Genome-wide analysis of the *SWEET* gene family mediating the cold stress response of *Prunus mume*

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The SWEET (Sugars Will Eventually be Exported Transporter) is a family of sugar transporters that plays an essential role in plant growth, reproduction, and biotic and abiotic stresses. *Prunus mume*, a considerable ornamental tree with high edible and medicinal values. However, the distribution area of *P. mume* is limited by low temperature , and there were no related studies about SWEET gene family in *P. mume*. Herein, we identified 17 *PmSWEET* genes in total, of which 16 genes were anchored on six chromosomes and one gene on the scaffold with four pairs of segmental gene duplications and two pairs of tandem genes duplications. Phylogenetic analysis suggested 230 SWEET genes from 11 species were divided into four groups. Cis-acting regulatory elements analysis indicated that the *PmSWEET* genes were presumably involved in the *P. mume* developmental procedure and responses to diversified stresses, such as circadian control, abscisic acid-responsive, light-responsive, low-temperature responsive and so on. We performed low-temperature treatment in ‘Songchun’ and ‘Zaolve’ and found that seven of 17 *PmSWEETs* expressed differently, 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 stresses.

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Abstract

The SWEET (Sugars Will Eventually be Exported Transporter) is a family of sugar transporters that plays an essential role in plant growth, reproduction, and biotic and abiotic stresses. *Prunus mume*, a considerable ornamental tree with high edible and medicinal values. However, the distribution area of *P. mume* is limited by low temperature, and there were no related studies about SWEET gene family in *P. mume*. Herein, we identified 17 *PmSWEET* genes in total, of which 16 genes were anchored on six chromosomes and one gene on the scaffold with four pairs of segmental gene duplications and two pairs of tandem genes duplications. Phylogenetic analysis suggested 230 SWEET genes from 11 species were divided into four groups. Cis-acting regulatory elements analysis indicated that the *PmSWEET* genes were presumably involved in the *P. mume* developmental procedure and responses to diversified stresses, such as circadian control, abscisic acid-responsive, light-responsive, low-temperature responsive and so on. We performed low-temperature treatment in 'Songchun' and 'Zaolve' and found that seven of 17 *PmSWEETs* expressed differently, 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 stresses.

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

1. Introduction

Sugars are an energy and carbon source that is necessary for plant growth and development, which is composite in the leaves during photosynthesis and after that transported by phloem sap to storage organs, such as roots, stems, flowers, seeds and fruits (Rennie and Turgeon, 2009; Lemoine et al., 2013). In addition, sugar is an important signal and resistance molecule for the normal growth of higher plants (Rolland et al., 2006; Chen et al., 2015). However, sugar 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: the monosaccharide transporters (MSTs), the 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 depicted in *Arabidopsis*(Chen et al., 2010). SWEET proteins act as a sugar transporter that intercedes 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 membrane (Maynard and Lucas, 1982; Lemoine, 2000), the SWEETs family promotes the diffusion of sugar across the concentration gradients on the cellular membrane or vacuolar membrane, regardless of the proton gradient or pH (Chen et al., 2012; Chen et al., 2015).

The SWEET proteins are featured by possessing conserved MtN3_saliva (MtN3_slv) transmembrane (TM) domains (Chen et al., 2012), also known as the PQ-loop-repeat (Eom et al., 2015; Feng and Frommer, 2015). The SWEETs in eukaryotes commonly consist of seven a-transmembrane helix (TMH), which contain a couple of 3-TMH repeats detached by an added helix (Xuan et al., 2013), and this structure has been depicted as the “3-1-3” TM SWEET structure (Chen et al., 2010). In opposition to the SWEET protein of eukaryotes, prokaryotes' SWEET protein, known as SemiSWEETs, comprises solely one 3-TMH (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, which suggests that SWEETs may be formed by direct fusion from SemiSWEETs (Jia et al., 2017). Besides, a novel extraSWEET protein consisting of 14 and 15 TMHs, has been reported from *Vitis vinifera* (Patil et al., 2015) and *Oryza punctate* (Jia et al., 2017); it is speculated that this extraSWEET may be duplicated from 7 TMHs. Recent research on 3249 SWEET proteins also ascertained superSWEET with > 18 TMHs in oomycetes, which carry 5–8 repeats of a semiSWEET (Jia et al., 2017). Although semiSWEET, extraSWEET and superSWEET are not familiar, all the three types are found in eukaryotes. Yet, in a study of SWEET genes from 25 plant genomes, 140 of the 411 sugar transporters were semiSWEET, indicating that semiSWEET are not unusual in higher plants (Patil et al., 2015). According to phylogenetic analysis, the SWEET genes in *Arabidopsis* fell into four clades: Clade I (SWEET1–3) and Clade II (SWEET4–8) mainly transport glucose, meanwhile Clade I also transports hexose (Chen et al., 2010; Lin et al., 2014). Clade III (SWEET9–15) mainly transport sucrose (Chen et al., 2012; Eom et al., 2015) and Clade IV (SWEET16–17), which are located on the tonoplast membrane mainly transport fructose (Eom et al., 2015). The phylogeny SWEET genes of plants hereafter described 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 on. Meanwhile, 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, in *A. thaliana*, overexpression of *AtSWEET16* and *AtSWEET17* increases cold tolerance, although plants that overexpress *AtSWEET16* cannot accumulate fructose (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); *AtSWEET11* and *AtSWEET12* are

