to measure sugar content and analyze gene expression. At each sampling site, at least three fruits (excluding transgenic strains) or three mature leaves (from the same location on each plant) were randomly selected and collected together. All samples were immediately frozen in liquid nitrogen and kept at -80℃ until analysis was performed.

Determination of sugar content and enzyme activity

The contents of fructose, glucose, sucrose, and sorbitol in plum fruit were determined using high-performance liquid chromatography (HPLC) [25]. The activities of CWINV, VINV, NINV, SUSY, and SPS were determined using enzyme-linked immunosorbent assay (ELISA). The ELISA kit was obtained from Shanghai Zike Biotechnology Co., Ltd.

Identification of *SWEET* gene family and chromosomal localization of 'fengtang' Plum

The genome sequences of *Prunus salicina* Lindl. were obtained from the Rosaceae Genome Database (https://www.rosaceae.org/). Two methods were used to identify members of the *SWEET* family in 'Fengtang' plums: hidden Markov model search (HMMER) (http://pfam.xfam.org/) and BLASTp search using the MtN3/Saliva domain (PF03083) from the PFAM database, downloaded from the TAIR database (https://www.arabidopsis.org/) using *Arabidopsis thaliana* with an E-value < 1.0 as the query. The obtained candidate protein sequences were submitted to PFAM (http://pfam.xfam.org/) to verify the structure of the MtN3/Saliva domain. The SWEET genes identified from the *Prunus salicina* genome and those screened from the 'Fengtang' plum RNA-seq data (determined by Professor Huaming An's research group at Guizhou University) were used as criteria for selecting candidate *SWEET* gene family members. ProtParam tools (http://www.expasy.org/tools) were used to analyze the physical and chemical properties of *SWEET* genes, including molecular weight and theoretical isoelectric point. Plant-mPLoc was used for subcellular localization prediction (http://www.csbio.sjtu.edu.cn/bioinf/plant-multi/). The chromosomal locations of *SWEET* genes in *Prunus salicina* were identified from the genome database (https://www.rosaceae.org/). The positions of *SWEET* genes on the chromosomes of *Prunus salicina* were mapped using TBtools software (v1.113) [26] and used for collinearity analysis within the species.

Phylogenetic analysis of the *SWEET* gene family in *Arabidopsis thaliana*,*Prunus persica*,*Prunus avium*, and 'fengtang' Plum

The *Arabidopsis thaliana* genome was downloaded from the TAIR database (https://www.arabidopsis.org/), and *Prunus persica* and *Prunus avium* SWEET protein sequences were obtained from the NCBI database (https://www.ncbi.nlm.nih.gov/guide/). MEGA 7 was used to construct the phylogenetic tree of the *SWEET* gene family with the following parameters: multiple alignments of SWEET protein sequences were generated using Clustal W, and the evolutionary tree was constructed using 1000 bootstrap replicates based on the neighbor-joining (NJ) method. The resulting file was submitted to the EvolView V2 website (http://www.evolgenius.info/evolview/) to visualize the evolutionary tree.

Conserved motif and gene structure analysis of *SWEET* genes in 'fengtang' Plum

TBtools v1.113 was used to map the exon–intron structure of the *SWEET* gene family members. The MEME online tool (https://meme-suite.org/meme/tools/meme) was used to predict highly conserved *SWEET* sequences, and TBtools was used for visualization.

Sequence and protein structure analysis of the *SWEET* gene family in 'fengtang' Plum

Online tool TMHMM (https://services.healthtech.dtu.dk/service.php) was used to predict the transmembrane domain of *SWEET* proteins, and MTN3/salivary predictive structure analysis of *SWEET* proteins was performed using TBtools v1.113.

Construction of the expression vector

Using primers (forward: 5'-GAGTAAGGTTACCGAATTCCAGTTCTCAGGCATTCCGTAT (EcoR I)-3', reverse: 5'-TGAGCTCGGTACCGGATCCCTTGGTTCTGATCACTGTGC (BamH I)-3'), we amplified a 357 bp cDNA fragment of *PsSWEET1a* (112–468 bp); Using primers (forward: 5'-GAGTAAGGTTACCGAATTCCAAATAGCGTGCTGGTG (EcoR I)-3', reverse: 5'-TGAGCTCGGTACCGGATCCCAAGAATGGCATAGAGTGTC (BamH I)-3'), we amplified a 360 bp cDNA fragment of *PsSWEET17b* (187–547 bp). The pTRV2 construct was prepared and digested with EcoR I and BamH I, and the vector and amplified fragment were ligated using the pEASY-Basic Seamless Cloning and Assembly

Kit (TransGen Biotech, Beijing, China). *Agrobacterium* transformation was then performed. *Agrobacterium* strain GV3101 containing pTRV1, pTRV2, or the pTRV2 derivative pTRV2-*PsSWEET1a*/*PsSWEET17b* was used for virus-induced gene silencing (VIGS). The full-length open reading frames of *PsSWEET1a* and *PsSWEET17b* were amplified from the cDNA of 'Fengtang' plum. Primers with restriction sites were designed using SnapGene software. For transient overexpression of *PsSWEET1a*, the primers were: forward 5'-GAACACGGGGGACGAGCTCGGTACCATGCATATTCTCAAGGTCTTCTTTGG (Kpn I)-3', reverse 5'-GCCAAATGTTTGAACGATCCTGCAGCTAGGGCTGCCCATCTTG (Pst I)-3'; For transient expression of *PsSWEET17b*, the primers were: forward 5'-GAACACGGGGGACGAGCTCGGTACCATGGCAATAAATTTCGTTGTCATTTTTGG (Kpn I)-3', reverse 5'-GCCAAATGTTTGAACGATCCTGCAGTCACTCTGCAATGATGGGTTTTGAAG (Pst I)-3'. The overexpression construct pCambia1301-GFP was prepared, and the plasmid was isolated and digested with restriction enzymes Kpn I and Pst I. The digested vector and amplified fragment were ligated using the pEASY-Basic Seamless Cloning and Assembly Kit (TransGen Biotech, Beijing, China), and then transformed into *Agrobacterium*. *Agrobacterium* strain GV3101 containing pCambia1301-GFP or the pCambia1301-GFP derivative pCambia1301-GFP-*PsSWEET1a*/*PsSWEET17b* was used for transient gene overexpression.

RNA extraction and qRT-PCR validation

Total RNA was extracted from the samples using a polysaccharide and polyphenol plant total RNA extraction kit (Tian Gen, Beijing, China), and genomic DNA contamination was removed using DNase I (Takara, Dalian, China). Complementary DNA (cDNA) was synthesized using the PrimeScript RT reagent kit with gDNA Eraser (Takara, Dalian, China) according to the manufacturer's instructions. Amplification was performed using the Applied Biosystems StepOnePlus Real-Time PCR system (Life Technologies Corporation, Carlsbad, CA, USA) in a total volume of 20 μL. The reaction mixture contained: TB Green Premix (2X) 10 μL; upstream primer (10 μmol/L) 0.4 μL; downstream primer (10 μmol/L) 0.4 μ; template 2 μL; ddH_2O 7.2 μL. Candidate gene primers were designed using Primer5, and the clathrin adaptor complex medium subunit family protein (*CAC*) was used as the internal reference gene [27]. The tomato housekeeping gene *ACTIN* was used as an internal control (Table S1). The primers were synthesized by Shanghai Shenggong Biotechnology Co., Ltd. The following conditions were used for the PCR procedures: pre-denaturation at 94℃ for 30 s, 94℃ for 5 s, 58℃ for 30 s, 72℃ for 1 min, for 40 cycles; final extension at 72℃ for 10 min. Three biological replicates were performed per sample. Relative quantitative analysis of the data was performed using the $2^{-\triangle\triangle CT}$ method.

Subcellular localization of *PsSWEET1a* and *PsSWEET17b* proteins

The coding regions of the *PsSWEET1a* and *PsSWEET17b* genes (without stop codons) with Kpn I and Pst I restriction sites were cloned into a pCAM35-GFP expression vector. The resulting constructs were then transformed into *Agrobacterium tumefaciens* strain GV3101 and used to infect *Arabidopsis* protoplasts. After 3–4 weeks, *Arabidopsis* seedlings were soaked in an enzymatic solution, vacuum infiltrated, and subjected to enzymatic hydrolysis at 23 °C for 3 h under low light. The protoplasts were filtered through 40 μm nylon mesh, washed several times with W5 solution, and diluted. For transfection: 10 μL target gene plasmid + 10 μL marker gene plasmid + 100 μL protoplast + 120 μL 40% PEG4000 solution. The mixture was gently inverted in a 22.5 °C water bath for 15–20 min. The protoplasts were then diluted with W5 solution, washed, centrifuged twice, resuspended in W5 solution, and cultured overnight at 23 °C under low light. Laser confocal microscopy (Laser confocal microscopy, Nikon, A1 HD25) was used for observation and imaging.

Genetic transformation of tomato

The CDS sequence of the candidate gene was cloned into the pCAMBIA-1301 vector after the removal of the stop codon. The recombinant plasmid was transformed into *Agrobacterium* strains by electroporation and preserved at -8℃. The method for *Agrobacterium*-mediated genetic transformation was as follows: Tomato (*Solanum lycopersicum* L. 'Micro-Tom') seeds were disinfected and grown in a sterile environment. The seeds were cultured in the dark for 3 days before being transferred to light. Remove the root, take the cotyledon with the backside up, and culture in darkness on KCMS solid medium with filter paper. Cotyledons were immersed in an *Agrobacterium* suspension containing 35 S::*PsSWEET1a* or 35 S::*PsSWEET17b* constructs, shaken for 2 min, and the bacterial suspension was poured out. The cotyledons were blotted dry with sterile filter paper to remove residual liquid, then returned to KCMS solid medium for 2 days in the dark. The cotyledons were then placed on co-culture medium and incubated in darkness for 3 days. The cotyledons were cleaned with cephalosporins and transferred to a screening medium under light conditions until callus formation. When the seedlings reached 4–5 cm in height, they were transferred to rooting medium. After 3 weeks of rooting, the seedlings were transplanted into the growing medium. Both wild-type and transgenic tomato plants were grown in greenhouses with a 16-hour/8-hour light/dark cycle, a temperature of 25 °C, and a relative humidity of 50–70%. The transgenic

lines were verified by PCR. T2 transgenic tomato plants were used for analysis.

Statistical analysis

Data are expressed as mean ± SD (standard deviation). SPSS (v27, IBM, Armonk, NY) was used for significance testing, and the Student's t-test was applied. The Pearson correlation coefficient (r) was used to analyze the correlation. Line charts were generated using Origin 9.0, and heat maps were produced using TBtools software.

Results

Identification and characterization of SWEET gene family members in 'fengtang' Plum

According to the Pfam database (ID: PF03083), 18 *SWEET* members were identified by combining the transcriptome with the genome of *Prunus salicina* Lindl. The physicochemical properties of the members of the *SWEET* gene family were determined via the online website ProtParam (Table. S2). The 18 *SWEET* genes varied in length, ranging from 364 bp (*PsSWEET1c*) to 901 bp (*PsSWEET10* and *PsSWEET12*), and encoded amino acids ranging from 120 to 293. The molecular weight ranged from 13,595.40 Da (*PsSWEET1c*) to 33,080.55 Da (*PsSWEET10* and *PsSWEET12*). The theoretical isoelectric points (pIs) of the presumed *SWEET* family members ranged from 5.97 (*PsSWEET10/12*) to 9.68 (*PsSWEET1a*), with 16 members having pIs higher than 7.0, indicating that these proteins were weakly alkaline, while *PsSWEET10* and *PsSWEET1c* were acidic. Further analysis of the presumed subcellular localization revealed that *SWEETs* were located in the cell membrane. The TMHMM software was used to predict the transmembrane regions of *SWEETs*. The encoding proteins of 13 members had seven transmembrane regions. Two members had five transmembrane regions. *PsSWEET1b/1c* had only two or three transmembrane regions. Ten *SWEET* proteins (*PsSWEET1a/1b/1c/2b/5d/10/14/17a/17b/17c*) had an instability index of more than 40, indicating that they were not stable. The hydrophilicity of plum *SWEET* proteins was greater than 0, indicating that they were hydrophobic proteins.

Thirteen *SWEET* genes were distributed on five chromosomes (LG 01, LG 02, LG 03, LG 04 and LG 08), and five *SWEET* genes were located on the unplaced scaffolds Contig 1 and Contig 20. Among these chromosomes, LG 08 has up to six *SWEET* genes, including *PsSWEET1a,PsSWEET1b,PsSWEET1c,PsSWEET5a,PsSWEET5b*, and *PsSWEET14*. In addition, LG 03, LG 02, and LG 04 each have two *SWEET* genes, while LG 01 has only one *SWEET* gene on its chromosome (Fig. 1A). Gene duplication events the amplification of gene family members in the process of plant evolution plays an important role (Fig. 1B). Intra-species collinearity analysis showed that there were 3 collinear gene pairs, *PsSWEET2a* and *PsSWEET2b* fragment repeat events. *PsSWEET1b* and *PsSWEET1c,PsSWEET5a* and *PsSWEET5b* are thought to have evolved from tandem repeating events.

