# Genome-wide identification of the *SWEET* gene family mediating the cold stress response in *Prunus mume*

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The SWEET (Sugars Will Eventually be Exported Transporter) gene family encodes a family of sugar transporters that plays an essential role in plant growth, reproduction, and biotic and abiotic stresses. *Prunus mume* is a considerable ornamental wood plant with high edible and medicinal values; however, low temperature has severely limited its geographical distribution. To investigate the putative *SWEET* genes responsible for the cold response, we identified 17 *SWEET* genes in *P. mume* and divided them into four groups. Sixteen of these genes were anchored on six chromosomes, and one gene was anchored on the scaffold with four pairs of segmental gene duplications and two pairs of tandem gene duplications. Cis-acting regulatory element analysis indicated that the *PmSWEET* genes were presumably involved in the *P. mume* developmental procedure, such as circadian control, abscisic acid-responsive and light-responsive, and responses to diversified stresses, such as low-temperature responsive, and drought-inducibility. We performed low-temperature treatment in the cold-tolerant cultivar ‘Songchun’ and cold-sensitive cultivar ‘Zaolve’ and found that seven of 17 *PmSWEETs* expressed upregulated or downregulated with prolonged treatment times, which indicated that these genes were prospective for cold resistance in *P. mume*. Our study provides the basis for further investigation into the role of *SWEET* proteins in the development of *P. mume* and its responses to cold resistance.

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## Abstract

The SWEET (Sugars Will Eventually be Exported Transporter) gene family encodes a family of sugar transporters that plays an essential role in plant growth, reproduction, and biotic and abiotic stresses. *Prunus mume* is a considerable ornamental wood plant with high edible and medicinal values; however, low temperature has severely limited its geographical distribution. To investigate the putative *SWEET* genes responsible for the cold response, we identified 17 *SWEET* genes in *P. mume* and divided them into four groups. Sixteen of these genes were anchored on six chromosomes, and one gene was anchored on the scaffold with four pairs of segmental gene duplications and two pairs of tandem gene duplications. Cis-acting regulatory element analysis indicated that the *PmSWEET* genes were presumably involved in the *P. mume* developmental procedure, such as circadian control, abscisic acid-responsive and light-responsive, and responses to diversified stresses, such as low-temperature responsive, and drought-inducibility. We performed low-temperature treatment in the cold-tolerant cultivar 'Songchun' and cold-sensitive cultivar 'Zaolve' and found that seven of 17 *PmSWEETs* expressed upregulated or downregulated with prolonged treatment times, which indicated that these genes were prospective for cold resistance in *P. mume*. Our study provides the basis for further investigation into the role of *SWEET* proteins in the development of *P. mume* and its responses to cold resistance.


**Keywords: SWEET, gene family, expression pattern, *Prunus mume*, cold response.**

## 1. Introduction

Sucrose is the main carbohydrate in most plants; it is synthesized in the leaves during photosynthesis and then transported by phloem sap to storage organs, such as roots, stems, flowers, seeds and fruits (Rennie and Turgeon, 2009; Lemoine et al., 2013). Sucrose provides energy and carbon sources for plants and act as an important signal and resistance molecule that participates in the normal growth of higher plants (Chen et al., 2015). However, these sugars must be assisted by appropriate sugar transporters and not transported independently to the storage organs (Ainsworth and Bush, 2011). At present, three transporter families have been identified as essential sugar transporters: monosaccharide transporters (MSTs), sucrose transporters (SUTs), and Sugar Will Eventually be Exported transporters (SWEETs) (Chen et al., 2010; Chen et al., 2015; Eom et al., 2015). Of these three families, *SWEETs* were the final ones to be depicted and first identified by Chen et al. in *Arabidopsis* (Chen et al., 2010). SWEET proteins act as sugar transporters that mediate the inflow or outflow of phloem parenchyma sugar into the phloem apoplast (Slewinski, 2011; Braun, 2012; Chen, 2014). Unlike the SUT and MST families, which require energy to transport sugar across the plasma membrane (Maynard and

Lucas, 1982; Lemoine, 2000), the SWEET family promotes the diffusion of sugar across concentration gradients on the cellular membrane or vacuolar membrane, regardless of the proton gradient or pH (Chen et al., 2012; Chen et al., 2015).

SWEET proteins are characterized by conserved MtN3_saliva (MtN3_slv) transmembrane (TM) domains (Chen et al., 2012), also known as PQ-loop repeats (Eom et al., 2015; Feng and Frommer, 2015). SWEETs in eukaryotes commonly consist of seven transmembrane helices (TMHs), which contain a pair of 3-TMH repeats detached by an added helix (Xuan et al., 2013), and this structure has been described as the "3-1-3" TM SWEET structure (Chen et al., 2010). In contrast to the SWEET protein of eukaryotes, prokaryote SWEET proteins, known as SemiSWEETs, comprise only three TMHs (Xuan et al., 2013). In eukaryotes, proteins that contain 6 or 7 TMHs are prevalent, but SemiSWEETs with 3 or 4 TMHs have also been detected in plant genomes. In a study of SWEET genes from 25 plant genomes, 140 of the 411 sugar transporters were semiSWEET; these 140 semiSWEETs either lack the first or second 3-TM domain or exist only in partial form (Patil et al., 2015), which indicates that semiSWEET is not unusual in higher plants and that SWEETs may be formed by direct fusion from SemiSWEETs (Jia et al., 2017). In addition, a novel extraSWEET protein consisting of 14 and 15 TMHs has been reported from *Vitis vinifera* (Patil et al., 2015) and *Oryza punctata* (Jia et al., 2017); it is speculated that this extraSWEET may be duplicated from the interior of 7 TMHs. Recent research on 3, 249 SWEET proteins also ascertained superSWEET with > 18 TMHs in oomycetes, which carry 5–8 repeats of a semiSWEET (Jia et al., 2017). According to phylogenetic analysis, the SWEET genes in *Arabidopsis* divided into four clades: Clade I (SWEET1–3) and Clade II (SWEET4–8) mainly transport glucose, while Clade I also transports hexose (Chen et al., 2010; Lin et al., 2014). Clade III (SWEET9–15) mainly transports sucrose (Chen et al., 2012; Eom et al., 2015), and Clade IV (SWEET16–17), which are located on the tonoplast membrane, mainly transports fructose (Eom et al., 2015). The phylogenetic SWEET genes of the plants described hereafter are all based on *Arabidopsis*.

Advances in whole-genome sequencing enabled genome-wide identification of SWEET genes that have been reported in numerous species. These include important crops, fruits and vegetables, such as rice (*Oryza sativa*) (Yuan and Wang, 2013), sorghum (*Sorghum bicolor*) (Mizuno et al., 2016), soybean (*Glycine max*) (Patil et al., 2015), apple (*Malus domestica*) (Wei et al., 2014), grape (*Vitis vinifera*) (Chong et al., 2014), banana (*Musa acuminate*) (Miao et al., 2017), tomato (*Solanum lycopersicum*) (Feng et al., 2015), rapeseed (*Brassica napus*) (Jian et al., 2016), potato (*Solanum tuberosum*) (Li et al., 2020), valencia sweet orange (*Citrus sinensis*) (Yao et al., 2021) and so forth. Additionally, many SWEET genes have been confirmed to play diverse and complex roles in physiological processes, such as nectar secretion (Ge et al., 2000; Lin et al., 2014), pollen development (Chen et al., 2015), senescence (Quirino et al., 1999), and seed filling (Sosso et al., 2015). Moreover, SWEET genes are also involved in biotic and abiotic

stress responses (Yuan and Wang, 2013), including the reaction of plants to stress at low temperatures. For example, overexpression of *AtSWEET16* and *AtSWEET17* increases cold tolerance (Chardon et al., 2013; Klemens et al., 2013; Guo et al., 2014); overexpression of *AtSWEET4* increases plant size and frost resistance (Chong et al., 2014; Liu et al., 2016); and *AtSWEET11* and *AtSWEET12* are also related to stress caused by cold or dehydration (Le Hir et al., 2015; Durand et al., 2016). *AtSWEET15* is also known as SAG29 (senescence-associated gene); however, its transcription level gradually increases at low temperature, high salinity, and drought during natural leaf senescence (Quirino et al., 1999). Cold stress significantly inhibited the expression of *CsSWEET2, 3,* and *16* in *Camellia sinensis*, while the expression of *CsSWEET1* and *CsSWEET17* increased sharply (Yue et al., 2015). The functional study of *CsSWEET16* in *C. sinensis* indicates that it is located in the vacuolar membrane and regulates the cold resistance of *A. thaliana* (Wang et al., 2018). The transcriptional activity of many *SlSWEET* genes is increased under low-temperature stress in *S. lycopersicum* (Feng et al., 2015). Studies have shown that the *MaSWEET* gene of bananas is upregulated in response to low temperature, salt, and osmotic stress (Miao et al., 2017). Using genome-wide analysis of the *BoSWEET* gene in *Brassica oleracea* var. *capitata*, five possible candidate genes were found to promote sugar transport and thereby enhance chilling tolerance in cabbage (Zhang et al., 2019).

*Prunus mume* is a traditional flower native to southwest China and the middle and lower reaches of the Yangtze River. In northern China, low temperatures severely limit growth and distribution. Even though SWEET sugar transporters have been associated with responses to cold in other species, little is known about the role of *PmSWEETs* in cold responses in *P. mume*. This study aimed to conduct a genome-wide analysis of the SWEET gene family in *P. mume*, providing the basis for the further study of *PmSWEETs*.

## 2. Materials and Methods

### 2.1 *Arabidopsis*, rice and other Species Genomic Resources

To explore the phylogeny of the SWEET genes in *P. mume* and other species, we downloaded SWEET proteins from two model plants (*Arabidopsis thaliana* and *Oryza sativa*, representing dicotyledons and monocotyledons) and eight other Rosaceae species. The protein sequences of 17 *AtSWEETs* and 21 *OsSWEETs* were downloaded from the TAIR 10 database (http://www.arabidopsis.org/) and TIGR (http://rice.plantbiology.msu.edu/), respectively. The *P. mume* genome sequence and annotation files were obtained from the *P. mume* genome project (http://prunusmumegenome.bjfu.edu.cn/); eight other Rosaceae genomes, *Malus domestica* (Daccord et al., 2017), *P. avium* (Shirasawa et al., 2017), *P. persica* (Verde et al., 2013), *P. yedoensis* (Baek et al., 2018), *Pyrus communis* (Linsmith et al., 2019), *Rosa chinensis* (Raymond et al., 2018), *P. salicina* (Liu et al., 2020), and *P. armeniaca* (Jiang et al., 2019), were downloaded from the Genome Database for Rosaceae databases (https://www. rosaceae.org/).

### 2.2 Identification of *SWEET* Genes in *P. mume* and other species

The Hidden Markov Model (HMM) profiles of the MtN3_slv domain for the SWEET gene family (PF03083) were downloaded from the Pfam database (http://pfam.xfam.org/) and used as queries to search for SWEET proteins in *P. mume* and other species proteomes with HMMER software (version 3.1b2, http://hmmer.org/) (Finn et al., 2015). To ensure confidence, the E-value cutoff was set at $10^{-5}$. Then, all putative SWEET proteins were screened to confirm the presence of the MtN3_slv domain by SMART (http://smart.embl-heidelberg.de/), the Pfam database (http://pfam.xfam.org/) and NCBI-CDD (https://www.ncbi.nlm.nih.gov/cdd), and sequences with MtN3_slv domain were retained.

The SWEET genes were named based on their location information in the genome. In addition, the number of amino acids, molecular weight (MW) and isoelectric point (pi) were calculated using the online ExPasy program (https://web.expasy.org/cgi-bin/protparam/protparam). The distributions of TM helices were predicted by TMHMM Server v. 2.0 (http://www.cbs.dtu.dk/services/TMHMM/) .