also related to stress caused by cold or dehydration (Le Hir et al., 2015; Durand et al., 2016). The *AtSWEET15* is also known as SAG29 (senescence-associated genes). However, its transcription level gradually increases in low temperature, high salinity, and drought during natural leaf senescence (Quirino et al., 1999). Cold stress significantly inhibited the expression of *CsSWEET2, 3, 16* in *Camellia sinensis*, while the expression of *CsSWEET1* and *CsSWEET17* increased sharply (Yue et al., 2015). The functional study of *CsSWEET16* in *Camellia sinensis* indicates that it is located in the vacuolar membrane and regulates the cold resistance of *A. thaliana* (Wang et al., 2018). Many *SlSWEET* genes are increased under low-temperature stress in tomatoes (Feng et al., 2015). Studies have shown that the *MaSWEET* gene in bananas is up-regulated in response to low temperature, salt, and osmotic stress (Miao et al., 2017). Genome-wide analysis of the *BoSWEET* gene in cabbage discovered five possible candidate genes, which promote sugar transport, thereby further improving the plant's cold tolerance (Zhang et al., 2019).

Prunus mume is a famous flower native to southwest China and the middle and lower reaches of the Yangtze River. In northern China, low temperatures severely limit the growth and distribution. Sugar plays a vital role in improving the cold tolerance of plants under low-temperature stress (Yamada et al., 2010); however, little is known about the characteristics of *PmSWEETs*. This study aimed to conduct a genome-wide analysis of the SWEET gene family in *P. mume*, laying the basis for the further function study of *PmSWEETs*.

2. Materials and Methods

2.1 *Arabidopsis, rice SWEET* Family, and Nine-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 nine Rosaceae species. Download 17 *AtSWEETs* and 21 *OsSWEETs* protein sequences from TAIR 10 (http://www.arabidopsis.org/) and TIGR (http://rice.plantbiology.msu.edu/) respectively. The *P. mume* genome sequence and annotation files were obtained from Zhang et al. (Zhang et al., 2012); other eight 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 GDR 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 SWEET domain (PF03083) were extracted from the Pfam database (http://pfam.xfam.org/) and used to search for the SWEET proteins in the *P. mume* and other eight species proteome with HMMER software (Finn et al., 2015). To ensure confidence, the E-value cut-off was set at 10^{-5}. Then, use SMART (http://smart.embl-heidelberg.de/), the Pfam database (http://pfam.xfam.org/) and NCBI-CDD

(https://www.ncbi.nlm.nih.gov/cdd) to screen all putative SWEET proteins to verify the presence of the SWEET domain.

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

2.3 Multiple Sequence Alignments and Phylogenetic Analysis

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

2.4 Protein Conserved Motif and Gene Structure Analysis

Use MEME v5.3.3 (https://meme-suite.org/meme/tools/meme) (Bailey et al., 2009) to predict the conserved motifs of each *PmSWEET*, the maximum of motifs for the conserved domains was set to ten, and the residuals were designated as the default parameters. Extract gene structure data from the *P. mume* genome annotations gff file, use Tbtools for visualization (Chen et al., 2020) and beautify in AI CS6 software.