Phylogenetic tree analysis of *SWEET* genes in *Arabidopsis thaliana,Prunus persica,Prunus avium*, and 'fengtang' Plum

To explore the phylogenetic relationships among *SWEET* gene family members in plants, we analyzed 17 *SWEET* genes from *Arabidopsis thaliana*, 19 from *Prunus persica* L., 13 from *Prunus avium* L., and 18 from 'Fengtang' plum. The amino acid sequences of 67 *SWEET* proteins were compared, and an evolutionary tree was constructed (Fig. 2; Table S3). Based on studies of *AtSWEET* in *Arabidopsis thaliana*, the *SWEET* members of these four species were divided into four subfamilies (I, II, III, and IV). The majority of *PsSWEET* members in 'Fengtang' plum were grouped in subfamily II, with 6 *PsSWEET* members; subfamily I followed with 5 *PsSWEET* members; and there were 4 and 3 *PsSWEET* proteins in subfamilies III and IV, respectively. As shown by the evolutionary tree, most 'Fengtang' plum *SWEET* genes were more closely related to those in *Prunus persica* than to those in *Prunus avium* and *Arabidopsis thaliana*.

Sequence and motif analysis of *SWEET* genes in 'fengtang' Plum

We constructed a phylogenetic tree using 18 PsSWEET proteins in 'Fengtang' plum and divided them into four classes (Fig. 3A), consistent with the results in Fig. 1. Nine highly conserved motifs were identified in PsSWEET proteins (Fig. 3B and D). *PsSWEET1b* and *PsSWEET5a* contained the fewest conserved motifs, with only four, while *PsSWEET4* and *PsSWEET5d* contained nine motifs each. Motifs 2, 3, and 6 were present in all PsSWEET proteins. Introns, a characteristic feature of eukaryotic genes, provide genetic diversity through alternative splicing (Fig. 3C). Exon-intron structure analysis revealed that most *PsSWEET* genes had five or six exons, while *PsSWEET1c* contained only three exons and *PsSWEET17c* contained seven exons.

SWEET proteins in plant species contain seven transmembrane (TM) helices, and the presence of these seven TM regions in all *SWEET* members suggests that *SWEET* proteins function as membrane receptor proteins. Conserved domain analysis using NCBI-CDD confirmed that all *SWEET* proteins contained 1–2 MtN3/saliva domains (CDD accession No. pfam03083) or PQ-loop superfamily domains (CDD accession No. cl21610). These MtN3/saliva domains ranged from 70 to 91 amino acids, with most being approximately 85 amino acids in length. The positions of the MtN3/saliva domains in the protein are shown in Fig. S1. To obtain more detailed information about the *PsSWEET1a* and *PsSWEET17b* proteins, we

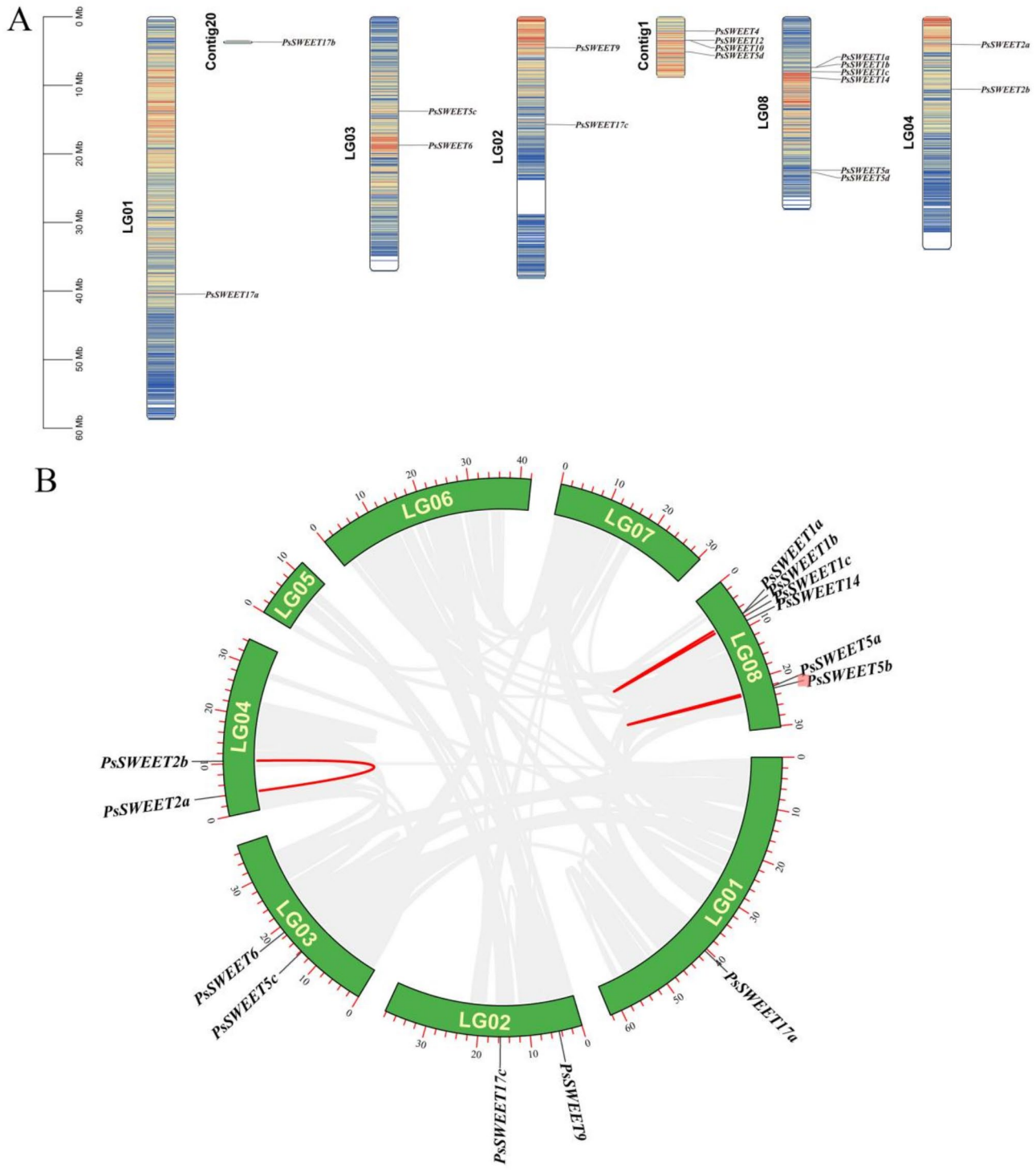

Fig. 1 Chromosome localization and collinearity analysis in *SWEET* genes. (**A**) The relative locations of *SWEET* genes are marked on the chromosomes. Chromosome numbers are displayed on the left side of each chromosome, and a ruler on the left shows the physical distance of the chromosomes. (**B**) Collinearity analysis of *SWEET* genes. Gray lines represent all the syntenic blocks in the *Prunus salicina* Lindl. genome, and the red lines represent duplicated *SWEET* gene pairs

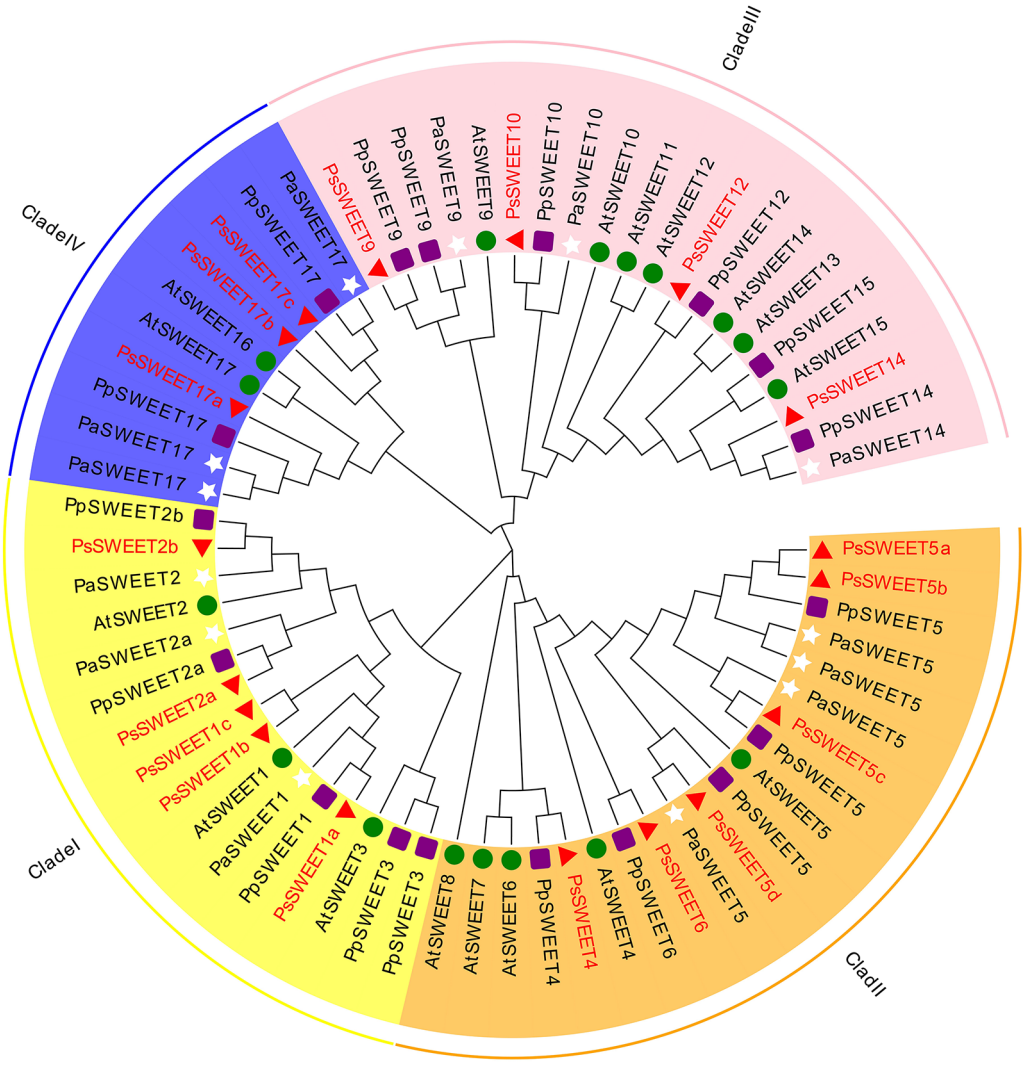

Fig. 2 Phylogenetic relationship among *Arabidopsis thaliana,Prunus persica,Prunus avium* and 'Fengtang' plum. Based on the analysis of *PsSWEET* members in *Arabidopsis thaliana*, 18 *PsSWEET* are divided into four branches, labeled I, II, III, and IV, denoted by yellow, orange, pink, and blue, respectively. In the image, *Arabidopsis thaliana* is represented by a green circle; *Prunus persica* by a purple square; *Prunus avium* by a white star and the 'Fengtang' plum by a red triangle

performed multiple sequence alignments with *SWEET* sugar transporters from *Arabidopsis thaliana*. The results indicated that the conserved domains of *PsSWEET1a* and *PsSWEET17b* were highly consistent with those of the *SWEET* proteins in *Arabidopsis thaliana*, supporting their role in sugar transport (Fig. S2).

Analysis of *cis*-acting elements in *SWEET* genes

Cis-acting elements were analyzed in the 2 kb upstream sequences of *SWEET* genes (Fig. 4), and the results indicated that the *cis*-acting elements of the 18 *SWEET* members could be classified into five categories: plant hormone response elements, light response elements, plant growth and development-related elements, abiotic stress response elements, and transcription factor recognition and binding sites. Among these, light response elements constituted the highest proportion. Additionally, component analysis revealed that *PsSWEET1a* contains 6 photoresponsivity elements, 2 MeJA-responsive elements, 1 MYB-responsive element, and 1 gibberellin-responsive element. The *PsSWEET17b* gene contains 16 light-responsive elements, 1 MYB-responsive element, and 1 low-temperature-responsive element.

Analysis of *SWEET* gene expression patterns during the development of 'Fengtang' plum fruit based on RNA-seq.