### 2.3 Phylogenetic and Conserved Domains Analysis

To examine the phylogeny between SWEET genes in *P. mume* and other species, full-length SWEET protein sequence alignment from three species (*P. mume*, *A. thaliana*, and *O. sativa*) and eight Rosaceae species was performed by using Mafft software with the FFT-NS-1 strategy (Katoh and Standley, 2013). Subsequently, maximum likelihood (ML) phylogenetic trees were constructed using FastTree (version 2.1.11) (Price et al., 2010) with default parameters. Then, iTols v4.0 (https://itol.embl.de/itol.cgi) (Letunic and Bork, 2019) and AI CS6 software were used to annotate and embellish the phylogenetic tree.

### 2.4 Conserved Motif and Gene Structure Analysis

The conserved motifs of *PmSWEETs* were predicted by MEME Suite Version 5.3.3 (https://meme-suite.org/meme/tools/meme) (Bailey et al., 2009) , the maximum number of motifs for the conserved domains was set to 10, motif width was set to 6-50, and the residuals were designated as the default parameters. Gene structure data were extracted from the *P. mume* genome gff file, visualized using Tbtools software (Chen et al., 2020), and then beautified in AI CS6 software.

### 2.5 Chromosome Location, Duplications and Synteny Analysis

The location and chromosome length information of *PmSWEETs* were obtained from the gff file. A chromosomal location figure was drawn using the online tool MG2C (http://mg2c.iask.in/mg2c_v2.0/). Gene tandem and segment replication events were analyzed

using the Multiple Collinearity Scan Toolkit (MCScanX) and Circos in Tbtools, respectively, using default parameters. The synteny of the *PmSWEETs* with *A. thaliana*, *P. armeniaca*, and *P. salicina* was mapped using MCScanX in Tbtools. The Ks and Ka values for duplicated gene pairs were calculated based on the coding sequence alignments using the Ka/Ks calculator in Tbtools. According to two ordinary rates (λ) of $1.5 \times 10^{-8}$ or $6.1 \times 10^{-9}$ substitutions per site per year (Lynch and Conery, 2000; Blanc and Wolfe, 2004), the formula $t = Ks/2\lambda \times 10^{-6}$ Mya was used to calculate the divergence time.

### 2.6 Cis-Acting Element in *PmSWEET* Gene Promoter Analysis

The upstream sequences (2.0 kb) of the *PmSWEETs* were retrieved from the genomic sequence data in Tbtools and then submitted to the PlantCARE database (http://bioinformatics.psb.ugent. be/webtools/plantcare/html/) (Lescot et al., 2002) for cis-acting analysis. We finally selected 12 elements, including those induced by hormones, such as MeJA (methyl jasmonate)-responsive, abscisic acid-responsive, and stress-responsive elements; the stress-responsive factors included defense and stress, low temperature, and light. Combined with phylogenetic tree information (nwk file), the map was constructed by Tbtools and beautified by AI CS6 software.

### 2.7 *PmSWEET* Genes Expression Analysis

To investigate the function of *PmSWEETs* involved in tissue development and cold tolerance, we used the root, stem, leaf, bud and fruit data of RNA sequencing (Zhang et al., 2012) to analyze the *PmSWEET* expression patterns in different tissues and then used the flower bud dormancy data of RNA sequencing of *P. mume* (‘Zaolve’) (Zhang et al., 2018) to analyze the *PmSWEET* response to low temperature from November to February. Furthermore, we explored the expression of the stem in *P. mume* (‘Songchun’) in three different places (Beijing (BJ, N39°54′, E116°28′), Chifeng (CF, N42°17′, E118°58′) and Gongzhuling (ZGL, N43°42′, E124°47′)) and three different periods (cold exercise (October, autumn), the final period of endo-dormancy (January, winter), and deacclimation (March, spring). Tbtools (Chen et al., 2020) was used to create the heatmap.

### 2.8 qRT–PCR Analysis of *PmSWEET* Genes

To examine the effect of *PmSWEET* response to low temperature, the annual branches of the cold-sensitive cultivar ‘Zaolve’ and the cold-tolerant cultivar ‘Songchun’ were collected. Before chilling treatment, the shoots were incubated overnight at 22 °C and then transferred to 4 °C for 0, 1, 4, 6, 12, 24, 48, and 72 h under long-day conditions (16-h light/8-h dark). The stems were collected immediately and stored in liquid nitrogen at -80 degrees Celsius for complete RNA isolation. Each treatment had three biological replicates.

Total RNA of each sample was extracted using the RNAprep Pure Plant Plus Kit (Tiangen, Beijing, China). Complementary cDNA was synthesized using ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan). The specific primers were designed by Primer 3 (https://bioinfo.ut.ee/primer3-0.4.0/) based on the cDNA sequences (Table S1). The expression levels of *PmSWEETs* at low temperature were analyzed using quantitative real-time polymerase chain reaction (qRT–PCR) with a PikoReal real-time PCR system (Thermo Fisher Scientific, CA, USA) with SYBR® PremixEx*Taq* TM (TaKaRa, Dalian, China). The reactions were performed in a 10 μL volume, including 5 μL SYBR®Green Premix *Pro Taq* HS qPCR Kit, 0.5 μL each of forward and reverse primers, 1μL cDNA and 3 μL dd$H_2O$. The reactions were performed according to the following procedure: 95 °C for 30 s, 40 cycles of 95 °C for 5 s and 60 °C for 30 s. With the phosphatase 2A gene of *P. mume* as the reference gene, the relative expression was calculated by the $2^{-\Delta\Delta Ct}$ method. The final data were subjected to an analysis of variance test.

## 3. Results

### 3.1 Identification of the *SWEET* Gene Family

A total of 17 nonredundant *PmSWEETs* were detected in the *P. mume* genome (sequence information is shown in Supplement File S1), and 175 SWEETs were detected in the other eight species of Rosaceae, including 16 *SWEET* genes in *P. armeniaca*, 19 in *P. avium*, 19 in *P. persica*, 19 in *P. salicina*, 16 in *P. yedoensis*, 21 in *P. communis*, 29 in *M. domestica*, and 36 in *R. chinensis* with rigorous filtering. All the newly identified SWEET genes were named according to their location on the chromosome (Table 1 and Table S2). We determined that candidates with at least one MtN3_slv domain were "genuine" *SWEETs*, all *SWEETs* contained MtN3_slv domains (domain architecture of *PmSWEETs* is shown in Supplement File S2). The number of amino acids, molecular weight (MW), and isoelectric point (pI) were calculated on the basis of the protein sequences. As exhibited in Table 1, the predicted *PmSWEET* proteins ranged from 105 (*PmSWEET14*) to 580 (*PmSWEET8*) in amino acids length, with relative molecular weights ranging from 15.96 kDa (*PmSWEET11*) to 63.43 kDa (*PmSWEET8*), and theoretical pIs from 8.30 (*PmSWEET4*) to 9.76 (*PmSWEET3*), except *PmSWEET14*, its pI and MW cannot be computed, because its sequence contains four consecutive undefined AA (Table 1). Through prediction and analysis of TMHs of putative *PmSWEET* proteins, we found that this family includes 2–7 TMHs, and seven genes possess 7 TMHs. Detailed location information of the TMH is shown in Table S3 and Figure S1.

### 3.2 Phylogeny Analysis and Classification of *SWEET* Genes

To better understand the evolution of homologous *SWEET* genes, we used the ML method to create a phylogenetic tree of all *SWEET* sequences from *A. thaliana* (dicots), *O. sativa*

(monocots), and *P. mume*. According to the previously reported *AtSWEETs* and *OsSWEETs* (Chen et al., 2010; Yuan and Wang, 2013), 17 *PmSWEETs* were divided into four clades (i.e., Clade I, Clade II, Clade III, and Clade IV) (Figure S2). To investigate the evolutionary relationships between *PmSWEETs* and other species, an ML phylogenetic tree of *SWEETs* from 11 species was constructed, including 8 other Rosaceae species. All members of the *SWEET* gene family in 11 species were divided into four clades (Figure 1). The largest clade was Clade III, which comprised five *OsSWEET* genes, seven *AtSWEET* genes, and 68 Rosaceae *SWEET* genes; the specific number of genes is shown in Table S4. The smallest clade was Clade IV, which consisted of only two *A. thaliana SWEET* genes, one *O. sativa* gene, and 18 Rosaceae *SWEET* genes (Table S4), indicating that SWEETs were distributed unevenly in the different clades. The number of Clade I, II and III genes varied greatly, suggesting that SWEET gene families expanded, especially in Clades I, II and III, during Rosaceae evolution. The SWEETs from the Rosaceae genus were distributed uniformly in every small clade, whereas SWEETs from *O. sativa* tended to cluster together. The *PmSWEETs*, *PpSWEETs*, and *PavSWEETs* were clustered together and had similar distributions in the phylogenetic tree.

### 3.3 Conserved Motif and Gene Structure Analysis

To explore the sequence features of *PmSWEET* proteins, MEME software and Tbtools were used to predict and draw conserved domains. As a consequence, ten distinct motifs were detected in *SWEET* proteins (Figure 2B), and a schematic diagram of *PmSWEET* protein motifs is shown in Figure S3. Motifs 3, 1, 2, 6 and 4 were highly conserved and present in 16, 15, 13, 12 and 11 *PmSWEET* proteins, respectively, while motifs 7, 8 and 10 were relatively evolutionary and were only present in 4, 2 and 2 *PmSWEET* proteins, respectively. Aside from some unusual proteins, most *PmSWEETs* contain 4–6 conserved motifs. For instance, *PmSWEET14* in Clade III only had one motif. Intriguingly, *SWEET* members of the same clade, particularly the closest members, have comparable conservative motifs, suggesting that they might have similar functions.

To elucidate the structural characteristics of the *PmSWEETs*, the exon-intron structure was further analyzed. As shown in Figure 2C, *PmSWEETs* in Clade II (except *PmSWEET10*) contained four introns. *PmSWEET1, 9,* and *15* in Clade III had five introns, *PmSWEET8* contained the largest 12 introns, and *PmSWEET14* contained only one intron. All *PmSWEETs* in Clade IV had five introns. The number of introns in clade I varied from just two to ten. These results indicated that introns in the same phylogenetic clade were relatively evenly distributed.

### 3.4 Chromosomal Distribution and Tandem Duplication (TD) of the *PmSWEET* Gene Family

According to gene loci information, the 17 *PmSWEETs* on chromosomes were mapped, showing that 16 *PmSWEETs* were located on chromosomes, and one *PmSWEET* gene was

located on scaffold54 (Figure 3). *PmSWEETs* on chromosomes 6 and 7 were clustered in the center of chromosomes, and all contained four *PmSWEETs*. Two genes were distributed on chromosomes 2, 3, 4 and 5. *PmSWEET11* and *PmSWEET12*, *PmSWEET14* and *PmSWEET15* were clustered into two tandem duplication events on chromosomes 6 and 7. Based on the above results, some *PmSWEETs* were probably generated by gene tandem duplication.