2.5 Chromosome Location, Duplications and Synteny Analysis

Retrieve information about the location and chromosome length of *PmSWEETs* from the gff file. Use the online tool MG2C (http://mg2c.iask.in/mg2c_v2.0/) to draw chromosomal location figures. Analyze gene tandem and segment replication events using 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 the MCScanX in Tbtools. Use the Ka/Ks calculator in Tbtools to align the coding sequences and calculate duplicate gene pairs' Ks and Ka values. According to two ordinary rates (λ) of 1.5×10^{-8} or 6.1×10^{-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

Obtain the upstream sequences (2.0 kb) of the *PmSWEETs* from the genetic sequence data in Tbtools and then send them to the PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/) (Lescot et al., 2002) for cis-acting analyze. We finally selected 12

ingredients, including those caused by hormones, such as MeJA (methyl jasmonate)-responsive, abscisic acid-responsive, and stress-responsive elements; the factors of stress-responsive included defence and stress, low temperature, and light. Combined with phylogeny 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 *PmSWEET* genes involved in tissue development and cold tolerance, we use the root, stem, leaf, bud and fruit data of RNA sequencing (Zhang et al., 2012), analyzed the *PmSWEET* gene expression patterns in different tissues, and then use the flower bud dormancy data of RNA sequencing of *P. mume* ('Zaolve') (Zhang et al., 2018) analyzed the *PmSWEET* genes' expression patterns response to the 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 de acclimation (March, spring). Use the Tbtools (Chen et al., 2020) to create the heat map.

2.8 qRT-PCR Analysis of *PmSWEET* Genes

To examine the effect of *PmSWEET* genes on the response to low temperature, the annual branches of the cold-sensitive cultivar 'Zaolve' and the cold-tolerant cultivar 'Songchun' were collected. Before cold stress treatment, the shoots were incubated overnight at 22°C, 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 minus 80 degrees Celsius for complete RNA isolation. Each treatment had three biological replicates.

Use RNAprep Pure Plant Plus Kit (Tiangen, Beijing, China) to isolate total RNA from each sample. The complementary cDNA was synthesized using the ReverTra Ace® qPCR RT Master Mix with gDNA Remover (Toyobo, Osaka, Japan). Use Primer 3 (https://bioinfo.ut.ee/primer3-0.4.0/) to design specific primers based on the cDNA sequences (Table S1). The expression levels of *PmSWEETs* during the artificial 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 implemented 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 ddH_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 protein phosphatase 2A gene of *P. mume* as the reference gene, use the $2^{-\Delta\Delta Ct}$ method to calculate the relative expression. Perform an analysis of variance test on the final data.

3. Results

3.1 Identification of the *SWEET* Gene Family

A total of 17 non-redundant *PmSWEETs* were detected in the *P. mume* genome and 175 SWEETs 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 as "genuine" *SWEETs*. Calculate the number of amino acids, Molecular weight (MW), and Isoelectric point (pI) based on the protein sequences. As exhibited in Table 1, the predicted *PmSWEET* proteins diversified from 105 (*PmSWEET14*) to 580 (*PmSWEET8*) in amino acids length, relative molecular weights going from 15.96 kDa (*PmSWEET11*) to 63.43 kDa (*PmSWEET8*). Theoretical pIs from 8.30 (*PmSWEET4*) to 9.76 (*PmSWEET3*), except *PmSWEET14*, it's 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 six genes possess 7 TMHs.

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 phylogeny 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 S1). To investigate the evolutionary relationships between *PmSWEETs* and other species, an ML phylogeny tree of *SWEETs* from 11 species was constructed, including 8 Rosaceae species. All members of the *SWEET* gene family in 11 species were segmented into four clades (Figure1). The maximal 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 S3. The smallest clade was Clade IV, which contains only two *A. thaliana SWEET* genes, one *O. sativa* gene, and 18 Rosaceae *SWEET* genes (Table S3), reflecting that SWEETs were maldistribution in the different clades. The number of Clade I, II and III genes varied notably, suggesting that the *SWEET* gene expanded in these clades during Rosaceae evolution. Rosaceae *SWEETs* were evenly distributed in each tiny clade, while *O. sativa SWEETs* tended to gather together. *PmSWEET*s, *PpSWEET*s, and *PavSWEET*s were similarly grouped and placed in the phylogeny tree.