Candidate genes were identified from transcriptome data using standard gene names and synonyms in functional annotations. A total of 18 *SWEET* genes showed different expression patterns (Fig. 5A). Among them, *PsSWEET1b,1c,5d,5c,6,9*, and *12* were not expressed at any of the three stages of fruit development. *PsSWEET2a,4,10,14*, and *17a* expression gradually decreased

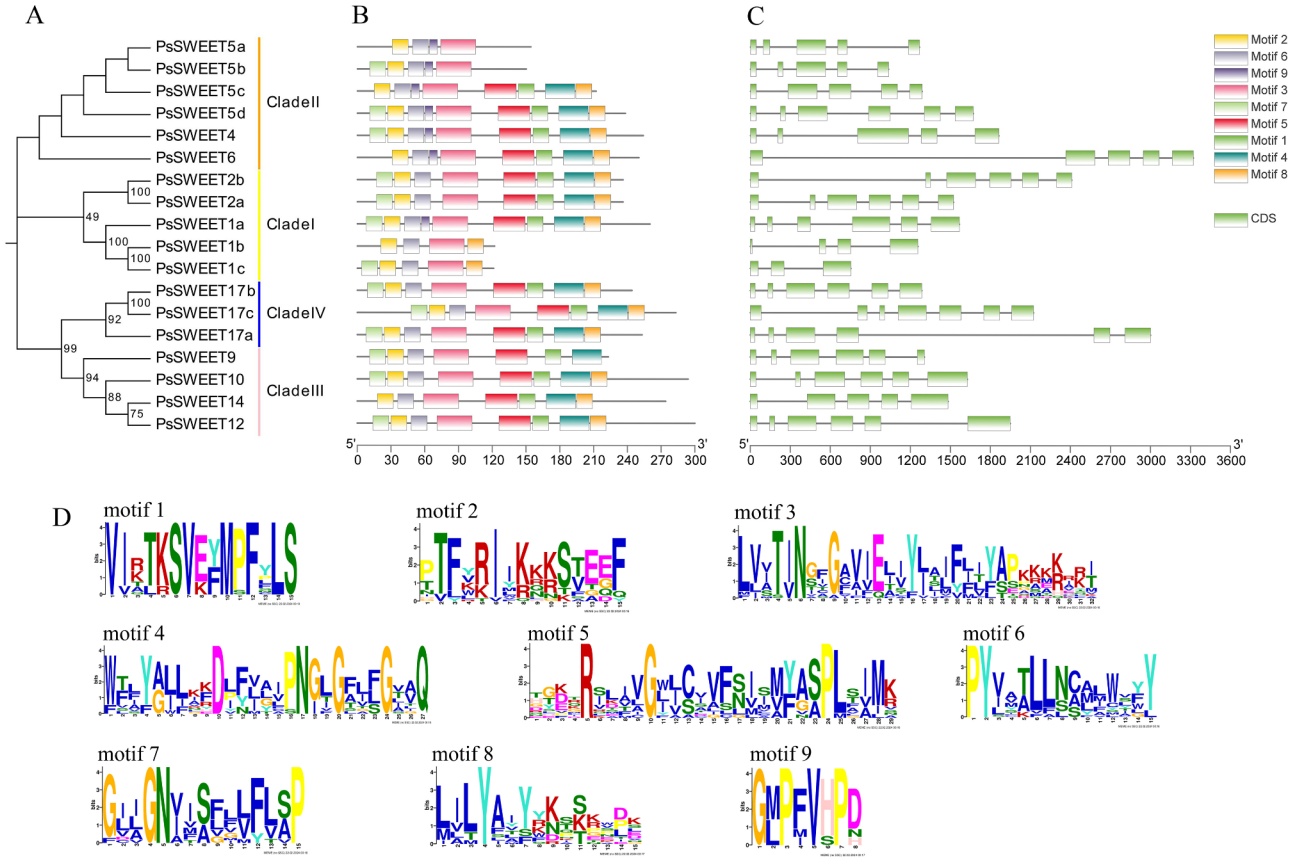

Fig. 3 Analysis of conserved motifs and gene structure in the evolutionary tree of the *SWEET* gene family. (**A**) Evolutionary tree of *PsSWEET* genes. Different clades are marked with different colors. Yellow, orange, pink, and blue boxes represent branches I, II, III, and IV. (**B**) Predicted conserved motifs in SWEET proteins. The nine motifs are represented by squares of different colors, numbered 1–9. (**C**) Exon-intron structure of *SWEET* genes. Green bars: exons; Line: Intron. (**D**) Motif components found in PsSWEET proteins

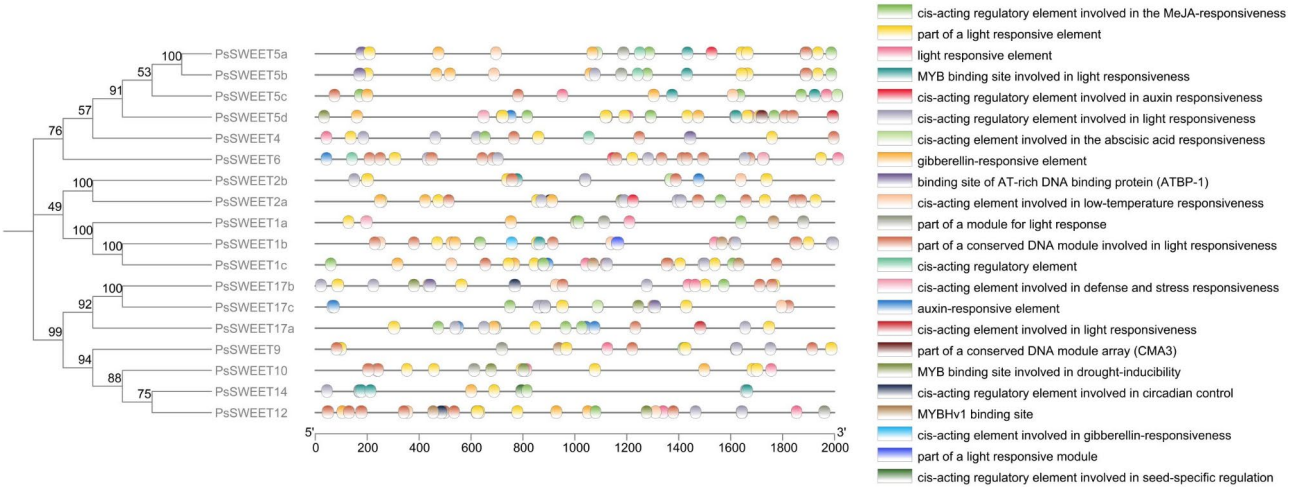

Fig. 4 Analysis of *cis*-acting elements in *PsSWEET* genes. The 2000 bp upstream codon of *PsSWEET* translation initiation was analyzed using plant-CARE online analysis software

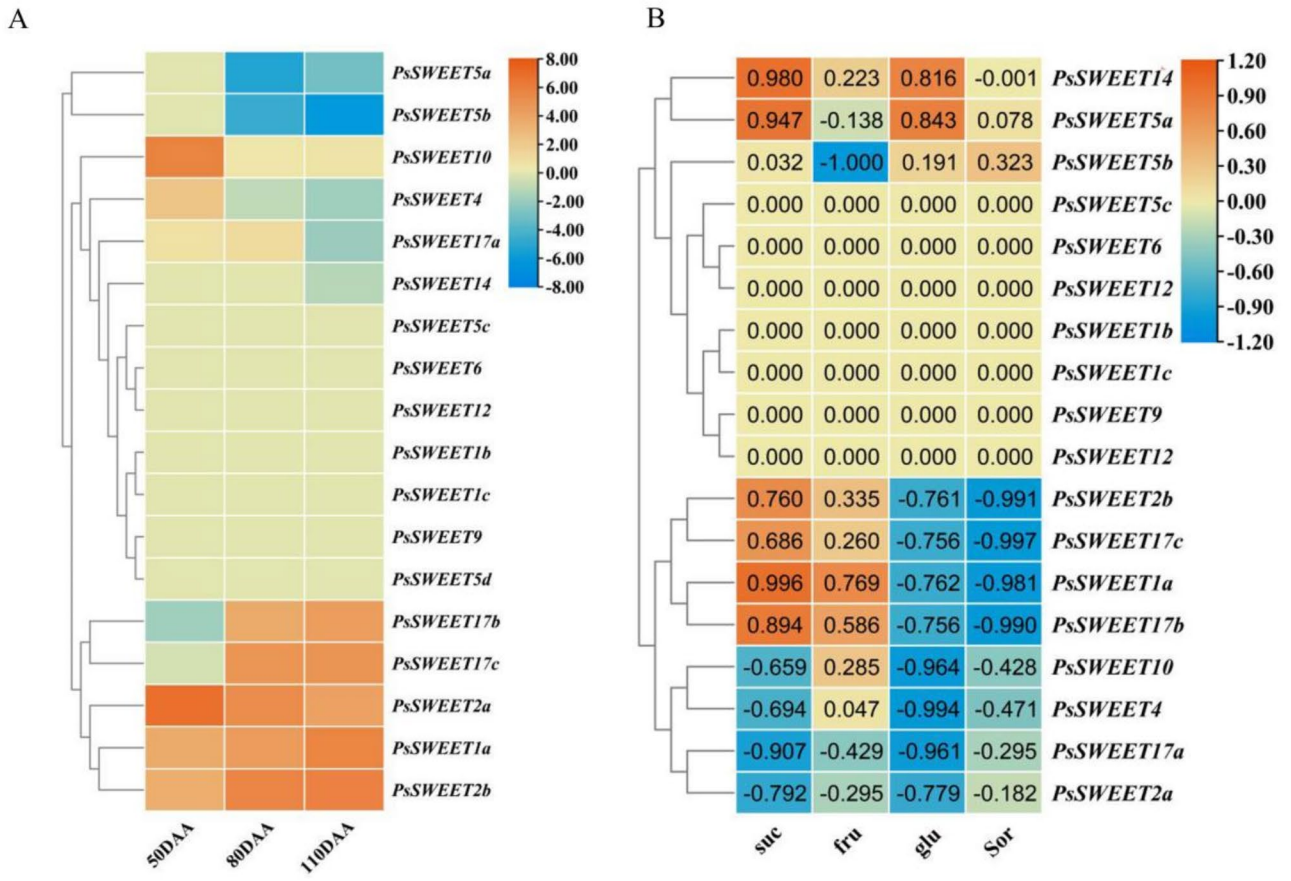

Fig. 5 Analysis of the expression and correlation of *PsSWEET* genes in 'Fengtang' plum fruit based on RNA-seq and targeted metabolomics. RNA-seq and targeted metabolomics data were sampled from April (average temperature 8–18°, relative humidity 54–85%) to July (average temperature 21–28°, relative humidity 46–80%) 2021. (**A**) Heatmap of *SWEET* genes expression profile in 'Fengtang' plum fruit. Different colors represent standardized processing values for relative expression levels, ranging from low (blue) to high (orange). (**B**) Correlation analysis of *SWEET* genes and corresponding sugar components. High correlation corresponds to orange, low correlation corresponds to blue

with fruit ripening, while *PsSWEET1a,2b,17b*, and *17c* expression steadily increased. *PsSWEET5a* showed high expression early in development. By combining RNA-seq data and metabolome data (Fig. 5B), *SWEET* genes highly correlated with plum sugar accumulation were identified as *PsSWEET1a,2a,2b,4,7a,10*, and *17b*.

qRT-PCR validation of *SWEET* genes during the development of 'fengtang' Plum fruit

To verify the reliability of the RNA-seq data, seven *SWEET* genes were selected for qRT-PCR verification. The gene expression patterns of these *SWEETs* in qRT-PCR exhibited trends consistent with the FPKM values observed in the RNA-seq data (Fig. 6A and B), indicating the reliability of the transcriptomic data. To investigate the relationship between *SWEET* gene expression and sugar accumulation, we further analyzed the correlation between *SWEET* gene expression and sugar components (Fig. 6C). *PsSWEET1a,2b,10*, and *17b* exhibited a strong positive correlation with sucrose and sorbitol, whereas *PsSWEET4,2a*, and *17a* displayed a strong negative correlation with these sugars. The expression of these genes was closely associated with sucrose and sorbitol content. These results suggest that *SWEET* genes may play a role in sugar accumulation during the development of 'Fengtang' plum.