### 3.5 Segmental Duplication and Synteny of the *PmSWEET* Gene Family

The synteny analysis of *PmSWEETs* was determined using the Circos program of Tbtools, four segmental duplication events (*PmSWEET1*/*PmSWEET14*, *PmSWEET5*/*PmSWEET8*, *PmSWEET6*/*PmSWEET9* and *PmSWEET6*/*PmSWEET16*) were detected, and they were located on di□erent chromosomes, as indicated with red lines in Figure 4, indicating that some *PmSWEETs* were probability generated by gene segmental duplication. In addition, the selection pressure and divergence time of the duplicated events were estimated by the substitution rate of Ka (nonsynonymous) and Ks (synonymous). In the evolutionary process, the ratio Ka/Ks > 1 means positive selection (adaptive evolution), ratio = 1 means neutral evolution (drift), and ratio < 1 means negative selection (conservation). Only one pair of segmental duplication *PmSWEETs* (*PmSWEET6/9*) had a Ka/Ks ratio of 0.45, which was significant and indicated a synonymous change and was purified and selected during evolution. The differentiation period of the *PmSWEET6/9* gene pair was 55.34~136.07 Mya.

To further emphasize the specific retention of *PmSWEETs*, their collinearity relationship with *AtSWEETs*, *PaSWEETs*, and *PsSWEETs* was detected using the MCScanX procedure of Tbtools. A total of 16 homologous gene pairs were detected in *P. mume* and *A. thaliana*. Similarly, 16 pairs of homologous genes between *P. mume* and *P. armeniaca* and 20 between *P. mume* and *P. salicina* were detected (Figure 5, Table S5). The collinear complexity of *P. mume* and *P. salicina* was much higher than that of *P. armeniaca* and *A. thaliana*. These results suggested that *P. mume* was relatively distantly related to *A. thaliana* and *P. armeniaca* and was close to *P. salicina.*

### 3.6 Prediction Analysis of Cis-Acting Elements within *PmSWEETs*

To further investigate the possible regulatory mechanism of *PmSWEETs* in the process of growth and the defense reaction, in particular in response to abiotic stress, such as low temperature, we submitted the 2.0 kb upstream sequence from the translation start site of the *PmSWEETs* to the PlantCARE database to detect the cis-elements. The *PmSWEET* promoters comprised several conserved regulatory elements in response to plant hormones and environmental stress, and twelve of them were analyzed (Figure 6, Table S6). Elements related to light response, anaerobic induction, and abscisic acid (ABA) response were widespread in the promoter areas of 17, 17 and 16 SWEET genes, respectively. According to the regulatory

elements in their promoters, 14, 12, 11, 10, and 9 *PmSWEETs* were sensitive to drought inducibility, MeJA, gibberellin, low temperatures and auxin. Combined with the results of phylogenetic analysis, it was found that gene members of the same clade had similar cis-elements. These results indicated that *PmSWEET* genes were involved in the regulatory mechanisms of various stress responses.

### 3.7 Expression pattern analysis of *PmSWEETs*

To investigate the role of *PmSWEETs* in development and response to low temperature, the expression patterns of roots, stems, leaves, buds, fruits and flower bud dormancy in different stages were examined based on the RNA-seq dataset, and their RPKM values are shown in Tables S7 and S8. As exhibited in Figure 7A, 14 of the *PmSWEET* genes were expressed in at least one tissue, whereas three (*PmSWEET5*, *10*, *11*) were not detected. Among them, five *PmSWEETs* presented relatively higher expression levels in fruits (*PmSWEET9*, *6*, *17*, *1*, *12*) and buds (*PmSWEET13*, *16*, *15*, *3*, *14*). Two *PmSWEETs* showed higher expression levels in roots (*PmSWEET4*, *7*) and stems (*PmSWEET2*, *8*). Additionally, several genes were expressed in leaves, but their expression levels were low.

Most *PmSWEETs* were expressed during the bud dormancy period (except *PmSWEET5*, *16*) and expressed specifically during certain developmental stages (Figure 7B). Ten *PmSWEET* genes exhibited specifically higher expressions in the Natural flush (NF) stage (February), *PmSWEET9* was preferentially expressed in the Endo-dormancy I (EDI) stage (November), *PmSWEET10*, *12* showed the highest level of expression in the Endo-dormancy II (EDII) stage (December), *PmSWEET3*, *6*, *1*, *13*, *12* were upregulated in the Endo-dormancy III (EDIII) stage (January). Among these upregulated genes, eight *PmSWEETs* (*PmSWEET6*, *7*, *10*, *11*, *13*, *14*, *15*, *17*) (Table S6) all contained low temperature response elements.

To further investigate the expression patterns of *PmSWEETs* under cold response, we analyzed the stems of the cold-tolerant cultivar *P. mume* 'Songchun' from three regions, and their FPKM values are displayed in Table S9. The expression of six *PmSWEET* genes (*PmSWEET11*, *17*, *6*, *16*, *5*, *14*) was not detected. Among the other 11 *PmSWEET* genes, seven *PmSWEETs* (*PmSWEET8*, *2*, *9*, *3*, *7*, *4*, *1*) showed higher expression in spring (3.2~5.3 °C). *PmSWEET13* was upregulated in autumn (6.1~7.9 °C) and winter in Beijing (-5.4 °C) and Chifeng (-11.4 °C), but downregulated in spring; *PmSWEET15*, *10*, and *12* increased significantly in winter in Beijing (-5.4 °C) (Figure 8A). Among these upregulated genes, four *PmSWEETs* (*PmSWEET7*, *10*, *13*, *15*) (Table S6) all contained low-temperature response elements. To compare the expression patterns of *PmSWEETs* in different periods, another heatmap was generated (Figure 8B). As shown in Figure 8B, *PmSWEETs* in Chifeng and Gongzhuling showed similar expression patterns in the same periods, while *PmSWEETs* in Beijing showed higher expression in winter (Figure 8B).

### 3.8 Expression Patterns of *P. mume* SWEETs under Cold Treatment

To investigate the role of *PmSWEETs* in the cold response, the expression patterns under deliberate hypothermia (4 °C) (0, 1, 4, 6, 12, 24, 48 and 72 h) were examined by qRT–PCR using the cold-sensitive cultivar 'Zaolve' and the cold-tolerant cultivar 'Songchun'. We performed a qRT–PCR assay on 17 *PmSWEET* genes, but only 11 *PmSWEETs* were detected, while the remaining 6 *PmSWEETs* (*PmSWEET5*, *6*, *9*, *11*, *15*, *16*) were not detected, consistent with the transcriptome data (Figure 7, 8). As displayed in Figure 9, the expression levels of 11 genes in the two cultivars changed in different patterns during artificial cold treatment. Within 'Songchun', *PmSWEET2*, *4*, *7*, *8*, *10* were dramatically downregulated with increased cold stress treatment time, while *PmSWEET10* was increased at 24 h. The expression levels of *PmSWEET13* were raised with the continuation of the treatment time, which rose approximately 11-fold after 6 h of cold treatment. *PmSWEET14* was quickly upregulated at 72 h. The expression levels of *PmSWEET3* and *17* changed only slightly. *PmSWEET1* and *12* were upregulated at 1 h and then downregulated with increasing treatment time, while they were upregulated after 48 h. Within 'Zaolve', *PmSWEET1* and *PmSWEET12* were rapidly up-regulated at 48 h and 72 h, respectively. *PmSWEET4* and *10* were upregulated within 6 h and then declined with extended treatment time. The expression of *PmSWEET14* was not apparent in the early stage, but it was upregulated at 24 h. *PmSWEET17* was highly expressed only at 4 h. *PmSWEET2*, *7*, and *8* were dramatically downregulated at early treatment and then increased slightly with increased treatment time. The expression levels of *PmSWEET13* increased rapidly with prolonged treatment time, rose approximately 9-fold after 1 h, and then increased nearly 80-fold after 72 h of cold treatment. The expression level of *PmSWEET3* also changed, but the change was not significant.

## 4. Discussion

SWEET is a sugar transporter family that supports the transportation of sugar, mainly sucrose, glucose and fructose (Chen et al., 2010; Chen et al., 2012; Feng and Frommer, 2015; Guo et al, 2014; Klemens et al., 2013; Le Hir et al., 2015), which plays a critical role in the growth and development of many plants and responses to biological and abiological factors (Lemoine et al., 2013; Li et al., 2017; Li et al., 2018; Zhao et al., 2018). Previous studies revealed that SWEET participates in cold stresses (Chardon et al., 2013; Klemens et al., 2013; Guo et al., 2014; Chong et al., 2014; Liu et al., 2016; Le Hir et al., 2015; Yue et al., 2015; Wang et al., 2018; Feng et al., 2015; Miao et al., 2017; Zhang et al., 2019) and that *P. mume* can blossom at lower temperatures; thus, it is a very good material to study the mechanism of cold responses. In this research, we detected a total of 17 *PmSWEETs* in *P. mume*, as much as in *Arabidopsis*, which is also similar to other species in *Prunus*, showing that SWEET genes are still relatively conserved in *Prunus*. The length of *PmSWEET* proteins ranges from 105 aa to 580

aa, and this range in length provides diversity in the number of TMHs (2–7). *PmSWEET*, except for *PmSWEET14*, has a theoretical pI larger than 8.0. As an important parameter of proteins, pI is determined by the relative contents of amino acid residues at different pH values, which affects the stability, activity and function of proteins (Gasteiger, 2005). *PmSWEET14* did not detect pI, which may be due to its short amino acid sequence.

By predicting TMH domains, we found that the number of TMHs in *PmSWEET* genes ranged from 2 to 7 (Table 1). Fewer than seven TMHs in the eukaryotic SWEET family were also found in wheat, walnut (Gao et al., 2018; Jiang et al., 2020) and soybean (Patil et al., 2015). To further validate the accuracy of the SWEET protein, we submitted the protein sequence to the NCBI-CDD and SMART to predict its conserved domains, and it was found that all of them contain the MtN3_slv domain and belong to the SWEET family. Genetic loss or amplification and the emergence of certain SWEETs with only two, three, four, five or six TMHs means that SWEET replication and fusion might take place in the *P. mume* genome.According to the phylogenetic evolutionary relationship of *AtSWEET* and *OsSWEET*, *PmSWEETs* were classified into four clades. In a total of 230 SWEET genes, the number of members in Clade III was larger than that in other clades, suggesting that Clade III may have expanded during evolution. In *P. mume*, Clades I, II and III have the same number of SWEET genes, and Clade IV has only two SWEET genes, indicating high conservation in the SWEET family in the process of evolution. Most of the closely related genes in the family exhibit similar motif compositions, suggesting that there are functional similarities in the SWEET family genes. Gene structural diversity and conserved protein motif divergence played key roles in the evolution of the *SWEET* gene family (Xu et al., 2012). Gene members in each clade harbored some unique conserved motif, suggesting functional diversity of the SWEET genes in *P. mume*.

Gene duplication, including tandem and segmental duplication events, is the origin of gene family extension and genomic evolution in plants (Cannon et al., 2004; Ganko et al., 2007). In this study, two pairs of *PmSWEETs* were detected as tandem duplications, and four pairs of *PmSWEETs* were segmental duplications. This outcome was consistent with other studies on *SWEET* duplication, including segmental and tandem duplications (Feng et al., 2015; Miao et al., 2017; Gao et al., 2018; Jiang et al., 2020).

The cis-elements in the promoter play an essential role in gene regulation. All *PmSWEETs* contain at least one light-responsive and anaerobic induction cis-element, suggesting that the two elements have an essential role in *PmSWEET* regulation. Moreover, 10 *PmSWEETs* contained one or more low-temperature responsive cis-elements (Table S6), indicating that these *PmSWEETs* may play considerable roles in the response to cold stress. However, whether and how these cis-elements work in *P. mume* requires further research.