3.3 Conserved Motif and Gene Structure Analysis

To explore the specific region of *PmSWEET* proteins, MEME software and Tbtools was used to predict and draw conserved domains. As a consequence, ten distinct motifs were detected in *SWEET* proteins (Figure2B). Motif 3 was detected in all *PmSWEETs* except *PmSWEET14*. Motif 1 was detected in all *PmSWEETs* except *PmSWEET11*, *17*. Motif 2 was detected in 14 *PmSWEETs*. Motif 6 was detected in 12 *PmSWEETs*. Motif 4 was detected in 11 *PmSWEETs*. Motif 7 was only detected in *PmSWEET5*, *6*, *10*, *16*. Motif 8 was only detected in *PmSWEET11*, *17*. Motif 10 was only detected in *PmSWEET3*, *15*. 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 standpat motifs, suggesting 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, most Clade II genes (except *PmSWEET10*) contained four introns. Meanwhile, Clade III was divided into two sub-clades; the three genes of Clade III had five introns, while the *PmSWEET8* contained the largest 12 introns, *PmSWEET14* contained only one intron. Of the two Clade IV genes, all had five introns. The number of introns in the clade I varied from two to ten. These results indicated that introns in the same phylogeny 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 plotted, displaying that 16 *PmSWEETs* were situated on chromosomes, and one *PmSWEET* gene was located on scaffold54 (Figure 3). *PmSWEET* genes were concentrated distributed on chromosomes 6 and 7, and both contained four *PmSWEETs*. Two genes were arranged on each of chromosomes 2, 3, 4 and 5. *PmSWEET11* and *PmSWEET12*, *PmSWEET*14 and *PmSWEET*15 were grouped into two tandem duplication incidents in chromosomes 6 and 7. The above results can be deducted directly that part of the *PmSWEETs* might be produced 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 situated on different chromosomes, as indicated with red lines in Figure 4, indicating that part of the *PmSWEET* genes can be produced by gene segmental duplication. In addition, the selection pressure and divergence time of the duplicated events was appraised by the substitution rate of Ka (non-synonymous) 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 *PmSWEETs* (*PmSWEET6/9*) has a Ka/Ks ratio of 0.45, which was significant and indicated a synonymous change. 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 S4). The collinear complexity of *P. mume* and *P. salicina* was much higher than its with *P. armeniaca* and *A. thaliana*. These results suggested that *P. mume* was relatively distantly related to *A. thaliana* and *P. armeniaca*, and was pretty close to *P. salicina*.

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

In order to further investigate the possible regulatory mechanism of *PmSWEETs* in the process of growth and the defence reaction, in particular in response to abiotic stress, such as low temperature, we have submitted the 2.0 kb upstream sequence of 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 S5). Elements related to light-responsive, anaerobic induction, and abscisic acid (ABA) responsive were widespread in the promoter areas of 17, 17 and nine SWEET genes, respectively. From the perspective of the regulatory elements, there were each 13 *PmSWEETs* sensitive to MeJA and drought-inducibility. On the contrary, low temperatures and circadian control affect a small number of *PmSWEETs*. Combined with the results of phylogeny analysis, it was found that gene members of the same clade had similar cis-elements. But most cis-elements in *PmSWEET* genes emerge a diversity distribution. 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 value is shown in Table S6 and S7. As exhibited in Figure 7A, 14 of the *PmSWEET* genes were detected to be expressed in at least one tissue, whereas three (*PmSWEET5*, *10*, *11*) were not detected. *PmSWEET9*, *6*, *17*, *1*, *12* showed the highest level of expression in fruits, *PmSWEET13*, *16*, *15*, *3*, *14* showed specifically higher expression in buds, *PmSWEET4*, *7* showed higher expression levels in roots, *PmSWEET2*, *8* showed the highest level of expression in stems. At the same time, 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 development stages (Figure 7B). Ten *PmSWEET* genes exhibited specifically higher expressions in the NF stage (February), *PmSWEET9* was preferentially expressed in the EDI stage (November), *PmSWEET10*, *12* showed the highest level of expression in the EDII stage (December), *PmSWEET3*, *6*, *1*, *13*, *12* were up-regulated in the EDIII stage (January).