Expression profiles of *SWEET* genes in different 'fengtang' Plum tissues and organs

To study the influence of *SWEET* genes on 'Fengtang' plum fruit development, we extracted RNA from various plum tissues and performed qRT-PCR. Seven *SWEET* genes were detected in seven tissues (Fig. 7). Most *SWEET* genes were expressed across the tissues and organs of the 'Fengtang' plum, but their expression levels varied. *PsSWEET1a* showed the lowest transcript levels in roots and leaves, while *PsSWEET2a,10*, and *17a* exhibited the highest transcript abundance in leaves. The expression levels of *PsSWEET1a* and *4* were dominant in flower organs compared to other tissues, suggesting that they may play a role in flower development. Additionally, *PsSWEET1a* and *PsSWEET17b* exhibited similar

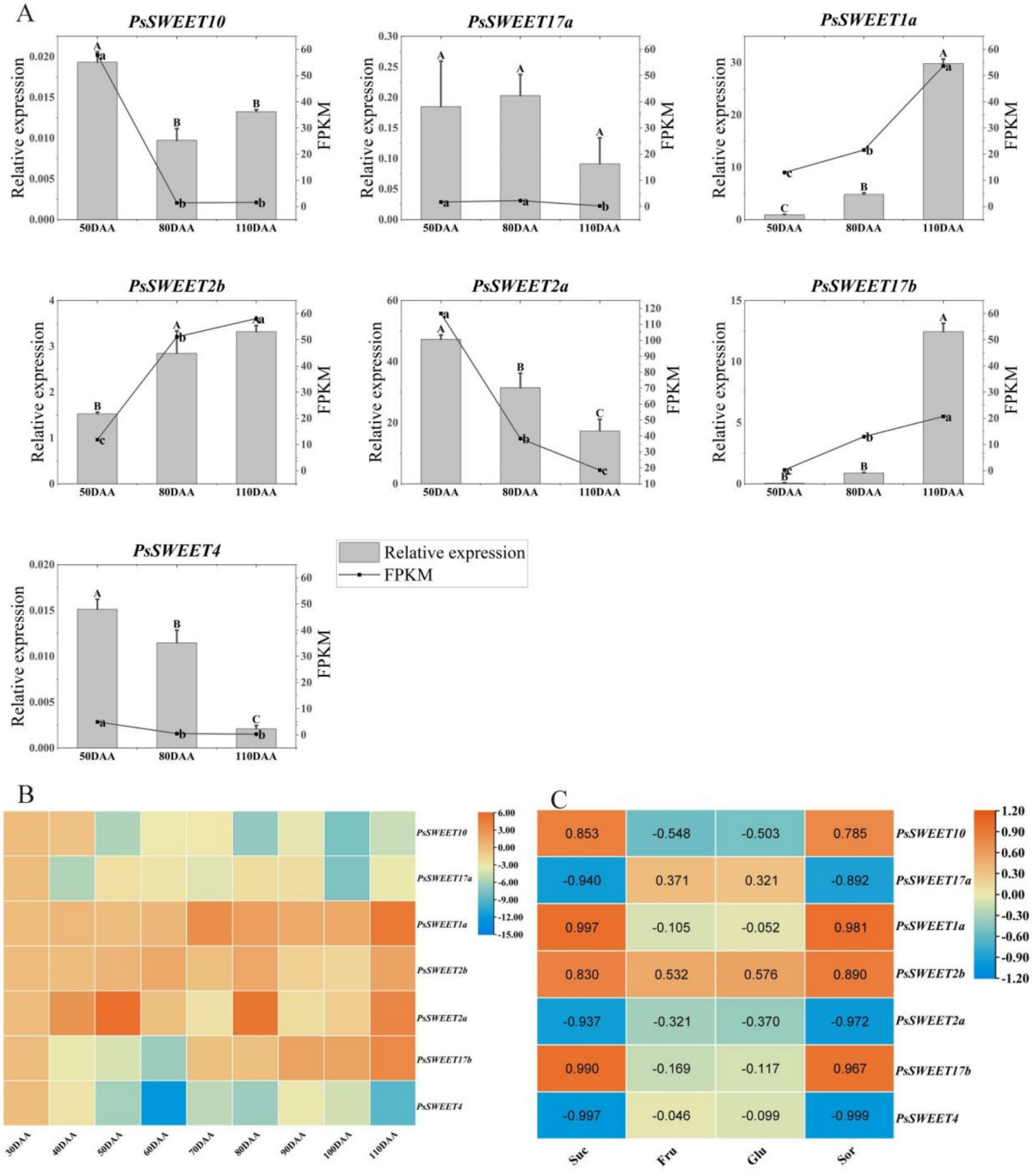

Fig. 6 Expression and correlation analysis of *SWEET* genes in 'Fengtang' plum fruit. (**A**) *SWEET* gene expression was verified by qRT-PCR analysis. The left Y-axis is the relative expression value of *SWEET* gene, and the expression amount of 30DAA samples is calculated as 1 (which can be seen in Fig. 6B), and the right Y-axis is FPKM. The error bars represent the standard deviation of three independent bioassays. Different uppercase letters showed significant differences in qRT-PCR $p \leq 0.05$, and different lowercase letters showed significant differences in RNA-seq $p \leq 0.05$. The error bar represents the standard deviation of the three biological replicates. (**B**) Heatmap of *SWEET* gene expression profiles in developing 'Fengtang' plum fruit. Different colors represent standardized processing values for relative expression levels, ranging from low (blue) to high (orange). (**C**) Correlation analysis of selected *SWEET* gene and corresponding sugar components. High correlation corresponds to orange, low correlation corresponds to blue

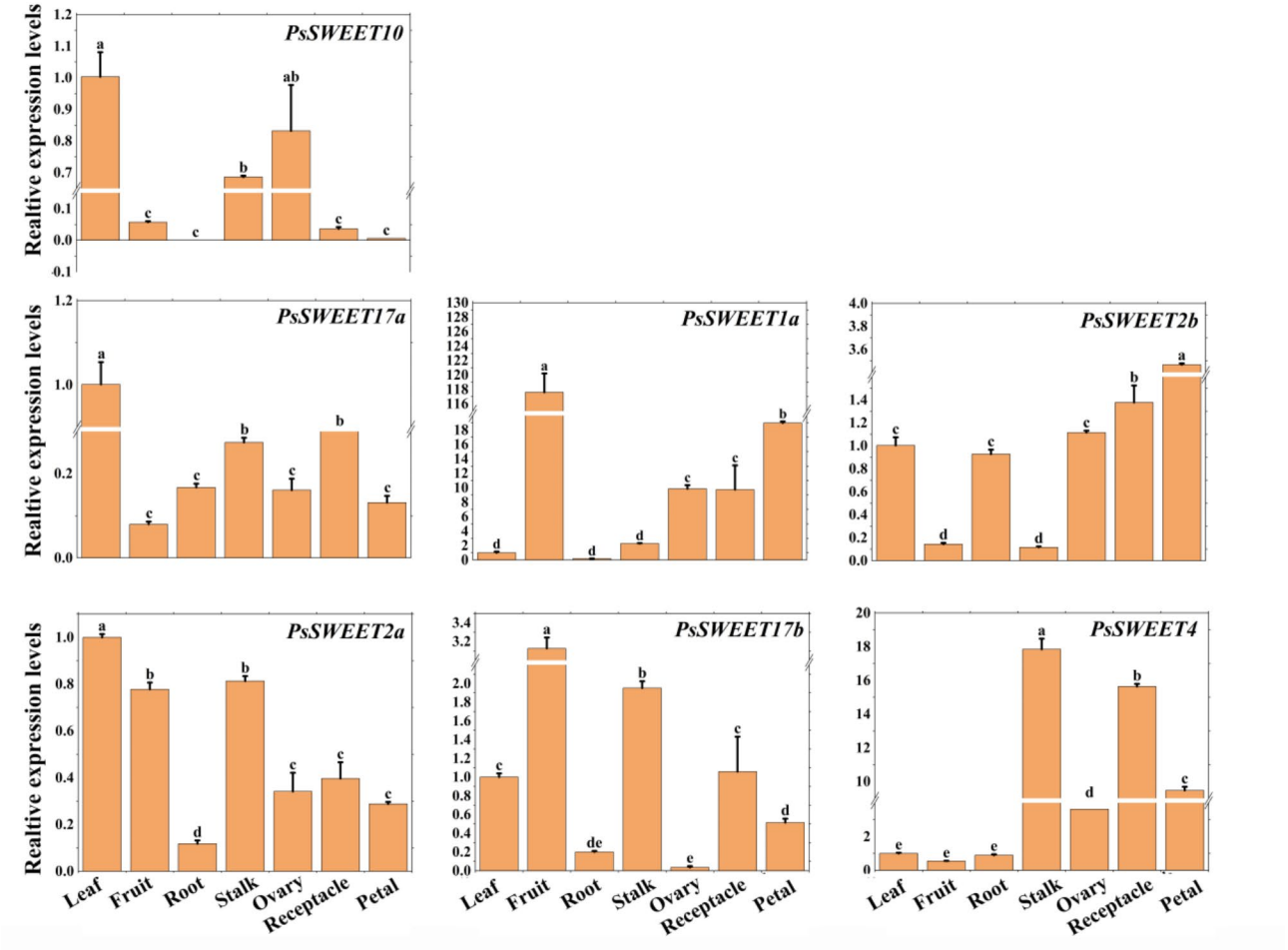

Fig. 7 The relative expression levels of 7 *PsSWEET* genes in different tissues of 'Fengtang' plum were analyzed by real-time fluorescence quantitative PCR with the expression level of 1 in leaves. The horizontal coordinates from left to right are leaf, fruit, root, stalk, ovary, receptacle and petal. Different letters indicate significant differences. The error bar represents the standard deviation of the three biological replicates

expression patterns during fruit development and were highly expressed in fruit, suggesting their involvement in sugar accumulation in 'Fengtang' plum fruit.

Subcellular localization of *PsSWEET1a* and *PsSWEET17b*

To further investigate the potential role of *PsSWEET1a* and *PsSWEET17b* in soluble sugar accumulation in plum fruit, we analyzed their subcellular localization. *PsSWEET1a*-GFP and *PsSWEET17b*-GFP fusion constructs were transiently expressed under the control of the *CaMV35S* promoter in *Arabidopsis thaliana* protoplasts (Fig. 8). The *PsSWEET1a*-GFP construct was co-expressed in *Arabidopsis* protoplasts with a nuclear marker protein (SV40 NLS). Fluorescent signals revealed that *PsSWEET1a* was localized to the nucleus and cytoplasm. The *PsSWEET17b*-GFP construct was co-expressed in *Arabidopsis thaliana* protoplasts with a cell membrane marker protein (OsRac3), and the GFP fluorescence signal was observed on the vacuolar membrane (Fig. 8d). This signal was distinct from those of chloroplasts (Chlorophyll) and the cell membrane marker OsRac3-mCherry, confirming that *PsSWEET17b* is localized to the vacuolar membrane.

Functional characterization of *PsSWEET1a* and *PsSWEET17b* in 'fengtang' Plum fruit

To further investigate the role of *PsSWEET1a* and *PsSWEET17b* in sugar accumulation in plum fruit, recombinant VIGS-*SWEETs* and OE-*SWEETs* vectors were infiltrated into live fruit using *Agrobacterium*-mediated fruit injection (Fig. 9A). In the control fruit (VIGS), significant impregnation of sugar was observed, whereas only weak impregnation of sugar was noted in VIGS-*PsSWEET1a* and VIGS-*PsSWEET17b*. Conversely, the control fruit (CK) exhibited weak impregnation of sugar, while OE-*PsSWEET1a* and OE-*PsSWEET17b* showed significant impregnation of sugar (Fig. 9B).

To confirm that *PsSWEET1a* and *PsSWEET17b* expression was inhibited at the molecular level, qRT-PCR analysis was performed on the silenced and control fruit.

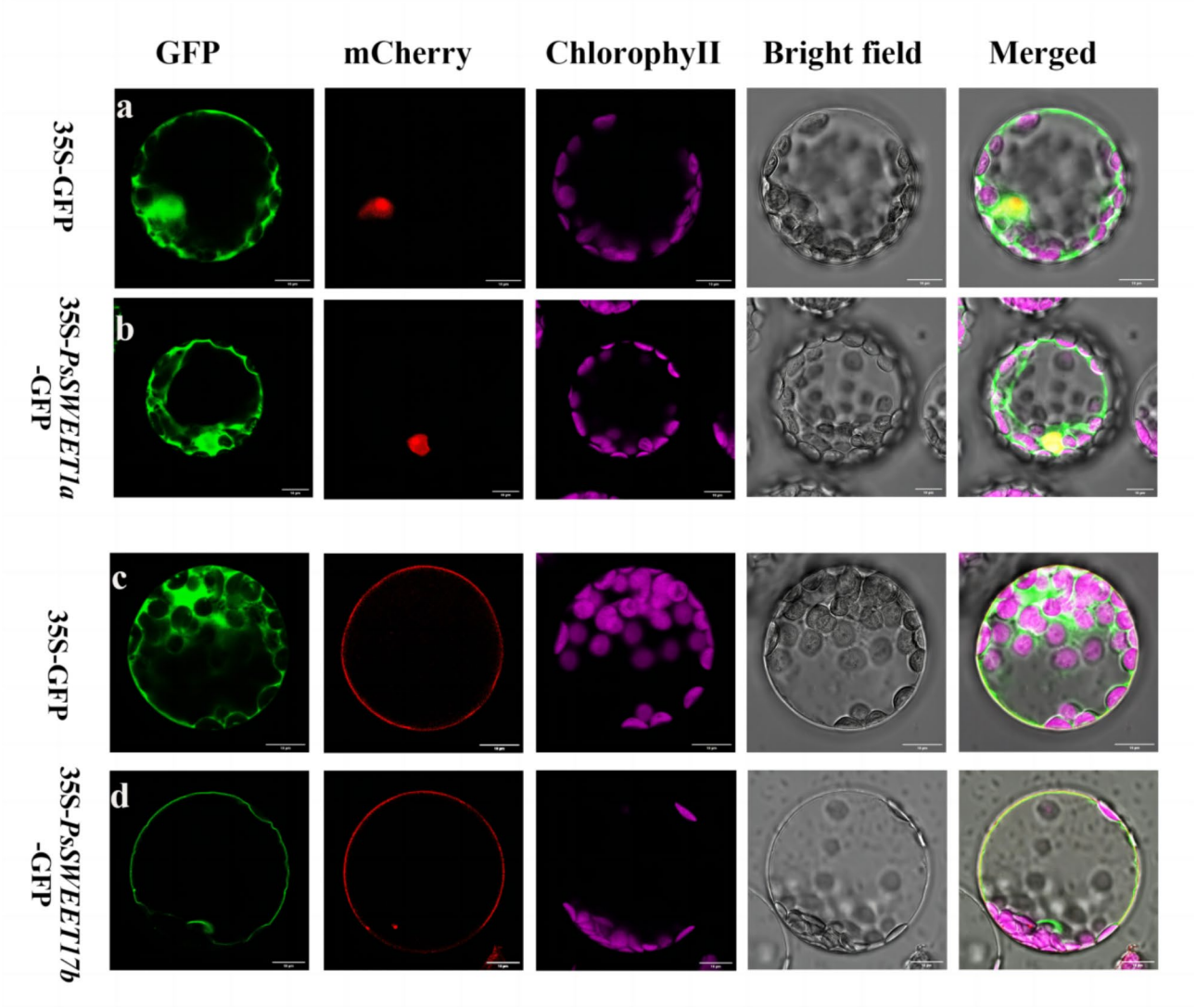

Fig. 8 Subcellular localization of green fluorescent protein (GFP) fusion proteins of *PsSWEET1a* and *PsSWEET17b* in *Arabidopsis thaliana* protoplasts. (**a**) 35 S-GFP, red marked by nuclear Marker: SV40 NLS, green marked by GFP signal. (**b**) 35 S::*PsSWEET1a*-GFP. (**c**) 35 S-GFP, marked in red by cell membrane Marker: OsRac3, marked in green by GFP signal. (**d**) 35 S::*PsSWEET17b*-GFP. Scale bar = 10 μm