Studies have shown that under low-temperature stress, the soluble sugar content in plants increases, and sugar transporters maintain the balance of osmotic potential through the balance and distribution of sugar, thus improving the cold tolerance of plants (Yamada et al., 2010). Numerous studies have also verified that SWEETs are involved in maintaining sugar homeostasis in plant organs and promoting plant adaptation to low temperatures (Seo et al., 2011; Chardon et al., 2013; Klemens et al. 2013; Chandran, 2015; Le Hir et al. 2015; Miao et al., 2017; Wang et al., 2018; Zhang et al., 2019; Zhang et al., 2020). Transcriptome analysis showed that *PmSWEETs* were differentially expressed in different tissues and during dormancy release and cold acclimation. *PmSWEET5* expression was not detected in any tissue/organ we used, indicating that its expression may be species-specific or time-specific. Some *PmSWEETs* in different organs had specific expression patterns (Figure 7A). For example, expression of *PmSWEET10* was only detected in 'Zaolve' buds on dormancy EDII and 'Songchun' stems in winter in Beijing; *PmSWEET11* was only detectable in 'Zaolve' buds dormancy release; *PmSWEET16* was only detected in *P. mume* buds; which indicates that the gene is only expressed in specific tissues or varieties. *PmSWEET1*, *9* (Clade III), *PmSWEET12*, *17* (Clade I) and *PmSWEET6* (Clade II) were strongly expressed in fruit, indicating that these genes may regulate sugar allocation during fruit ripening. *PmSWEET13*, *16* (Clade II), *PmSWEET14*, *15* (Clade III) and *PmSWEET3* (Clade I) were strongly expressed in the bud, indicating that they might play a part in the development of floral organs. *PmSWEET4* (Clade I) and *PmSWEET7* (Clade IV) were strongly expressed in roots. Previous studies have demonstrated that *SWEETs* in Clade IV were highly expressed in the root cortex and encode proteins as specific fructose uniporters in the root vacuole membrane (Guo et al., 2014). *PmSWEET2* (Clade IV) and *PmSWEET8* (Clade III) were strongly expressed in the stem, suggesting the potential roles of these genes in long-distance sugar transport.

The present results also show that most of the *PmSWEET* genes are expressed more strongly in different endo-dormancy stages of flower bud and fruit tissues than in other tissues and that these genes are differentially expressed during flower development (Fig. 7A, 7B). Together, these results sugest that the *P. mume* SWEET family is intimately associated with reproductive development and that different genes are specifically involved during different developmental stages. In rice, *Arabidopsis* and soybean, the expression of SWEET genes is also relatively higher in reproductive tissues than in other tissues (Yuan et al., 2014; Patil et al., 2015). *PmSWEETs* also have different expression levels during dormancy release on flower buds (from November to February). Thus, we speculate that these *PmSWEETs* may participated in the cold reaction at low temperatures to protect the flower bud. In addition, some *PmSWEETs* were expressed more at colder temperatures in the spring (3.2~5.3 °C) and at approximately -5 °C in the winter (Figure 8A), indicating that these two temperatures may trigger their cold stress response and increase *PmSWEET* expression to reduce stress injury.

The qRT–PCR analysis suggested that six of 17 *PmSWEET* genes (*PmSWEET5*, *6*, *9*, *11*, *15*, *16*) were not expressed in the stem, which was consistent with the transcriptome data. *PmSWEETs* were activated by low temperature (4 °C) and increased or decreased with the extension of treatment time (Figure 9). The expression levels of five *PmSWEETs* (*PmSWEET2*, *4*, *7*, *8*, *10*) in 'Songchun' and three *PmSWEETs* (*PmSWEET2*, *7*, *8*) in 'Zaolve' decreased with increasing treatment times (Figure 9), which suggested that these genes might be negatively regulated by low temperatures and increased cold sensitivity. The expression levels of two *PmSWEETs* (*PmSWEET13*, *14*) in 'Songchun' and four *PmSWEETs* (*PmSWEET1*, *12*, *13,14*) in 'Zaolve' increased with prolonged treatment times (Figure 9), which suggested that these genes might be positively regulated by cold stress responses and increased cold sensitivity. The discrepancy in expression patterns between *PmSWEET1, 4, 10, 12,* and *17* is potentially due to the species differences between 'Songchun' and 'Zaolve'.

## 5. Conclusions

In summary, our study is the first to show genome-wide identification and characterization of SWEETs in *P. mume*, including chromosomal location, duplicated genes, gene structure, phylogenetic relationships and conserved motifs. In addition, the expression profiles of the *PmSWEET* genes in different tissues and places were also examined based on the RNA-seq data. Furthermore, the expression profiles of these *PmSWEET* genes under cold stress conditions were analyzed by qRT–PCR assay. Our results could provide important information for further research on the biological functions of *PmSWEETs*.

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**Conflict of Interest**

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

**Author Contributions**

LS: conceptualization. PL and ML: data curation. ZW: formal analysis and software. LS, QZ and TC: funding acquisition and writing reviews and editing. ZW and JM: methodology. ZW: validation, visualization, and writing original draft. All authors contributed to writing, and approved the final manuscript.

**Data Availability Statement**

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author/s.

**Supplementary Material**

Supplemental information for this article can be found online at

Supplementary Figure 1 | Schematic representation of PmSWEET proteins.
Supplementary Figure 2 | Phylogenetic trees of *Arabidopsis thaliana*, *Prunus mume* and Rice
Supplementary Figure 3 | Schematic diagram of *PmSWEET* protein motifs
Supplementary Table 1 | Primer sequences used for qRT-PCR
Supplementary Table 2 | Information for the proteins used in the present study
Supplementary Table 3 | TM helix Locus of *PmSWEETs*Supplementary Table 4 | The specific number of genes in the Clades used in the present study
Supplementary Table 5 | Duplication events between *P. mume* and *A. thaliana*, *P. armeniaca* and *P. salicina*
Supplementary Table 6 | The data of cis-acting element in *PmSWEETs*promoters
Supplementary Table 7 | Expression profiles of 17 *PmSWEET* genes in five different tissues (root, stem, leaf, bud and fruit) (RPKM)
Supplementary Table 8 | Expression profiles of *PmSWEET* genes during the process of flower

490 bud dormancy release (RPKM)
491 Supplementary Table 9 | Expression profiles of 17 *PmSWEET* genes in different regions and
492 seasons (FPKM)
493 Supplementary Flie 1 | Protein sequences of *P. mume*
494 Supplementary Flie 2 | Domain architecture of *PmSWEETs*
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## References


Ainsworth, E.A., and Bush, D.R. (2011). Carbohydrate export from the leaf: A highly regulated process and target to enhance photosynthesis and productivity. *Plant Physiol.* 155(1)**,** 64-69. doi: 10.1104/pp.110.167684s

Baek, S., Choi, K., Kim, G.B., Yu, H.J., Cho, A., Jang, H., et al. (2018). Draft genome sequence of wild *Prunus yedoensis* reveals massive inter-specific hybridization between sympatric flowering cherries. *Genome Biol.* 19(1), 127. doi: 10.1186/s13059-018-1497-y

Bailey, T.L., Boden, M., Buske, F.A., Frith, M., Grant, C.E., Clementi, L., et al. (2009). MEME SUITE: tools for motif discovery and searching. *Nucleic Acids Res.* 37(Web Server issue), W202-208. doi: 10.1093/nar/gkp335

Blanc, G., and Wolfe, K.H. (2004). Widespread paleopolyploidy in model plant species inferred from age distributions of duplicate genes. *Plant Cell* 16(7)**,** 1667-1678. doi: 10.1105/tpc.021345.

Braun, D.M. (2012). SWEET! The pathway is complete. *Science* 335(6065), 173-174. doi: 10.1126/science.1216828

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

Chardon, F., Bedu, M., Calenge, F., Klemens, P.A.W., Spinner, L., Clement, G., et al. (2013). Leaf fructose content is controlled by the vacuolar transporter *SWEET17* in *Arabidopsis*. *Curr. Biol.* 23(8), 697-702. doi: 10.1016/j.cub.2013.03.021

Chandran, D. (2015). Co-option of developmentally regulated plant SWEET transporters for pathogen nutrition and abiotic stress tolerance. *IUBMB Life*. 67(7): 461-71. doi: 10.1002/iub.1394

Chen, C., Chen, H., Zhang, Y., Thomas, H.R., Frank, M.H., He, Y., et al. (2020). TBtools: An integrative toolkit developed for interactive analyses of big biological data. *Mol. Plant.* 13(8), 1194-1202. doi: 10.1016/j.molp.2020.06.009

Chen, L.Q. (2014). SWEET sugar transporters for phloem transport and pathogen nutrition. *New Phytol.* 201(4), 1150-1155. doi: 10.1111/nph.12445

Chen, L.Q., Hou, B.H., Lalonde, S., Takanaga, H., Hartung, M.L., Qu, X.Q., et al. (2010). Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* 468(7323), 527-532. doi: 10.1038/nature09606

Chen, L.Q., Cheung, L.S., Feng, L., Tanner, W., and Frommer, W.B. (2015). Transport of sugars. *Annu. Rev. Biochem.* 84, 865-894. doi: 10.1146/annurev-biochem-060614-033904

Chen, L.Q., Qu, X.Q., Hou, B.H., Sosso, D., Osorio, S., Fernie, A.R., et al. (2012). Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* 335(6065), 207-211. doi: 10.1126/science.1213351

Chong, J., Piron, M.C., Meyer, S., Merdinoglu, D., Bertsch, C., and Mestre, P. (2014). The SWEET family of sugar transporters in grapevine: *VvSWEET4* is involved in the interaction with Botrytis cinerea. *J. Exp. Bot.* 65(22), 6589-6601. doi: 10.1093/jxb/eru375

Daccord, N., Celton, J.M., Linsmith, G., Becker, C., Choisne, N., Schijlen, E., et al. (2017). High-quality de novo assembly of the apple genome and methylome dynamics of early fruit development. *Nat. Genet.* 49(7), 1099-1106. doi: 10.1038/ng.3886

Durand, M., Porcheron, B., Hennion, N., Maurousset, L., Lemoine, R., and Pourtau, N. (2016). Water deficit enhances C export to the roots in *Arabidopsis thaliana* plants with contribution of sucrose transporters in both shoot and roots. *Plant Physiol.* 170(3), 1460-1479. doi: 10.1104/pp.15.01926

Eom, J.S., Chen, L.Q., Sosso, D., Julius, B.T., Lin, I.W., Qu, X.Q., et al. (2015). SWEETs, transporters for intracellular and intercellular sugar translocation. *Curr. Opin. Plant Biol.* 25, 53-62. doi: 10.1016/j.pbi.2015.04.005

Feng, L., and Frommer, W.B. (2015). Structure and function of SemiSWEET and SWEET sugar transporters. *Trends Biochem. Sci.* 40(8)**,** 480-486. doi: 10.1016/j.tibs.2015.05.005.

Feng, C.Y., Han, J.X., Han, X.X., and Jiang, J. (2015). Genome-wide identification, phylogeny, and expression analysis of the SWEET gene family in tomato. *Gene* 573(2), 261-272. doi: 10.1016/j.gene.2015.07.055

Finn, R.D., Clements, J., Arndt, W., Miller, B.L., Wheeler, T.J., Schreiber, F., et al. (2015). HMMER web server: 2015 update. *Nucleic Acids Res.* 43(W1), W30-W38. doi: 10.1093/nar/gkv397

Ganko, E.W., Meyers, B.C., and Vision, T.J. (2007). Divergence in expression between duplicated genes in *Arabidopsis*. *Mol. Biol. Evol.* 24(10), 2298-2309. doi: 10.1093/molbev/msm158

Gao, Y., Wang, Z.Y., Kumar, V., Xu, X.F., Yuan, P., Zhu, X.F., et al. (2018). Genome-wide identification of the SWEET gene family in wheat. *Gene* 642, 284-292. doi: 10.1016/j.gene.2017.11.044

Gasteiger, E. (2005). Protein identification and analysis tools on the ExPASy server. *The proteomics protocols handbook*. Humana Press, pp. 571-607

Ge, Y.X., Angenent, G.C., Wittich, P.E., Peters, J., Franken, J., Busscher, M., et al. (2000). NEC1, a novel gene, highly expressed in nectary tissue of *Petunia hybrida*. *Plant J.* 24(6), 725-734. doi: 10.1046/j.1365-313x.2000.00926.x

Guo, W.J., Nagy, R., Chen, H.Y., Pfrunder, S., Yu, Y.C., Santelia, D., et al. (2014). *SWEET17*, a facilitative transporter, mediates fructose transport across the tonoplast of *Arabidopsis*roots and leaves. *Plant Physiol.* 164(2), 777-789. doi: 10.1104/pp.113.232751

Jia, B., Zhu, X.F., Pu, Z.J., Duan, Y.X., Hao, L.J., Zhang, J., et al. (2017). Integrative view of the diversity and evolution of SWEET and SemiSWEET sugar transporters. *Front. Plant Sci.* 8, 2178. doi: 10.3389/fpls.2017.02178

570 Jian, H., Lu, K., Yang, B., Wang, T., Zhang, L., Zhang, A., et al. (2016). Genome-wide analysis
571 and expression profiling of the SUC and SWEET gene families of sucrose transporters in
572 *Oilseed Rape* (*Brassica napus* L.). *Front. Plant Sci.* 7, 1464. doi: 10.3389/fpls.2016.01464
573 Jiang, F., Zhang, J., Wang, S., Yang, L., Yingfeng, L., Gao, S., et al. (2019). The apricot (*Prunus*
574 *armeniaca* L.) genome elucidates Rosaceae evolution and beta-carotenoid synthesis.
575 *Hortic. Res.* 6, 128. doi: 10.1038/s41438-019-0215-6
576 Jiang, S., Balan, B., Assis, R.d.A.B., Sagawa, C.H.D., Wan, X., Han, S., et al. (2020). Genome-
577 wide profiling and phylogenetic analysis of the SWEET sugar transporter gene family in
578 walnut and their lack of responsiveness to *Xanthomonas arboricola* pv. juglandis infection.
579 *Int. J. Mol. Sci.* 21(4), 1251. doi: 10.3390/ijms21041251
580 Katoh, K., and Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7:
581 improvements in performance and usability. *Mol. Biol. Evol.* 30(4), 772-780. doi:
582 10.1093/molbev/mst010
583 Klemens, P.A., Patzke, K., Deitmer, J., Spinner, L., Le Hir, R., Bellini, C., et al. (2013).
584 Overexpression of the vacuolar sugar carrier *AtSWEET16* modifies germination, growth,
585 and stress tolerance in *Arabidopsis*. *Plant Physiol.* 163(3), 1338-1352. doi:
586 10.1104/pp.113.224972
587 Le Hir, R., Spinner, L., Klemens, P.A.W., Chakraborti, D., de Marco, F., Vilaine, F., et al. (2015).
588 Disruption of the sugar transporters *AtSWEET11* and *AtSWEET12*affects vascular
589 development and freezing tolerance in *Arabidopsis*. *Mol. Plant* 8(11), 1687-1690. doi:
590 10.1016/j.molp.2015.08.007
591 Lemoine, R. (2000). Sucrose transporters in plants: update on function and structure. *BBA.*
592 *Bioenergetics* 1465(1-2), 246-262. doi: 10.1016/s0005-2736(00)00142-5
593 Lemoine, R., La Camera, S., Atanassova, R., Dedaldechamp, F., Allario, T., Pourtau, N., et al.
594 (2013). Source-to-sink transport of sugar and regulation by environmental factors. *Front.*
595 *Plant Sci.* 4, 272. doi: 10.3389/fpls.2013.00272
596 Lescot, M., Dehais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., et al. (2002).
597 PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in
598 silico analysis of promoter sequences. *Nucleic Acids Res.* 30(1), 325-327. doi:
599 10.1093/nar/30.1.325
600 Letunic, I., and Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new
601 developments. *Nucleic Acids Res.* 47(W1), W256-W259. doi: 10.1093/nar/gkz239
602 Li, H., Li, X., Xuan, Y., Jiang, J., Wei, Y., and Piao, Z. (2018). Genome wide identification and
603 expression profiling of SWEET genes family reveals its role during plasmodiophora
604 brassicae-induced formation of clubroot in *Brassica rapa*. *Front. Plant Sci.* 9, 207. doi:
605 10.3389/fpls.2018.00207
606 Li, J., Qin, M., Qiao, X., Cheng, Y., Li, X., Zhang, H., et al. (2017). A new insight into the
607 evolution and functional divergence of SWEET transporters in Chinese white pear (*Pyrus*
608 *bretschneideri*). *Plant Cell Physiol.* 58(4), 839-850. doi: 10.1093/pcp/pcx025

Li, L., Sheen, J. (2016). Dynamic and diverse sugar signaling. *Curr. Opin. Plant Biol*. 33, 116–125. doi: 10.1016/j.pbi.2016.06.018

Li, M., Xie, H., He, M., Su, W., Yang, Y., Wang, J., et al. (2020). Genome-wide identification and expression analysis of the *StSWEET* family genes in potato (*Solanum tuberosum* L.). *Genes Genom.* 42(2), 135-153. doi: 10.1007/s13258-019-00890-y

Lin, I.W., Sosso, D., Chen, L.Q., Gase, K., Kim, S.G., Kessler, D., et al. (2014). Nectar secretion requires sucrose phosphate synthases and the sugar transporter *SWEET9*. *Nature* 508(7497), 546-549. doi: 10.1038/nature13082

Linsmith, G., Rombauts, S., Montanari, S., Deng, C.H., Celton, J.M., Guerif, P., et al. (2019). Pseudo-chromosome-length genome assembly of a double haploid "Bartlett" pear (*Pyrus communis* L.). *Gigascience* 8(12), giz138. doi: 10.1093/gigascience/giz138

Liu, C., Feng, C., Peng, W., Hao, J., Wang, J., Pan, J., et al. (2020). Chromosome-level draft genome of a diploid plum (*Prunus salicina*). *Gigascience* 9(12), giaa130. doi: 10.1093/gigascience/giaa130

Liu, X., Zhang, Y., Yang, C., Tian, Z., and Li, J. (2016). *AtSWEET4*, a hexose facilitator, mediates sugar transport to axial sinks and affects plant development. *Sci. Rep.* 6, 24563. doi: 10.1038/srep24563

Lynch, M., and Conery, J.S. (2000). The evolutionary fate and consequences of duplicate genes. *Science* 290(5494), 1151-1155. doi: 10.1126/science.290.5494.1151

Maynard, J.W., and Lucas, W.J. (1982). Sucrose and glucose uptake into *Beta vulgaris*leaf tissues : A case for general (apoplastic) retrieval systems. *Plant physiol.* 70(5), 1436-1443. doi: 10.1104/pp.70.5.1436

Miao, H., Sun, P., Liu, Q., Miao, Y., Liu, J., Zhang, K., et al. (2017). Genome-wide analyses of SWEET family proteins reveal involvement in fruit development and abiotic/biotic stress responses in banana. *Sci. Rep.* 7(1), 3536. doi: 10.1038/s41598-017-03872-w

Mizuno, H., Kasuga, S., and Kawahigashi, H. (2016). The sorghum SWEET gene family: stem sucrose accumulation as revealed through transcriptome profiling. *Biotechnol. Biofuels* 9, 127. doi: 10.1186/s13068-016-0546-6

Patil, G., Valliyodan, B., Deshmukh, R., Prince, S., Nicander, B., Zhao, M., et al. (2015). Soybean (*Glycine max*) SWEET gene family: insights through comparative genomics, transcriptome profiling and whole genome re-sequence analysis. *BMC Genomics* 16(1), 520. doi: 10.1186/s12864-015-1730-y

Price, M.N., Dehal, P.S., and Arkin, A.P. (2010). FastTree 2-approximately maximum-likelihood trees for large alignments. *PloS One* 5(3), e9490. doi: 10.1371/journal.pone.0009490

Quirino, B.F., Normanly, J., and Amasino, R.M. (1999). Diverse range of gene activity during *Arabidopsis thaliana* leaf senescence includes pathogen-independent induction of defense-related genes. *Plant Mol.Biol.* 40(2), 267-278. doi: 10.1023/a:1006199932265

Raymond, O., Gouzy, J., Just, J., Badouin, H., Verdenaud, M., Lemainque, A., et al. (2018). The Rosa genome provides new insights into the domestication of modern roses. *Nat. Genet.* 50(6), 772-777. doi: 10.1038/s41588-018-0110-3

Rennie, E.A., and Turgeon, R. (2009). A comprehensive picture of phloem loading strategies. *P. Natl. Acad. Sci. U.S.A.* 106(33), 14162-14167. doi: 10.1073/pnas.0902279106

Seo, P.J., Park, J.M., Kang, S.K., Kim, S.G., and Park, C.M. (2011). An *Arabidopsi*s senescence-associated protein SAG29 regulates cell viability under high salinity. *Planta* 233(1), 189-200. doi: 10.1007/s00425-010-1293-8

Shirasawa, K., Isuzugawa, K., Ikenaga, M., Saito, Y., Yamamoto, T., Hirakawa, H., et al. (2017). The genome sequence of sweet cherry (*Prunus avium*) for use in genomics-assisted breeding. *DNA Res.* 24(5), 499-508. doi: 10.1093/dnares/dsx020

Slewinski, T.L. (2011). Diverse functional roles of monosaccharide transporters and their homologs in vascular plants: aphysiological perspective. *Mol. Plant* 4(4), 641-662. doi: 10.1093/mp/ssr051

Sosso, D., Luo, D., Li, Q.B., Sasse, J., Yang, J., Gendrot, G., et al. (2015). Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport. *Nat. Genet.* 47(12), 1489-1493. doi: 10.1038/ng.3422

Verde, I., Abbott, A.G., Scalabrin, S., Jung, S., Shu, S., Marroni, F., et al. (2013). The high-quality draft genome of peach (*Prunus persica*) identifies unique patterns of genetic diversity, domestication and genome evolution. *Nat. Genet.* 45(5), 487-494. doi: 10.1038/ng.2586

Wang, L., Yao, L., Hao, X., Li, N., Qian, W., Yue, C., et al. (2018). Tea plant SWEET transporters: expression profiling, sugar transport, and the involvement of *CsSWEET16* in modifying cold tolerance in *Arabidopsis*. *Plant Mol. Biol.* 96(6), 577-592. doi: 10.1007/s11103-018-0716-y

Wei, X., Liu, F., Chen, C., Ma, F., and Li, M. (2014). The *Malus domestica* sugar transporter gene family: identifications based on genome and expression prof ling related to the accumulation of fruit sugars. *Front. Plant Sci.* 5, 569. doi: 10.3389/fpls.2014.00569

Xu, G., Guo, C., Shan, H., and Kong, H. (2012). Divergence of duplicate genes in exon-intron structure. *Proc. Natl. Acad. Sci. U.S.A.* 109(4), 1187-1192. doi: 10.1073/pnas.1109047109

Xuan, Y.H., Hu, Y.B., Chen, L.Q., Sosso, D., Ducat, D.C., Hou, B.H., et al. (2013). Functional role of oligomerization for bacterial and plant SWEET sugar transporter family. *Proc. Natl. Acad. Sci. U.S.A.* 110(39), E3685-E3694. doi: 10.1073/pnas.1311244110

Yamada, K., Osakabe, Y., Mizoi, J., Nakashima, K., Fujita, Y., Shinozaki, K., et al. (2010). Functional analysis of an *Arabidopsis thaliana* abiotic stress-inducible facilitated diffusion transporter for monosaccharides. *J. Biol. Chem.* 285(2), 1138-1146. doi: 10.1074/jbc.M109.054288