When *PsSWEET1a* and *PsSWEET17b* were silenced in 'Fengtang' plum fruit, their expression levels were significantly lower than in the control group (Fig. 9C). Soluble sugar components were also measured, revealing that sucrose, fructose, glucose, and sorbitol contents were significantly reduced in comparison to the control (CK) (Fig. 9D). Notably, sucrose content in *PsSWEET1a-* and *PsSWEET17b*-silenced fruit was reduced from 34.14 mg/g to 13.01 mg/g and 12.50 mg/g, respectively, representing decreases of 61.9% and 63.4% compared to the control (CK). Additionally, the expression levels of sugar metabolism-related genes *PsSPS2,PsSUS4*, PsINV3, and *PsINV4* were significantly reduced in the silenced fruits (Fig. 9G). Transient overexpression of *PsSWEET1a* and *PsSWEET17b* in 'Fengtang' plum fruit was achieved using *Agrobacterium* tumefaciens-mediated transformation, with an empty vector pCAMBIA1301 as the control (CK). Higher expression levels of *PsSWEET1a* and *PsSWEET17b* were detected compared to the CK (Fig. 9E). Correspondingly, higher concentrations of sucrose, fructose, and glucose were observed compared to the CK (Fig. 9F). The expression of *PsSPS2,PsSUS4,PsINV3*, and *PsINV4* in fruits overexpressing *PsSWEET1a* and *PsSWEET17b* was significantly higher than in the CK (Fig. 9H). These results suggest that changes in *PsSWEET1a* and *PsSWEET17b* expression may affect the content of sucrose, glucose, and fructose by altering the expression levels of genes involved in sugar metabolism in 'Fengtang' plum.

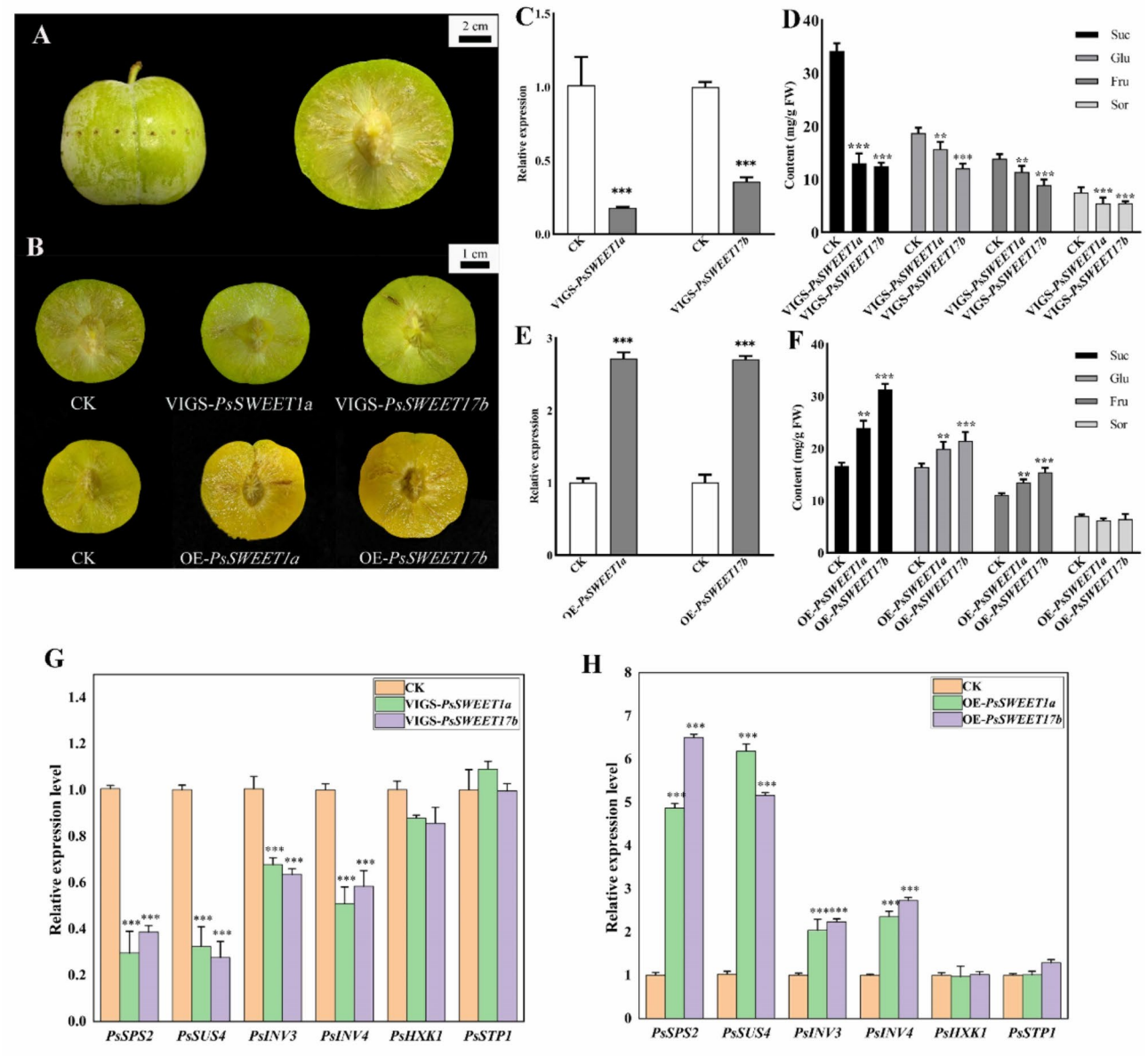

Fig. 9 Functional analysis of *PsSWEET1a* and *PsSWEET17b* in 'Fengtang' plum, respectively. (**A**) Location of injected fruit (left) and cross-section (right). (**B**) Silenced and overexpressed fruit phenotypes were observed in flesh: CK (silenced by injection of no-load TRV1 and TRV2, overexpressed by injection of no-load pCAMBIA1301), VIGS-*PsSWEET1a*, VIGS-*PsSWEET17b*, OE-*PsSWEET1a*,and OE-*PsSWEET17b* showed different degrees of impregnation of sugar. (**C**) Expression levels of *PsSWEET1a* and *PsSWEET17b*. (**D**) Soluble sugar content in fruit after gene silencing. (**E**) Expression levels of *PsSWEET1a* and *PsSWEET17b*. (**F**) Soluble sugar content in fruit after gene overexpression. (**G**) Relative expression levels of genes related to sugar accumulation in fruit after silencing *PsSWEET1a* and *PsSWEET17b*. (**H**) Relative expression levels of genes related to sugar accumulation in fruit after overexpression *PsSWEET1a* and *PsSWEET17b*. The error bar represents the standard deviation of the three biological replicates. "*", "**" and "***" indicate significant differences at $p < 0.05$, 0.01 and 0.001, respectively

Overexpression of *PsSWEET1a* and *PsSWEET17b* altered the phenotype and sugar content of tomato plants

To further understand the regulatory role of *PsSWEET1a* and *PsSWEET17b* in sugar accumulation, we expressed these genes heterologously in tomato plants and obtained four homozygous lines (OE*1a*-L8, OE*1a*-L15, OE*17b*-L4, and OE*17b*-L13). Wild-type Micro-Tom plants were used as a negative control (WT). Target bands were detected in all four transgenic lines (Fig. S3), indicating that *PsSWEET1a* and *PsSWEET17b* were successfully inserted into the tomato genome. Compared to WT plants, the transgenic tomato plants showed no significant changes in fruit size or vertical and horizontal length. However, the transgenic plants exhibited a significant dwarfing phenotype, characterized by reduced height, stem diameter, and internode length (Fig. 10; Table S4). These

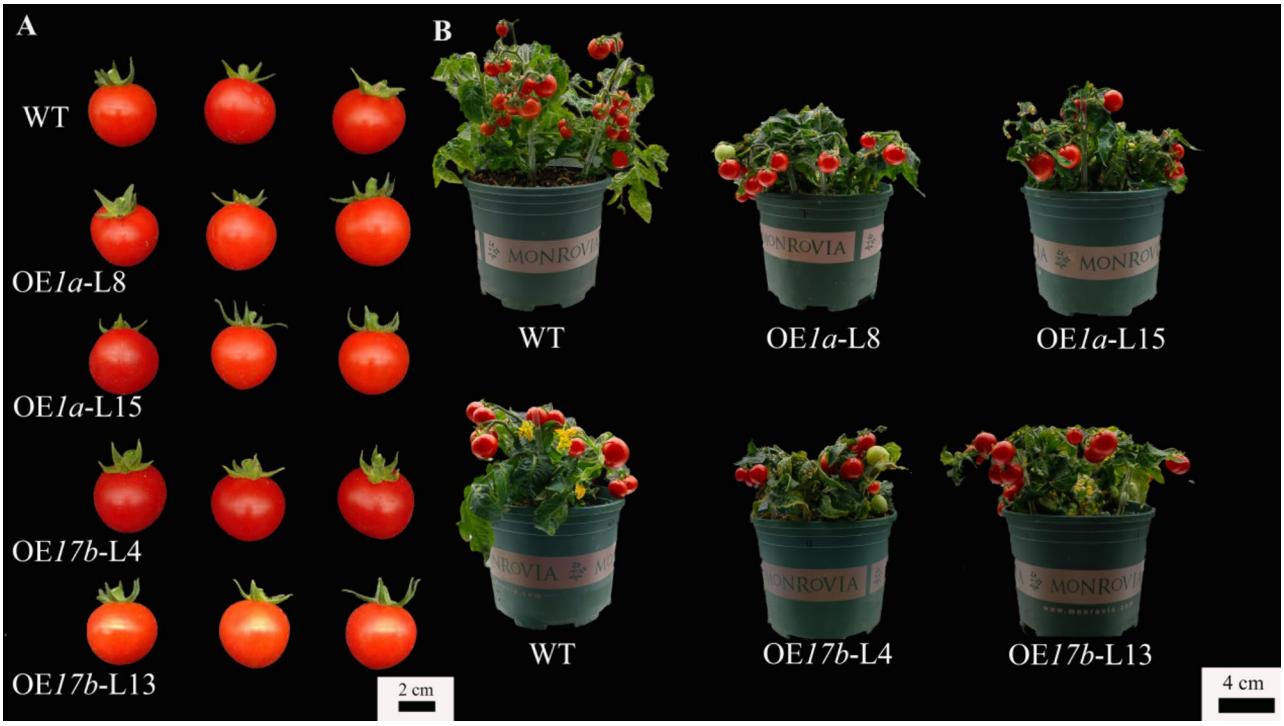

Fig. 10 Phenotypic comparison between wild type (WT) and transgenic tomato plants. (**A**) Phenotypes of ripe fruits from WT and transgenic tomato lines; The scale corresponds to 2 cm. (**B**) Phenotypes of plant structure in WT and transgenic tomato plants. The transgenic lines that overexpressed *PsSWEET1a* were OE*1a*-L8 and OE*1a*-15. The transgenic lines that overexpressed *PsSWEET17b* were OE*17b*-L4 and OE*17b*-L13

results suggest that overexpression of *PsSWEET1a* and *PsSWEET17b* can induce specific phenotype changes in tomato plants.