Yao, T., Xie, R., Zhou, Y., Hu, J., Gao, Y., and Zhou, C. (2021). Genome-wide identification of SWEET gene family and its response to abiotic stresses in valencia sweet orange. *Plant Mol. Biol. Rep*. doi: 10.1007/s11105-020-01268-1

Yuan, M., and Wang, S. (2013). Rice MtN3/Saliva/SWEET family genes and their homologs in cellular organisms. *Mol. Plant* 6(3), 665-674. doi: 10.1093/mp/sst035

Yuan, M., Zhao, J., Huang, R., Li, X., Xiao, J., and Wang, S. (2014). Rice MtN3/saliva/SWEET gene family: Evolution, expression profiling, and sugar transport. *J. Integr. Plant Biol.* 56(6), 559-570. doi: 10.1111/jipb.12173

Yue, C., Cao, H.L., Wang, L., Zhou, Y.H., Huang, Y.T., Hao, X.Y., et al. (2015). Effects of cold acclimation on sugar metabolism and sugar-related gene expression in tea plant during the winter season. *Plant Mol. Biol.* 88(6), 591-608. doi: 10.1007/s11103-015-0345-7

Zhang, Q., Chen, W., Sun, L., Zhao, F., Huang, B., Yang, W., et al. (2012). The genome of *Prunus mume*. *Nat. Commun.* 3, 1318. doi: 10.1038/ncomms2290

Zhang, W., Wang, S., Yu, F., Tang, J., Shan, X., Bao, K., et al. (2019). Genome-wide characterization and expression profiling of SWEET genes in cabbage (*Brassica oleracea* var. *capitata* L.) reveal their roles in chilling and clubroot disease responses. *BMC Genomics* 20(1), 93. doi: 10.1186/s12864-019-5454-2

Zhang, Z., Zhuo, X., Zhao, K., Zheng, T., Han, Y., Yuan, C., et al. (2018). Transcriptome profiles reveal the crucial roles of hormone and sugar in the bud dormancy of *Prunus mume*. *Sci. Rep.* 8(1), 5090. doi: 10.1038/s41598-018-23108-9

Zhao, L., Yao, J., Chen, W., Yan, L.I., Youjun, L., Guo, Y., et al. (2018). A genome-wide analysis of SWEET gene family in cotton and their expressions under different stresses. *J. Cotton Res.* 1(2), 1-15. doi: 10.1186/s42397-018-0007-9

## Table 1(on next page)

Table 1. The *PmSWEET* gene family members in *P. mume*.

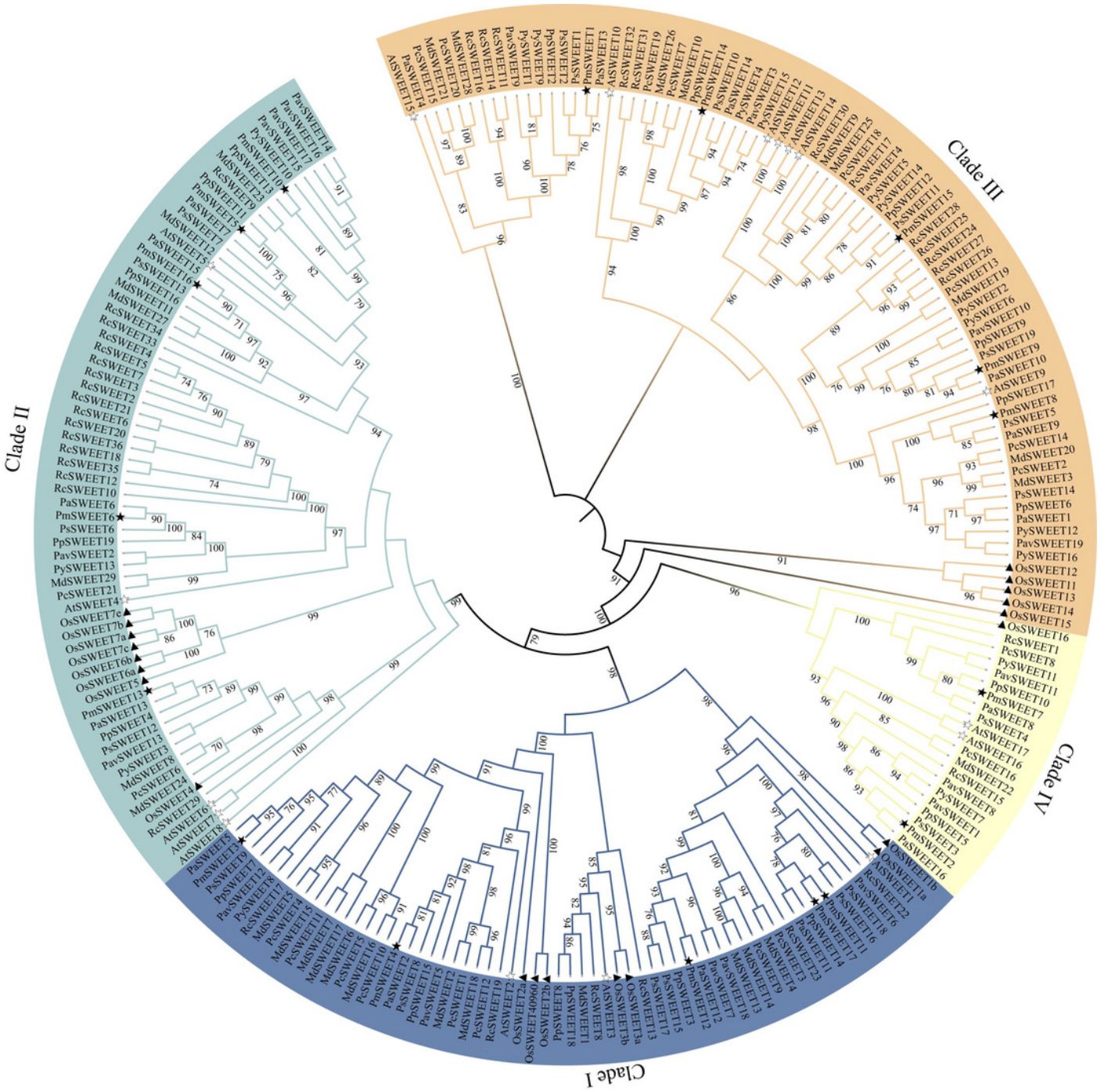
**Table 1. The *PmSWEET* gene family members in *P. mume*.**

| Name | Gene ID | Clade | CDS (bp) | No. of amino acids | Molecular weight (kDa) | Theoretical pI | TMHs | No. of MtN3/saliva domain | Locus |
|---|---|---|---|---|---|---|---|---|---|
| PmSWEET1 | Pm007067 | III | 849 | 282 | 31.38 | 8.34 | 7 | 2 | Pa2:21184396..21186332 |
| PmSWEET2 | Pm008206 | IV | 759 | 252 | 27.74 | 8.50 | 7 | 2 | Pa2:31718730..31721555 |
| PmSWEET3 | Pm010330 | I | 1248 | 415 | 46.25 | 9.76 | 8 | 2 | Pa3:3891190..3895205 |
| PmSWEET4 | Pm011260 | I | 708 | 235 | 26.45 | 8.30 | 7 | 2 | Pa3:9921623..9924001 |
| PmSWEET5 | Pm013198 | II | 519 | 172 | 19.42 | 8.97 | 5 | 1 | Pa4:2433448..2434735 |
| PmSWEET6 | Pm015728 | II | 708 | 235 | 25.67 | 9.21 | 5 | 2 | Pa4:21122646..21124537 |
| PmSWEET7 | Pm017566 | IV | 735 | 244 | 26.99 | 9.14 | 7 | 2 | Pa5:12327097..12328384 |
| PmSWEET8 | Pm018875 | III | 1743 | 580 | 63.43 | 8.34 | 6 | 2 | Pa5:20984940..20990591 |
| PmSWEET9 | Pm019954 | III | 828 | 275 | 30.68 | 9.20 | 7 | 2 | Pa6:436315..437664 |
| PmSWEET10 | Pm021931 | II | 708 | 235 | 26.60 | 8.59 | 6 | 2 | Pa6:12459796..12461199 |
| PmSWEET11 | Pm022695 | I | 417 | 138 | 15.96 | 9.74 | 3 | 1 | Pa6:19934418..19935334 |
| PmSWEET12 | Pm022696 | I | 651 | 216 | 23.21 | 8.78 | 5 | 2 | Pa6:19944525..19945680 |
| PmSWEET13 | Pm024167 | II | 780 | 259 | 28.66 | 9.37 | 6 | 2 | Pa7:10796671..10798904 |
| PmSWEET14 | Pm024554 | III | 318 | 105 | - | - | 2 | 1 | Pa7:13005181..13005663 |
| PmSWEET15 | Pm024555 | III | 891 | 296 | 33.14 | 8.61 | 7 | 2 | Pa7:13012731..13014646 |
| PmSWEET16 | Pm024712 | II | 639 | 212 | 23.95 | 8.37 | 5 | 2 | Pa7:13852243..13854234 |
| PmSWEET17 | Pm030352 | I | 510 | 169 | 19.26 | 9.14 | 4 | 1 | scaffold54:138478..139392 |

# Figure 1

Figure 1. Phylogenetic tree of SWEET sequences from *P. mume* and other plant species.

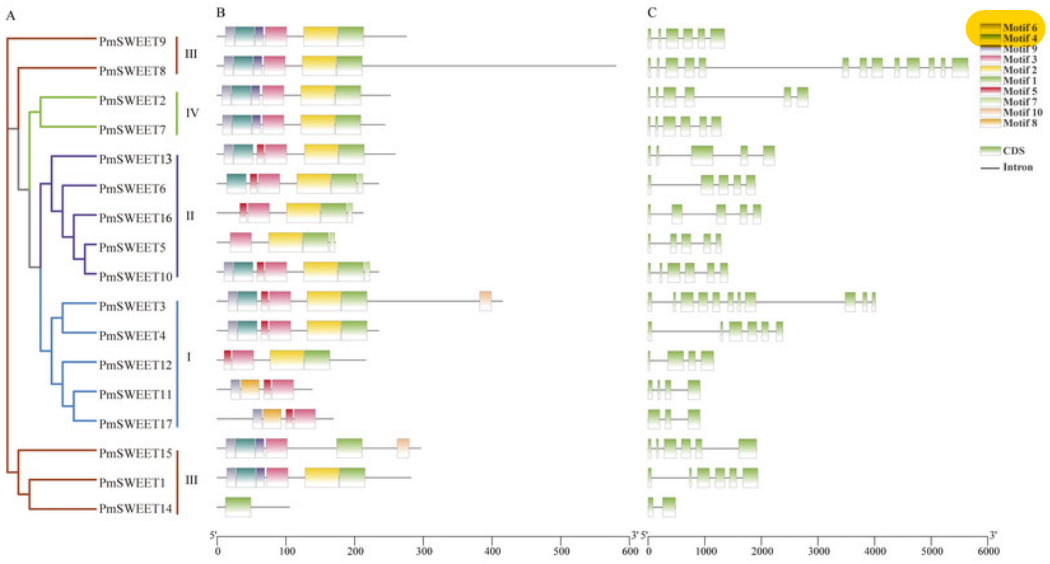
Clades I, II, III, and IV are indicated by blue, indigo, orange and pale yellow branch lines, respectively. At, *A. thaliana*; Os, *O. sativa*; Pa, *P. armeniaca*; Pav, *P. avium*; Pc, *P. communis*; Pm, *P. mume*;Pp, *P. persica*; Ps, *P. salicina*; Py, *P. yedoensis* var. *nudiflora*; Md, *M. domestica*; Rc, *R. chinensis*.