To further confirm the transcription levels of *PsSWEET1a* and *PsSWEET17b* in the fruits of transgenic strains, we performed quantitative real-time PCR (qRT-PCR) analyses (Fig. 11A, B). qRT-PCR confirmed high transcription levels in the four overexpressing transgenic lines OE*1a*-L8, OE*1a*-L15, OE*17b*-L4, and OE*17b*-L13 in red ripe fruit (RR). We analyzed the concentrations of soluble sugars (fructose, glucose, and sucrose) in tomato leaves overexpressing *PsSWEET1a* or *PsSWEET17b* (Fig. 11C). Compared to WT, sucrose content in the tomato leaves of transgenic lines OE*1a*-L15 and OE*17b*-L13 was significantly increased. Only transgenic line OE*17b*-L4 showed an increase in fructose levels, while the other transgenic lines showed no significant change. In the fruit tissue of RR, the content of soluble sugars (sucrose, fructose, and glucose) was significantly increased in all overexpressing lines compared to WT (Fig. 11D). Sucrose levels in fruit increased by 25.36%, 20.28%, 45.84%, and 33.17%. Fructose and glucose levels increased by 14.39–24.43% and 4.85–12.41%, respectively.

We studied both gene transcription and enzyme activity related to sugar metabolism to better understand changes in sucrose, fructose, and glucose content. We analyzed specific genes involved in tomato sugar metabolism, including sucrose synthase (*SISUS4*), hexokinase (*SIHXK*), invertase (*SIINV5* and *SIINV6*), sucrose phosphate synthase (*SISPS*), and fructokinase (*SIFK*). Transcription levels of all sugar-metabolizing genes were upregulated in transgenic lines compared to WT (Fig. 12A). To further understand the increase in sucrose, fructose, and glucose content in transgenic lines, we measured enzyme activities related to sugar metabolism. As shown in Fig. 12B, the activity changes of the three types of invertases (cell wall invertases, neutral invertases, and vacuolar invertases) were distinct. The activity of cell wall invertase (CWINV), which is involved in sucrose breakdown, was significantly increased in ripe tomato fruits of transgenic lines compared to WT. The activities of neutral invertase (NINV) and vacuolar invertase (VINV) were significantly decreased. The activities of sucrose phosphate synthase (SPS) and sucrose synthase (SUSY), both involved in intracellular sucrose synthesis, were significantly increased (Fig. 12B). These findings suggest that *PsSWEET1a* and *PsSWEET17b* regulate sucrose metabolism by modulating the expression of specific genes involved in sucrose degradation and synthesis, as well as by influencing related enzyme activities.

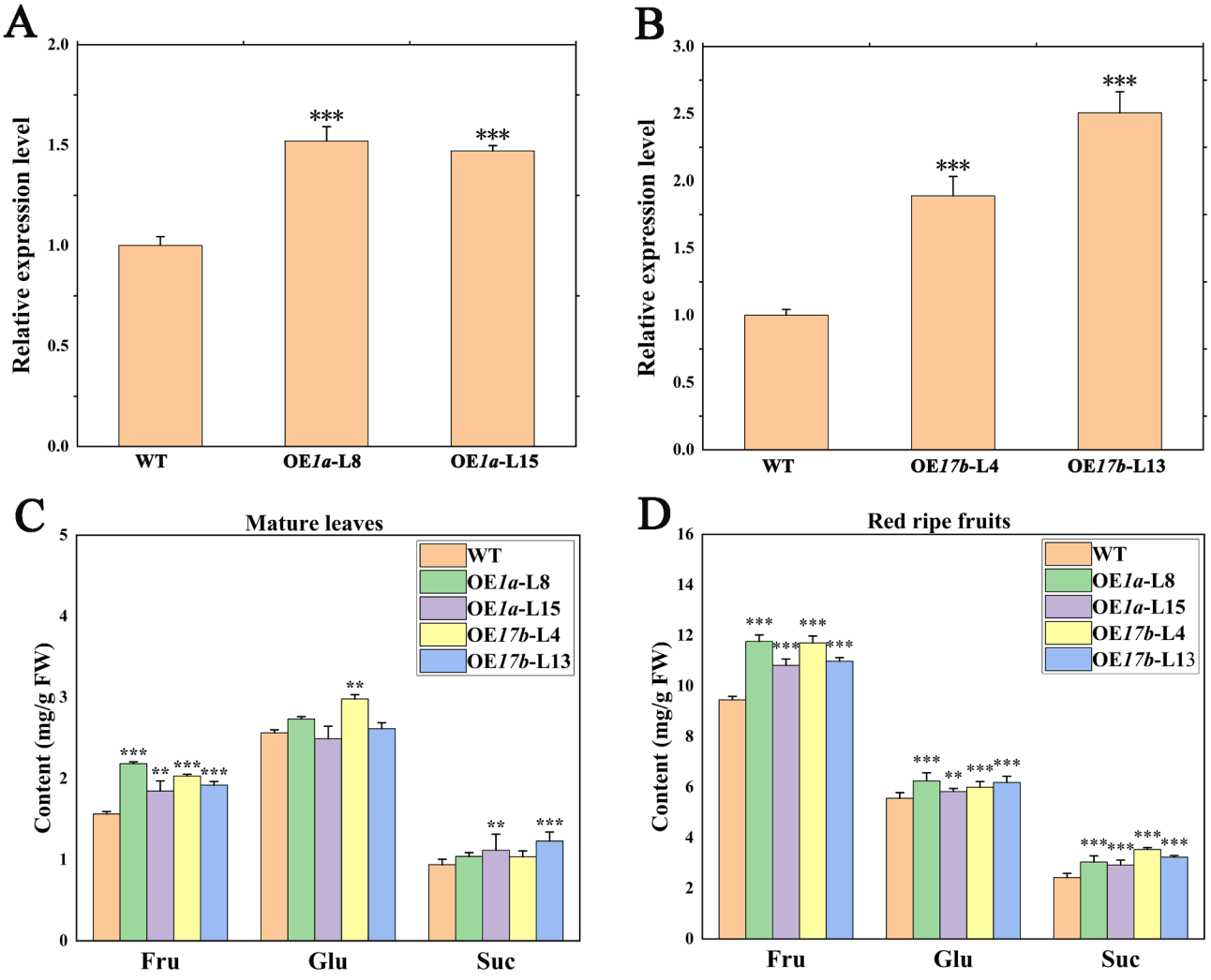

Fig. 11 Analysis of relative expression levels and sugar content between WT and transgenic tomato fruits. (**A**) Analysis of relative expression levels of *PsSWEET1a* in red ripe tomato fruits between WT and transgenic tomato plants. (**B**) Analysis of relative expression levels of *PsSWEET17b* in ripe tomato fruits between WT and transgenic tomato plants. The expression data of wild type (WT) plants were used as a control. (**C**) Changes in the content of sugar components in mature leaves between WT and transgenic tomato plants. (**D**) Changes in the content of sugar components in ripe tomato fruits between WT and transgenic tomato plants. The error bars represent the standard deviation of the three biological replicates. "*", "**", and "***" indicate significant differences at $p < 0.05$, 0.01, and 0.001, respectively

Discussion

The *PsSWEET* gene family of 'fengtang' Plum

Sucrose, glucose, and fructose are the predominant soluble sugars in plum fruit. Their content is a crucial determinant of the quality of 'Fengtang' plums, significantly influencing flavor. Sugar transporters control the loading and unloading of sugars in the phloem of plant tissues and play a key role in plant growth and development by regulating the translocation of sucrose, glucose, and fructose across the plasma membrane [28]. To date, the *SWEET* gene family has been identified in numerous plant species, including *Arabidopsis thaliana* [29], rice [10], wheat [30], European grape [31], cucumber [32], kale [15], and apple [17]. In this study, 18 members of the *SWEET* gene family were identified in the genome of *Prunus salicina* Lindl., which differs from the 15 members reported by Jiang [33]. This difference may be attributed to the varying criteria used in identifying candidate genes. Alternatively, the number of gene families identified in the genomes of different varieties may be inconsistent [34]. Studies have shown that variations in gene size are often caused by intron insertion [35]. *SWEET* genes exhibit structural differences, with minor variations in exon number and significant variations in intron length. Thirteen members of the *SWEET* gene family are unevenly distributed across five chromosomes. Collinearity analysis revealed that *SWEET* gene duplication events occurred during the evolution of the *SWEET* gene family in plum. *SWEET* proteins structurally contain seven transmembrane helices (TMHs), of

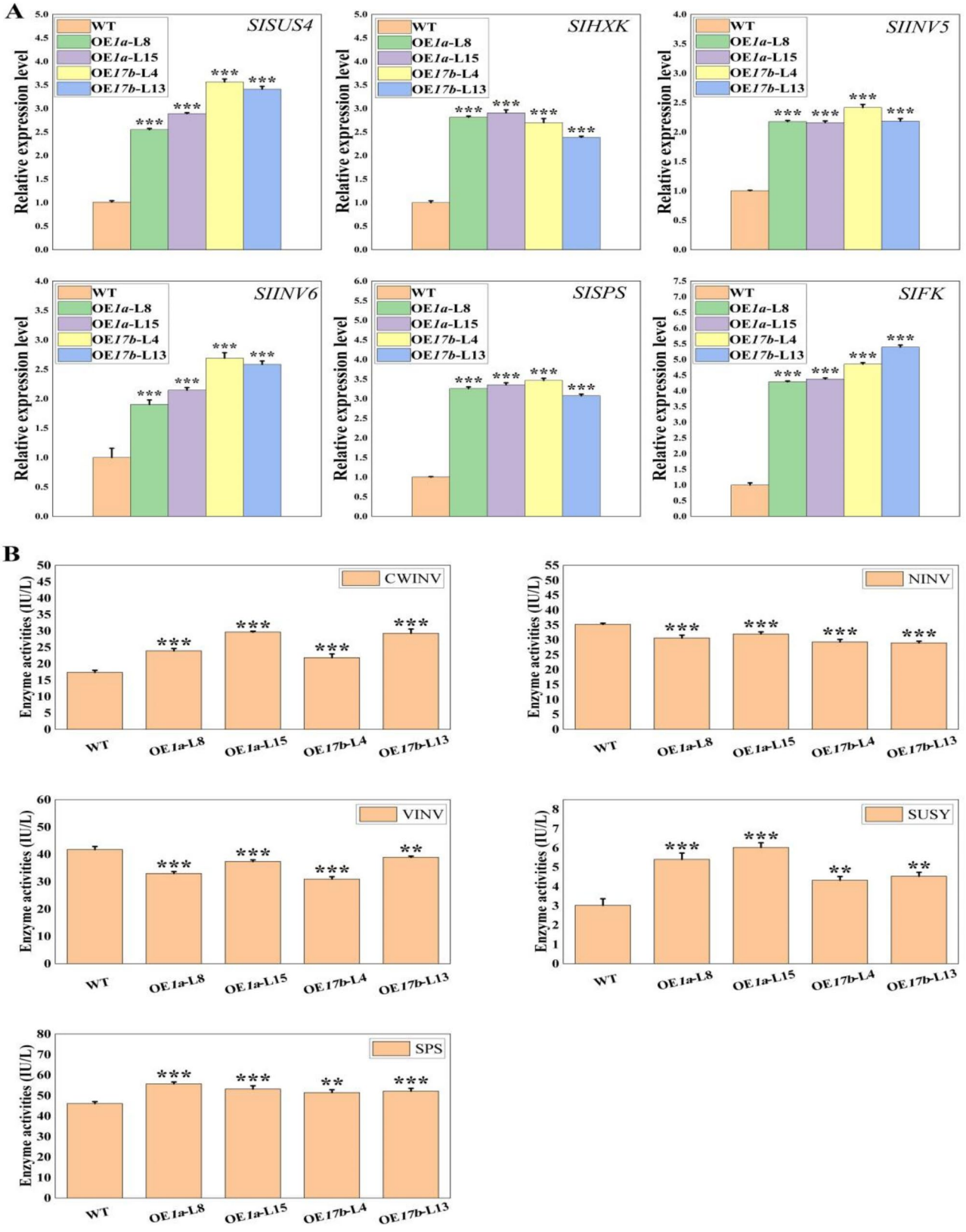

Fig. 12 (See legend on next page.)

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Fig. 12 Relative expression levels and enzyme activity of genes related to sugar accumulation in the ripe fruits of transgenic tomato plants overexpressing *PsSWEET1a* (OE*1a*-L8 and OE*1a*-L15) and *PsSWEET17b* (OE*17b*-L4 and OE*17b*-L13), respectively. (**A**) Relative expression levels of genes associated with sugar accumulation. (**B**) Enzyme activity determines ripe tomato fruit. CWINV cell wall invertase, NINV neutral invertase, VINV vacuole invertase, SUSY sucrose synthase (synthetic direction), SPS sucrose phosphate synthase. The data represent an average SDs of at least three biological replicates. "*", "**" and "***" indicate significant differences $p < 0.05$, 0.01 and 0.001, respectively

which four show low conservation and serve as linkers with the remaining three, forming a 3-1-3 structure [27]. The MtN3 subfamily is involved in glucose transport, while the PQ-loop subfamily is involved in amino acid transport [36, 37]. The conserved structure indicates that most *SWEET* proteins possess two MtN3 or PQ-loop superfamily domains, similar to most other plants. These results suggest that the *SWEET* gene family of plum is relatively conserved during evolution.