Clade I
Clade II
Clade III
Clade IV

# Figure 2

Figure 2. Phylogenetic relationship, conserved motif and gene structure analysis of *PmSWEET* genes.

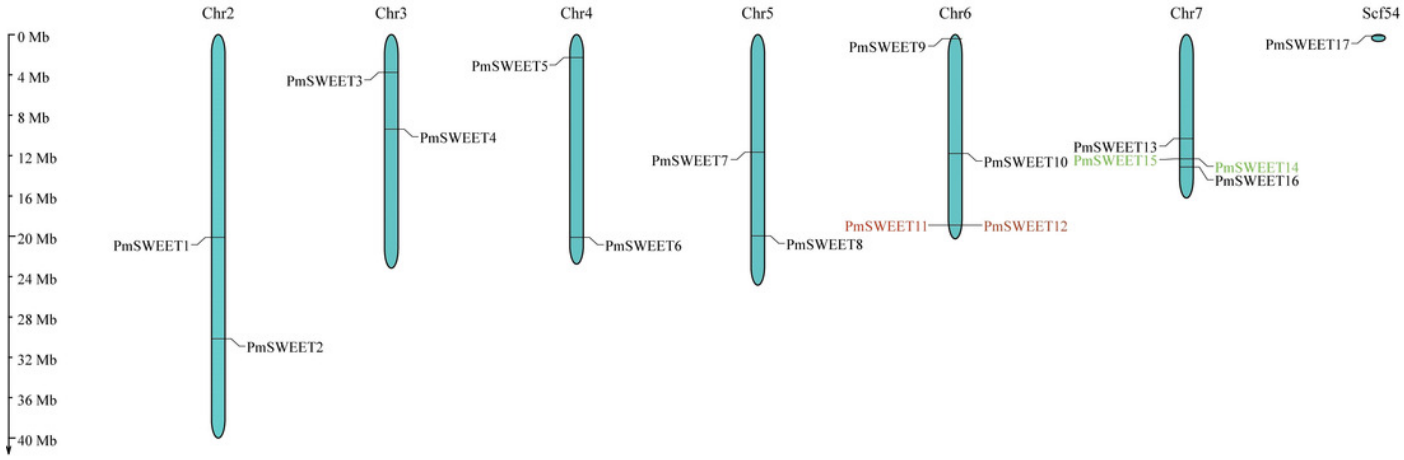
A: The ML phylogenetic tree of *PmSWEET* genes. The *SWEET* genes were classified into four clades, and blue, purple, red, and green represents Clades I, II, III, and IV, respectively. B: The motif composition of *PmSWEET* proteins. Ten motifs were displayed in different colored rectangles. C: Exon-intron organization of *PmSWEET* genes. Green and black correspond to exons and introns, respectively.



# Figure 3

Figure 3. Schematic representations of the chromosomal location of the *PmSWEET* genes.

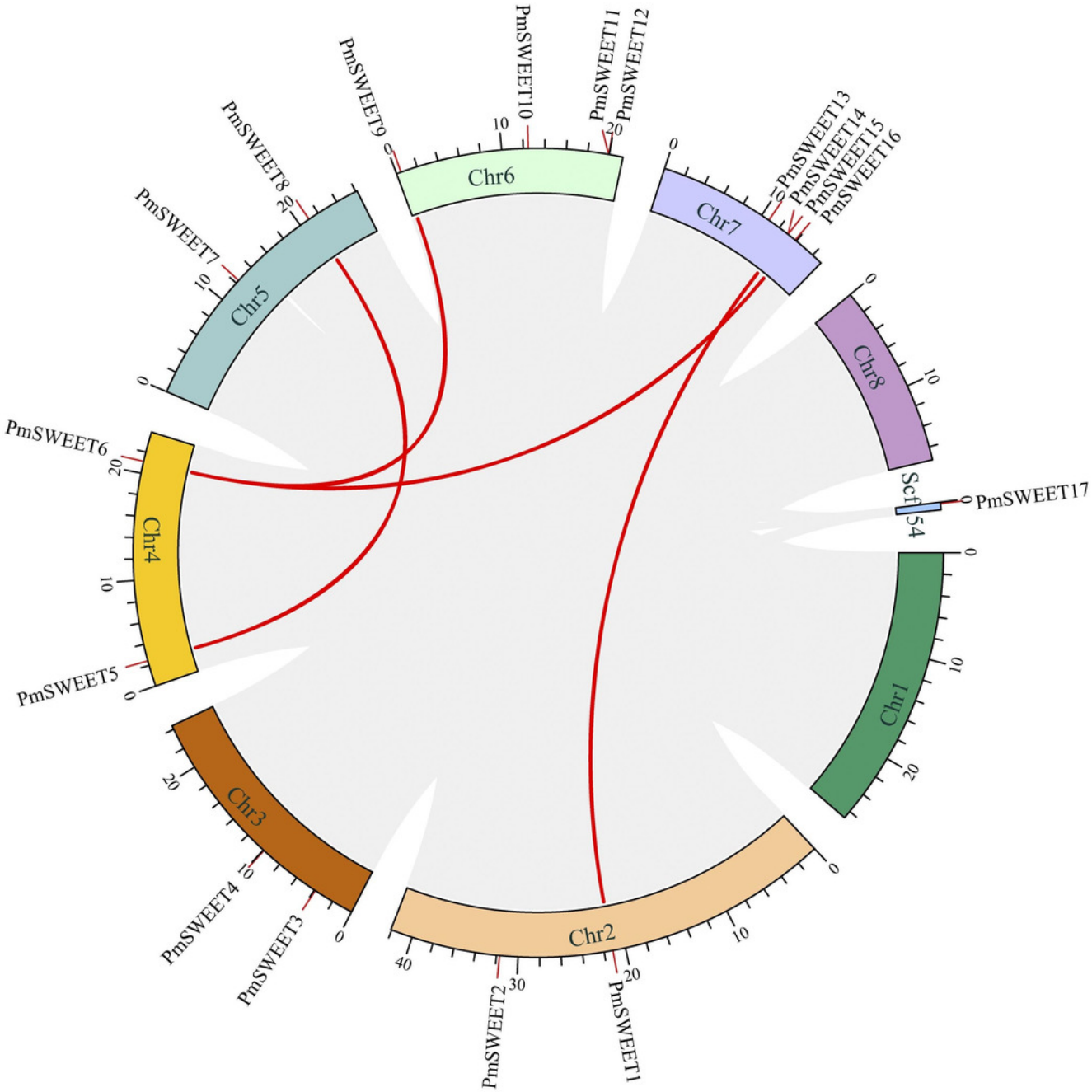
The chromosome number is indicated on the top of each chromosome and scaffold. Scf54 indicates scaffold54. Green and red gene names indicate tandem duplicated gene pairs.



# Figure 4

Figure 4. The Circos figure for *PmSWEET* segmental duplication links.

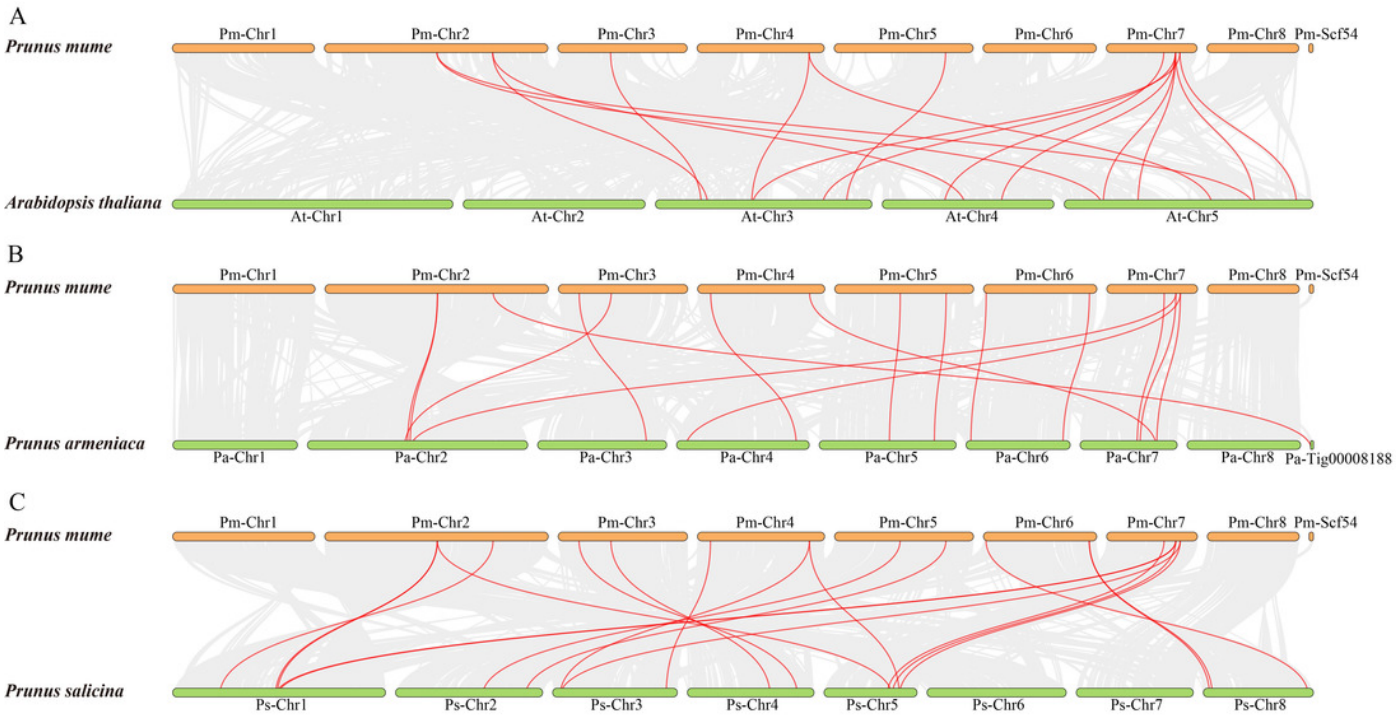
The red lines indicate segmented duplicated gene pairs.



# Figure 5

Figure 5. Synteny of SWEET genes in different genome of *P. mume*, *A. thaliana*, *P. armeniaca* and *P. salicina*.

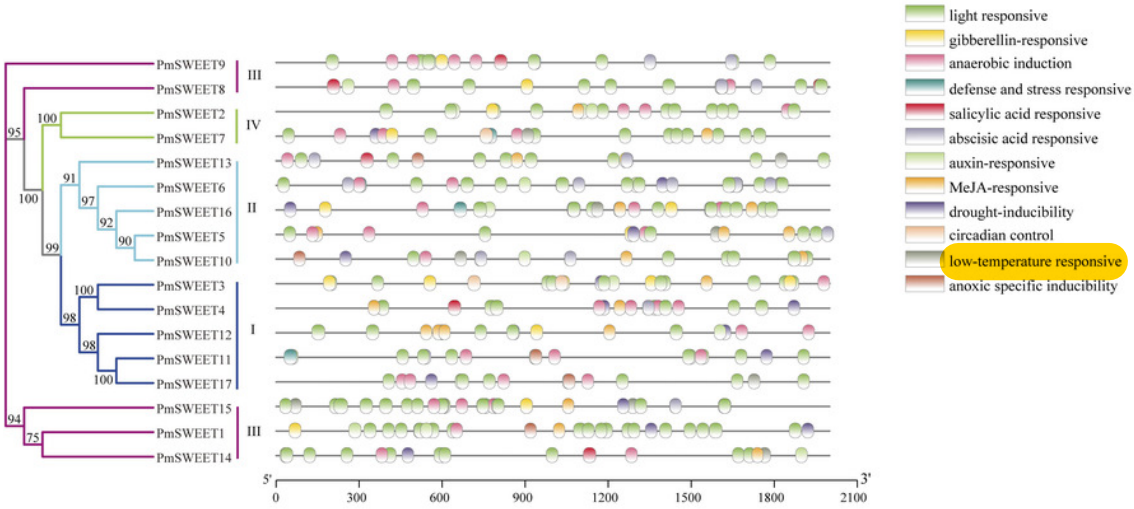
A: Synteny of *PmSWEET* and *AtSWEET* gene pairs. B: Synteny of *PmSWEET* and *PaSWEET* gene pairs. C: Synteny of *PmSWEET* and *PsSWEET* gene pairs.