Expression and functional diversity of *PsSWEET* genes in 'fengtang' Plum

Plant *SWEET* genes exhibit tissue-specific expression patterns and are involved in various sugar transport processes [38, 39]. Differential expression analysis of *SWEET* genes in 'Fengtang' plum enabled us to identify the specialized functions of each *SWEET* protein in sugar accumulation. In this study, *PsSWEET* genes were expressed at varying levels in leaves, fruits, roots, stems, and flower organs, with *PsSWEET1a* and *PsSWEET17b* showing high expression levels in fruits. This suggests that these genes play a crucial role in sugar accumulation in fruits. The role of *SWEET* genes in storage organ function, particularly in fleshy fruits, has been increasingly highlighted in recent studies. Tian et al. [40] discovered that *FanSWEET1,2a,3b,4c,5a,7a,9b*, and *17a* were predominantly expressed in leaves, while *FanSWEET10a,14*, and *15a* were mainly expressed in fruits. Temporal and spatial expression analyses revealed that *AnmSWEET5* and *AnmSWEET11* are important in the development of pineapple fruit [41]. The distribution and transmembrane transport of sugars between source and sink organs are crucial for sugar accumulation in fruit. Previous studies have shown that members of the *SWEET* gene family are primarily localized to the plasma membrane [42]. However, other localization patterns have been observed, such as *SWEET9* expression in the nectar gland and subcellular localization in the trans-Golgi network [43], as well as *SWEET6* in the endoplasmic reticulum [44]. In this study, *PsSWEET1a* was localized to the nucleus and cytoplasm, representing a novel finding that suggests it may have dual roles in regulation and transport. Potential post-translational modification sites in *SWEET* proteins were identified using computational tools, indicating that *SWEET* transporters may be regulated by phosphorylation [45]. *StSP6A* specifically binds to *StSWEET11*, which blocks sucrose leakage to exosomes and promotes plastid sucrose transport [46, 47]. In vacuolar sugar storage, *SWEET16* plays a crucial role as a fructose transporter within vacuoles [48]. Similarly, *PsSWEET17b* is located in the vacuolar membrane and is involved in intracellular sugar transport and accumulation, as well as in maintaining intracellular hexose homeostasis. These results suggest that the roles of *PsSWEET1a* and *PsSWEET17b* in sugar accumulation in Fengtang plum fruit warrant further investigation.

PsSWEET1a and *PsSWEET17b* participate in the accumulation of sugar in 'fengtang' Plum fruit

The *SWEET* gene family is among the most important sugar transporters, capable of enhancing the accumulation of sugar in fruit. Transient overexpression of *PsSWEET1a* and *PsSWEET17b* in Fengtang plum fruit resulted in an increase in soluble sugar content. Similar results were observed in loquat [49] and grape [50], further underscoring the important role of the *SWEET* gene family in enhancing fruit sweetness. The overexpression of *VvSWEET10* enhanced hexose accumulation in grape callus and tomato fruit. Overexpression of *MdSWEET12a* and *MdSWEET23* in apples led to a significant increase in sugar content. Silencing *MdSWEET12a* and *MdSWEET23* was associated with a reduction in sugar content [11, 51]. In this study, we observed a decrease in the concentration of soluble sugar in 'Fengtang' plum fruit following the silencing of *PsSWEET1a* or *PsSWEET17b*. Conversely, overexpression of *PsSWEET1a* or *PsSWEET17b* exhibited the opposite trend. *SWEET* genes are more likely to be involved in the translocation of the sugars. These results suggest that *PsSWEET1a* and *PsSWEET17b* directly or indirectly regulate the change of soluble sugar content.

Heterologous expression of *PsSWEET1a* and *PsSWEET17b* altered sugar concentration and metabolism in tomato fruit

Altered expression of *SWEET* genes has been reported to affect plant height. For example, rice plants exhibit dwarfism compared to wild-type rice plants when *OsSWEET14* is overexpressed [52]. In this study, we found that overexpression of *PsSWEET1a* and *PsSWEET17b* resulted in transgenic tomato plants exhibiting dwarfism and shorter internodes compared to wild-type plants, which may contribute to the observed increase in sugar content. Similarly, in tomatoes, *SlSWEET7a* and *SlSWEET14* are key regulators of sucrose discharge from tomato fruits, and their silencing leads to reduced plant

height [53]. In this study, heterologous overexpression of *PsSWEET1a* and *PsSWEET17b* in tomato significantly increased the sucrose, fructose, and glucose contents of tomato fruit. Previous studies have reported that silencing CWINV inhibitors leads to an increase in CWINV activity post-translation, resulting in elevated hexose levels in tomato fruits [54, 55]. In transgenic tomatoes, the expression of key CWINV protein-coding genes *SlINV5* and *SlINV6* was upregulated in tomato fruits, and the increase in CWINV activity in the cell wall space was consistent with the elevated fructose and glucose content in the fruit. Modifying a gene involved in sucrose metabolism often leads to corresponding changes in gene expression, ultimately affecting the plant's sugar content. Ma et al. [56] found that the sucrose transporter *MdSUT2* can affect the expression of sucrose metabolism genes. For example, overexpression of *MdSWEET17a* in tomato alters the expression of *SlLin5,Fk1,Fk2*, and other genes, thereby affecting the sugar content of the tomato [57]. In the current study, overexpression of *PsSWEET1a* and *PsSWEET17b* in Fengtang plum and tomato resulted in the upregulation of *SUSY,INV*, and *SPS* genes. In summary, our findings indicate that heterologous overexpression of *PsSWEET1a* and *PsSWEET17b* determines sugar content in fruit by modulating sugar transport, metabolism-related gene expression, and enzyme activity.

Conclusion

In this study, 18 candidate *PsSWEET* genes were identified, and the evolutionary relationships, conserved motifs, gene structure, and chromosome localization of these *PsSWEET* gene family members were characterized. *PsSWEET1a* and *PsSWEET17b* were highly expressed in 'Fengtang' plum fruits; *PsSWEET1a* was localized in the nucleus and cytoplasm, while *PsSWEET17b* was found in the vacuolar membrane. Transient overexpression of *PsSWEET1a* and *PsSWEET17b* in 'Fengtang' plum fruit and heterologous expression in tomato altered the expression of genes associated with sucrose metabolism, resulting in changes in soluble sugar content. These results strongly support the role of *PsSWEET1a* and *PsSWEET17b* in sugar accumulation in 'Fengtang' plum fruit and lay the groundwork for further elucidating the internal mechanisms of sugar transport mediated by the *PsSWEET* genes in 'Fengtang' plum fruit.

Abbreviations

SUT	Sucrose transporter
TST	Tonoplast sugar transporter
SPS	Sucrose phosphate synthase
SUSY	Sucrose synthase
STP	Sugar transporter protein
INV	Invertase
HXK	Hexokinase gene
DAA	Days after flowering
qRT-PCR	Quantitative real time PCR
HPLC	High performance liquid chromatography
cDNA	Complementary DNA
SWEETs	Sugar Will Eventually be Exported transporters

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12870-025-06407-y.

Supplementary Material 1: ***SWEETs*** sequence analysis.

Supplementary Material 2: Homologous sequence alignment of ***PsSWEET1a*** and ***PsSWEET17b***

Supplementary Material 3: PCR detection of transgenic tomato plants.

Supplementary Material 4

Supplementary Material 5

Supplementary Material 6

Supplementary Material 7

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Author contributions

H.C. constructed the project; H.C. and S.L. designed the experiments; S.L. X.S. and X.N. performed the experiments and analyzed the data; S.L. and X.S. wrote the paper. All authors have read and agreed to the published version of the manuscript.

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Data availability

The datasets supporting the conclusions of this article are available in the NCBI Bioproject repository, accession number: PRJNA968060 and was publicly released on May 7, 2024.

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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References

1. Chen LQ, Cheung LS, Feng L, Tanner W, Frommer WB. Transport of sugars. Annu Rev Biochem. 2015;84:865–94. https://doi.org/10.1146/annurev-biochem-060614-033904.
2. Wang ZY, Ma BQ, Yang NX, Jin L, Wang L, Ma SY, Ruan YL, Ma FW, Li MJ. Variation in the promoter of the sorbitol dehydrogenase gene MdSDH2 affects binding of the transcription factor MdABI3 and alters Fructose content in Apple fruit. Plant J. 2022;109(5):1183–98. https://doi.org/10.1111/tpj.15624.

3. Wen SY, Neuhaus HE, Cheng JT, Bie ZL. Contributions of sugar transporters to crop yield and fruit quality. J Exp Bot. 2022;73(8):2275–89. https://doi.org/10.1093/jxb/erac043.
4. Ren Y, Sun HH, Zong M, Guo SG, Ren ZJ, Zhao JY, Li MY, Zhang J, Tian SW, Wang JF, Yu YT, Gong GY, Zhang HY, He HJ, Li LG, Zhang XL, Liu F, Fei ZJ, Xu Y. Localization shift of a sugar transporter contributes to phloem unloading in SWEET watermelons. New Phytol. 2020;227(6):1858–71. https://doi.org/10.1111/nph.16659.
5. Ruan YL. Sucrose metabolism: gateway to diverse carbon use and sugar signaling. Annu Rev Plant Biol. 2014;65:33–67. https://doi.org/10.1146/annurev-arplant-050213-040251.
6. Slewinski TL. Diverse functional roles of monosaccharide transporters and their homologs in vascular plants: A physiological perspective. Mol Plant. 2011;4(4):641–62. https://doi.org/10.1093/mp/ssr051.
7. Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, Fernie AR, Frommer WB. Sucrose efflux mediated by *SWEET* proteins as a key step for phloem transport. Science. 2012;335(6065):207–11. https://doi.org/10.1126/science.1213351.
8. Chen LQ. *SWEET* sugar transporters for phloem transport and pathogen nutritio. New phytol. 2014; 201(4): 1150-5. https://doi.org/10.1111/nph.12445
9. Yuan M, Wang SP. Rice MtN3/saliva/SWEET family genes and their homologs in cellular organisms. Mol Plant. 2013;6(3):665–74. https://doi.org/10.1093/mp/sst035.
10. Xue XY, Wang J, Shukla D, Cheung LS, Chen LQ. When *SWEETs* turn tweens: updates and perspectives. Annu Rev Plant Biol. 2022;73:379–403. https://doi.org/10.1146/annurev-arplant-070621-093907.
11. Zhang B, Li YN, Wu BH, Yuan YY, Zhao ZY. Plasma membrane-localized transporter *MdSWEET12* is involved in sucrose unloading in Apple fruit. J Agric Food Chem. 2022;70(49):15517–30. https://doi.org/10.1021/acs.jafc.2c05102.
12. Lin IW, Sosso D, Chen LQ, Gase K, Kim SG, Kessler D, Klinkenberg PM, Gorder MK, Hou BH, Qu XQ. Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. Nature. 2014;508(7497):546–9. https://doi.org/10.1038/nature13082.
13. Sun MX, Huang XY, Yang J, Guan YF, Yang ZN. *Arabidopsis* RPG1 is important for primexine deposition and functions redundantly with RPG2 for plant fertility at the late reproductive stage. Plant Reprod. 2013;26(2):83–91. https://doi.org/10.1007/s00497-012-0208-1.
14. Cox KL, Meng F, Wilkins KE, Li F, Wang P, Booher NJ, Carpenter SCD, Chen LQ, Zheng H, Gao XQ, Zheng Y, Fei ZJ, Yu JY, Isakeit T, Wheeler T, Frommer WB, He P, Bogdanove AJ, Shan LB. TAL effector driven induction of a *SWEET* gene confers susceptibility to bacterial blight of cotton. Nat Commun. 2017;8:15588. https://doi.org/10.1038/ncomms15588.
15. Zhou Y, Liu L, Huang WF, Yuan M, Zhou F, Li XG, Lin YJ. Overexpression of *OsSWEET5* in rice causes growth retardation and precocious senescence. PLoS ONE. 2014;9(4):e94210. https://doi.org/10.1371/journal.pone.0094210.
16. Ho LH, Klemens PAW, Neuhaus HE, Ko HY, Hsieh SY, Guo WJ. *SlSWEET1a* is involved in glucose import to young leaves in tomato plants. J Exp Bot. 2019;70(12):3241–54. https://doi.org/10.1093/jxb/erz154.
17. Zhen QL, Fang T, Peng Q, Liao L, Zhao L, Owiti A, Han YP. Developing gene-tagged molecular markers for evaluation of genetic association of Apple *SWEET* genes with fruit sugar accumulation. Hortic Res. 2018;5(1):14. https://doi.org/10.1038/s41438-018-0024-3.
18. Klemens PAW, Patzke K, Deitmer J, Spinner L, Le HR, Bellini C, Bedu M, Chardon F, Krapp A, Neuhaus HE. Overexpression of the vacuolar sugar carrier *AtSWEET16* modifies germination, growth, and stress tolerance in *Arabidopsis*. Plant Physiol. 2013;163(3):1338–52. https://doi.org/10.1104/pp.113.224972.
19. Chardon F, Bedu M, Calenge F, Klemens PAW, Spinner L, Clement G, Chietera G, Léran S, Ferrand M, Lacombe B, Loudet O, Dinant S, Bellini C, Neuhaus HE, Daniel-Vedele F, Krapp A. Leaf Fructose content is controlled by the vacuolar transporter *SWEET17* in *Arabidopsis*. Curr Biol. 2013;23(8):697–702. https://doi.org/10.1016/j.cub.2013.03.021.
20. Liu B, Du HW, Rutkowski R, Gartner A, Wang XC. LAAT-1 is the lysosomal lysine/arginine transporter that maintains amino acid homeostasis. Science. 2012;337(6092):351–4. https://doi.org/10.1126/science.1220281.
21. Li XY, Guo W, Li JC, Yue PT, Bu HD, Jiang J, Liu WT, Xu YX, Yuan H, Li T, Wang A. Histone acetylation at the promoter for the transcription factor PuWRKY31 affects sucrose accumulation in Pear fruit. Plant Physiol. 2020;182(4):2035–46. https://doi.org/10.1104/pp.20.00002.
22. Wang S, Liu S, Wang J, Yokosho K, Zhou B, Yu YC, Liu Z, Frommer WB, Ma JF, Chen LQ. Simultaneous changes in seed size, oil content and protein content driven by selection of *SWEET* homologues during soybean domestication. Natl Sci Rev. 2020;7(11):1776–86. https://doi.org/10.1093/nsr/nwaa110.
23. Zhang Y, Li YQ, Xiao W, Wu JL, Cheng L. Breeding and cultivation techniques of a new Li variety 'fengtang' Plum. China North Fruits. 2018;47(6):146–8. https://doi.org/10.13938/j.issn.1007-1431.20180194.
24. Nie XS, Chen H, Wang QY, Lu M, An HM. Sugar composition and transcriptome analysis in developing 'fengtang' Plum (*Prunus salicina* Lindl.) reveal candidate genes regulating sugar accumulation. Plant Physiol Biochem. 2023;202:107955. https://doi.org/10.1016/j.plaphy.2023.107955.
25. Wang XH, Chen H, Dong XQ. The change of organic acid content and its relationship with malic acid metabolism related enzymes during the development of 'fengtang' Plum fruit. J Sci Food Agric. 2018;35(3):293–300. https://doi.org/10.1016/j.scienta.2009.11.001.
26. Chen C, Wu Y, Li J, Wang X, Zeng Z, Xu J, Liu Y, Feng J, Chen H, He Y, Xia R. TBtools-II: A one for all, all for one bioinformatics platform for biological big-data mining. Mol Plant. 2023;16:1733–42. https://doi.org/10.1016/j.molp.2023.09.010.
27. You YH, Zhang L, Li PM, Yang CQ, Ma FW. Selection of reliable reference genes for quantitative real-time PCR analysis in Plum (Prunus salicina Lindl.) under different postharvest treatments. Sci Hortic. 2016;210(10):285–93. https://doi.org/10.1016/j.scienta.2016.07.031.
28. Gao Y, Zhang C, Han X, Wang ZY, Ma L, Yuan DP, Wu JN, Zhu XF, Liu JM, Li DP, Hu YB, Xuan YH. Inhibition of *OsSWEET11* function in mesophyll cells improves resistance of rice to sheath blight disease. Mol Plant Pathol. 2018;19(9):2149–61. https://doi.org/10.1111/mpp.12689.
29. Chen LQ, Hou BH, Lalonde S, Takanaga H, Hartung ML, Qu XQ, Guo WJ, Kim JG, Underwood W, Chaudhuri B, Chermak D, Antony G, White FF, Somerville SC, Mudgett MB, Frommer WB. Sugar transporters for intercellular exchange and nutrition of pathogens. Nature. 2010;468(7323):527–32. https://doi.org/10.1038/nature09606.
30. Gautam T, Saripalli G, Gahlaut V, Kumar A, Sharma PK, Balyan HS, Gupta PK. Further studies on sugar transporter (*SWEET*) genes in wheat (*Triticum aestivum* L). Mol Biol Rep. 2019;46(2):2327–53. https://doi.org/10.1007/s11033-019-04691-0.
31. Chong J, Piron MC, Meyer S, Merdinoglu D, Bertsch C, Mestre P. The *SWEET* family of sugar transporters in grapevine: *VvSWEET4* is involved in the interaction with botrytis cinerea. J Exp Bot. 2014;65(22):6589–601. https://doi.org/10.1093/jxb/eru375.
32. Li Y, Feng S, Ma S, Sui X, Zhang Z. Spatiotemporal expression and substrate specificity analysis of the cucumber *SWEET* gene family. Front Plant Sci. 2017;8:1855. https://doi.org/10.3389/fpls.2017.01855.
33. Jiang C, Zeng S, Yang J, Wang X. Genome-Wide identification and expression profiling analysis of *SWEET* family genes involved in fruit development in Plum (*Prunus salicina* Lindl). Genes. 2023;14(9):1679. https://doi.org/10.3390/genes14091679.
34. Lin W, Pu Y, Liu S, Wu Q, Yao Y, Yang Y, Zhang X, Sun W. Genome-Wide identification and expression patterns of *AcSWEET* family in pineapple and *AcSWEET11* mediated sugar accumulation. Int J Mol Sci. 2022;23(22):13875. https://doi.org/10.3390/ijms232213875.
35. Hu W, Hua X, Zhang Q, Wang J, Shen Q, Zhang X, Wang K, Yu Q, Lin YR, Ming R, Zhang JS. New insights into the evolution and functional divergence of the *SWEET* family in saccharum based on comparative genomics. BMC Plant Biol. 2018;18(1):270. https://doi.org/10.1186/s12870-018-1495-y.
36. Jeena GS, Kumar S, Shukla RK. Structure, evolution and diverse physiological roles of *SWEET* sugar transporters in plants. Plant Mol Biol. 2019;100(4–5):351–65. https://doi.org/10.1007/s11103-019-00872-4.
37. Liu DD, Chao WM, Turgeon R. Transport of sucrose, not hexose, in the phloem. J Exp Bot. 2012;63(11):4315–20. https://doi.org/10.1093/jxb/ers127.
38. Eom JS, Chen LQ, Sosso D, Julius BT, Lin IW, Qu XQ, Braun DM, Frommer WB. *SWEETs*, transporters for intracellular and intercellular sugar translocation. Curr Opin Plant Biol. 2015;25:53–62. https://doi.org/10.1016/j.pbi.2015.04.005.
39. Verdier V, Triplett LR, Hummel AW, Corral R, Cernadas RA, Schmidt CL, Bogdanove AJ, Leach JE. Transcription activator-like (TAL) effectors targeting *OsSWEET* genes enhance virulence on diverse rice (*Oryza sativa*) varieties when expressed individually in a TAL effector-deficient strain of Xanthomonas oryzae. New Phytol. 2012;196(4):1197–207. https://doi.org/10.1111/j.1469-8137.2012.04367.x.
40. Tian R, Xu J, Xu Z, Li J, Li H. Genome-Wide identification and expression analysis of *SWEET* gene family in strawberry. Horticulturae. 2024;10:191. https://doi.org/10.3390/horticulturae10020191.
41. Guo C, Li H, Xia X, Liu X, Yang L. Functional and evolution characterization of *SWEET* sugar transporters in Ananas comosus. Biochem Biophys Res Commun. 2018;496(2):407–14. https://doi.org/10.1016/j.bbrc.2018.01.024.

42. Han L, Zhu YP, Liu M, Zhou Y, Lu GY, Lan L, Wang XP, Zhao YF, Zhang XJ. Molecular mechanism of substrate recognition and transport by the *AtSWEET13* sugar transporter. Proc Natl Acad Sci U S A. 2017;114(38):10089–94. https://doi.org/10.1073/pnas.1709241114.
43. Lin IW, Sosso D, Chen LQ, Gase K, Kim SG, Kessler D, Klinkenberg PM, Gorder MK, Hou BH, Qu XQ, Carter CJ, Frommer WB. Nectar secretion requires sucrose phosphate synthases and the sugar transporter *SWEET9*. Nature. 2014; 508: 546–49. https://doi.org/10.1038/nature13082
44. Lee J, Lee H, Kim J, Lee S, Kim DH, Kim S, wang I. Both the hydrophobicity and a positively charged region flanking the C-terminal region of the transmembrane domain of signal-anchored proteins play critical roles in determining their targeting specificity to the Endoplasmic reticulum or endosymbiotic organelles in *Arabidopsis* cells. Plant Cell. 2011;23:1588–607. https://doi.org/10.1105/tpc.110.082230.
45. Anjali A, Fatima U, Manu MS, Ramasamy S, Senthil-Kumar M. Structure and regulation of *SWEET* transporters in plants: an update. Plant Physiol Biochem. 2020;156:1–6. https://doi.org/10.1016/j.plaphy.2020.08.043.
46. Abelenda JA, Bergonzi S, Oortwijn M, Sonnewald S, Du M, Visser RG, Sonnewald U, Bachem CW. Source-sink regulation is mediated by interaction of an FT homolog with a *SWEET* protein in potato. Curr Biol. 2019;29(7):1178–e11861176. https://doi.org/10.1016/j.cub.2019.02.018.
47. Herik V, Bergonzi S, Bachem CW, Ten Tusscher K. Modelling the physiological relevance of sucrose export repression by an flowering time homolog in the long-distance phloem of potato. Plant Cell Environ. 2021;44(3):792–806. https://doi.org/10.1111/pce.13977.
48. Klemens PA, Patzke K, Deitmer JW, Spinner L, Le Hir R, Bellini C, Bedu M, Chardon F, Krapp A, Neuhaus HE. Overexpression of the vacuolar sugar carrier *AtSWEET16* modifies germination, growth and stress tolerance in *Arabidopsis Thaliana*. Plant Physiol. 2013;163:1338–52. https://doi.org/10.1104/pp.113.224972.
49. Li J, Chen D, Jiang G, Song H, Tu M, Sun S. Molecular cloning and expression analysis of *EjSWEET15*, encoding for a sugar transporter from Loquat. Sci Hortic. 2020;272:109552. https://doi.org/10.1016/j.scienta.2020.109552.
50. Zhang Z, Zou LM, Ren C, Ren F, Wang Y, Fan P, Li SH, Liang ZC. *VvSWEET10* mediates sugar accumulation in grapes. Genes(Basel). 2019;10(4):255. https://doi.org/10.3390/genes10040255.
51. Nie P, Wang L, Li M, Lyu D, Qin S, Xue X. *MdSWEET23*, a sucrose transporter from Apple (*Malus* ×*domestica* Borkh.), influences sugar metabolism and enhances cold tolerance in tomato. Front Plant Sci. 2023;14:1266194. https://doi.org/10.3389/fpls.2023.1266194.
52. Kim P, Xue CY, Song HD, Gao Y, Feng L, Li Y, Xuan YH. Tissue-specific activation of DOF11 promotes rice resistance to sheath blight disease and increases grain weight via activation of *SWEET14*. Plant Biotechnol J. 2021;19(3):409–11. https://doi.org/10.1111/pbi.13489.
53. Zhang XS, Feng CY, Wang MN, Li TL, Liu X, Jiang J. Plasma membrane-localized *SlSWEET7a* and *SlSWEET14* regulate sugar transport and storage in tomato fruits. Hortic Res. 2021;8(1):186. https://doi.org/10.1038/s41438-021-00624-w.
54. Jin Y, Ni DA, Ruan YL. Posttranslational elevation of cell wall invertase activity by Silencing its inhibitor in tomato delays leaf senescence and increases seed weight and fruit hexose level. Plant Cell. 2009;21(7):2072–89. https://doi.org/10.1105/tpc.108.063719.
55. Wan H, Wu L, Yang Y, Zhou G, Ruan YL. Evolution of sucrose metabolism: the dichotomy of invertases and beyond. Trends Plant Sci. 2018;23(2):163–77. https://doi.org/10.1016/j.tplants.2017.11.001.
56. Ma QJ, Sun MH, Lu J, Zhu XP, Gao WS, Hao YJ. Ectopic expression of Apple *MdSUT2* gene influences development and abiotic stress resistance in tomato. Sci Hortic. 2017;220(36):259–66. https://doi.org/10.1016/j.scienta.2017.04.013.
57. Lu J, Sun MH, Ma QJ, Kang H, Liu YJ, Hao YJ, You CX. *MdSWEET17*, a sugar transporter in Apple, enhances drought tolerance in tomato. J Integr Agric. 2019;18(9):2041–51. https://doi.org/10.1016/s2095-3119(19)62695-x.

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