# Figure 6

Figure 6. Cis-promoters analysis involved in the stress response.

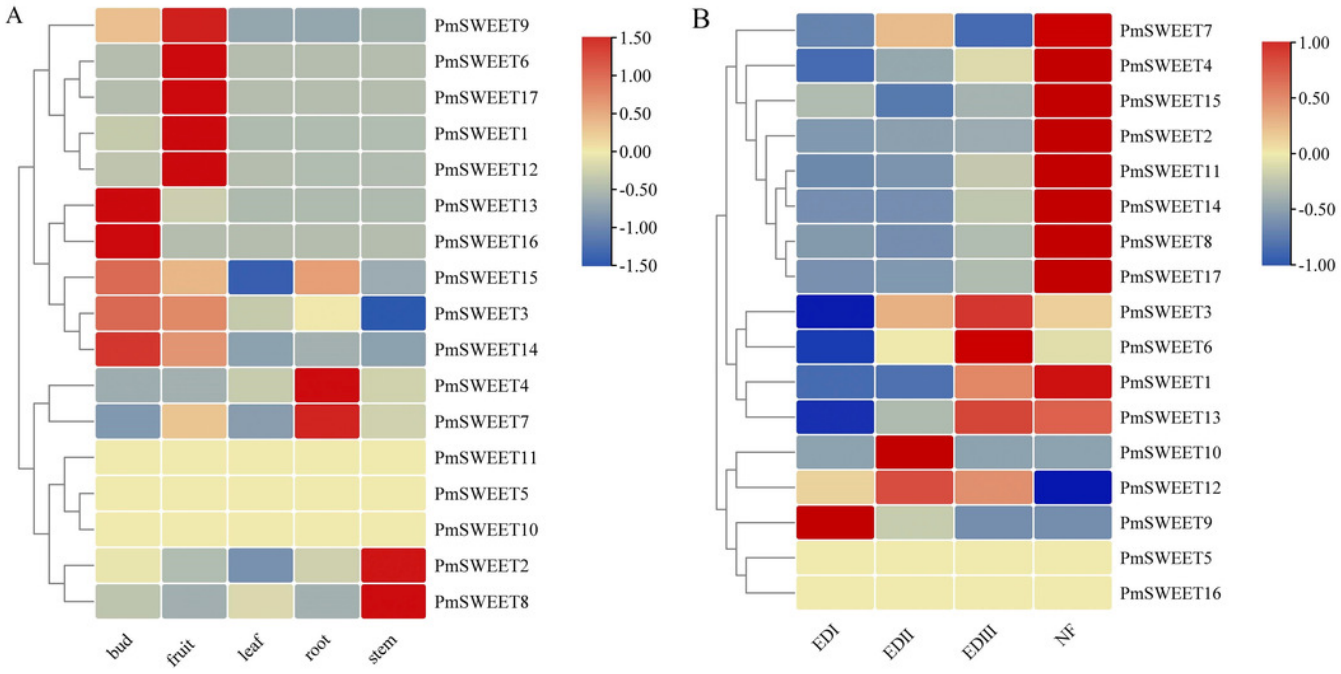
The *SWEET* genes are classified into four clades, and blue, indigo, purple red, and green represent Clades I, II, III, and IV, respectively.



# Figure 7

Figure 7 Expression profiles of PmSWEET genes in different tissues and different flower buds stage

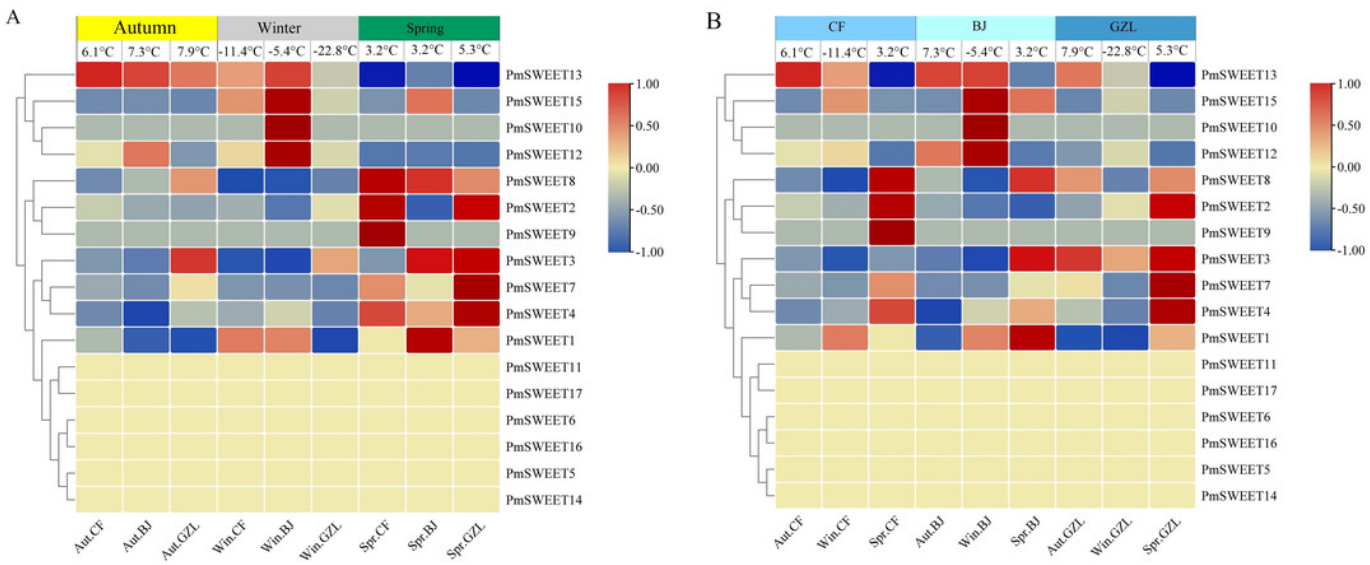
A: Expression profiles of *PmSWEETs* in different tissues. B: Expression profiles of *PmSWEETs* in the flower bud during dormancy. EDI: Endo-dormancy I, November; EDII: Endo-dormancy II, December; EDIII: Endo-dormancy III, January; NF: Natural flush, February.



# Figure 8

Figure 8 Expression profiles of PmSWEET genes under different conditions

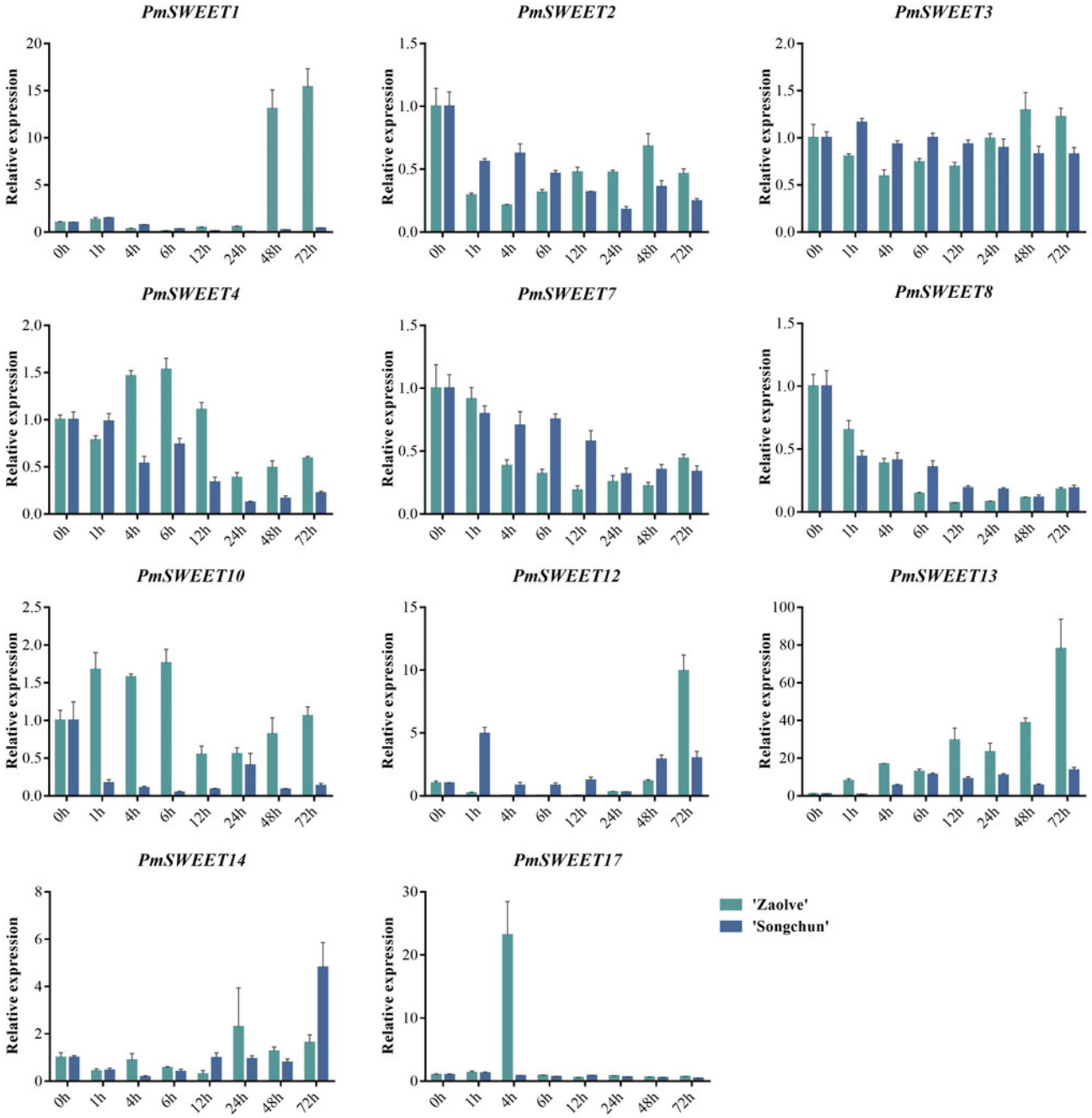
A. Expression profifiles of *PmSWEET*s in stems of ‘Songchun’ in different regions (Beijing, Chifeng and Gongzhuling) and seasons (autumn, winter and spring). B. Comparison of differential expression profifiles of stems in Beijing, Chifeng and Gongzhuling during different seasons.



# Figure 9

Figure 9. Expression analyses of 11 PmSWEETs

The relative quantification method (2-ΔΔCt) was used to evaluate quantitative variation. Error bars represent percentage error for three replicates.


PmSWEET1
PmSWEET2
PmSWEET3
PmSWEET4
PmSWEET7
PmSWEET8
PmSWEET10
PmSWEET12
PmSWEET13
PmSWEET14
PmSWEET17
Relative expression
0h
1h
4h
6h
12h
24h
48h
72h
'Zaolve'
'Songchun'