To further investigate the expression patterns of *PmSWEETs* under cold stress, we analyzed the stems of cold-tolerant cultivar *P. mume* 'Songchun' from three places and periods, and their FPKM value was displayed in Table S8. The expression of six *PmSWEET* genes (*PmSWEET11*, *17*, *6*, *16*, *5*, *14*) was not detected. In the other 11 *PmSWEET* genes, *PmSWEET13* was up-regulated in autumn and winter; *PmSWEET15*, *10*, *12* increased significantly in winter in Beijing (-5.4 °C). However, *PmSWEET*8, 2, 9, 3, 7, 4, 1 showed higher expressions in spring (3.2~5.3 °C). As shown in Figure 7C, *PmSWEET* genes in different regions show similar expression patterns in the same periods, except winter (Figure 7C).

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

To investigate the role of *PmSWEETs* played in cold tolerance, the expression patterns under deliberate hypothermia (4 °C) (0, 1, 4, 6, 12, 24, 48 and 72 h) were examined by qRT-PCR using 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, which was almost consistent with the transcriptome data (Figure 7). As displayed in Figure 8, the expression levels of 11 genes in two cultivars were changed in different patterns during artificial cold treatment. Within 'Songchun' (Figure 8A), *PmSWEET2*, *4*, *7*, *8*, *10* were dramatically down-regulated 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 about 11-fold after 6 h of cold treatment. While *PmSWEET14* was quickly up-regulated at 72 h. The expression levels of *PmSWEET3*, *17* only changed slightly. *PmSWEET1*, *12* were up-regulated at 1 h, then down-regulated with the treatment time increase, while up-regulated moderately after 48 h. Within 'Zaolve' (Figure 8B), *PmSWEET1* and *PmSWEET12* were rapidly up-regulated at 48 h and 72 h, respectively. *PmSWEET4*, *10* were up-regulated within 6 h, and then declined with the extended treatment time. The expression of *PmSWEET14* was not apparent in the early stage, but it was extremely expressed at 24 h. *PmSWEET17* was only highly expressed at 4 h. *PmSWEET2*, *7*, *8* were dramatically down-regulated at the early treatment and then increased slightly with increased treatment time. The expression levels of *PmSWEET13* were increased with prolonging the treatment time; the most remarkable expression level (about 80-fold) was detected after 72 h cold treatments. The expression level of *PmSWEET3* also changed, but the change was not significant.

4. Discussion

Sugar plays an essential role in the physiological metabolism, growth, development and reproduction of plants. It is not only a source of energy and carbon for plant growth and cell metabolism, but also an essential signalling molecule in cells (Liu et al., 2019). Sugar transporters act as membrane proteins that transport various sugar components regulate the supply and distribution of sugar in and out of cells and between organelles, play a critical role in the growth and development of many plants and responses to biological and non-biological factors (Lemoine et al., 2013; Li et al., 2017; Li et al., 2018; Zhao et al., 2018). The SWEET is a sugar transporter family that supports the transportation of sugar, and different genes in the SWEET family play different roles in sugar transport (Ayre, 2011; Yuan and Wang, 2013). The researchers found that it mainly transports sucrose, glucose and fructose (Chen et al., 2012; Feng and Frommer, 2015). With the completion of genome sequencing of plants, most studies have been conducted on the SWEET gene family. However, no one has studied the *P. mume* SWEET gene family. In this research, we detected a total of 17 SWEET family genes in *P. mume*, as much as in *Arabidopsis*, which is also similar to other species in the *Prunus*, such as 16 in *P. yedoensis*, 19 in *P. avium*, 19 in *P. persica*, and 19 in *P. salicina*. It is also similar to what has been reported in Rosaceae, such as 20 in strawberry (Liu et al., 2019), 18 in pear (Li et al., 2017), which shows that SWEET genes are still reasonably conservative in Rosaceae. The diversity of TMHs (2–7) strongly depends on the wide range of *PmSWEET* proteins from 105 aa to 580 aa. Except for *PmSWEET14*, the theoretical pI values of *PmSWEETs* are all greater than 8.0. As a fundamental parameter of proteins, pI depends on amino acid residue levels at different pH values, which affects protein stability and the physiological function or activity (Gasteiger, 2005). *PmSWEET14* did not detect pI, which may be due to its short amino acids sequence.

SWEET genes harbouring two conserved MtN3/Saliva or MtN3_slv domains, which were later named PQ rings, each consisting of 3-transmembrane helix (TMH) in eukaryotes, the fourth TMHs are suggested to take connection function (Chen et al., 2010). In prokaryotes, MtN3_slv domain has only a single 3-transmembrane helix (TMH) domain. The SWEETs protein locates on the membrane of the phloem parenchyma cells and is responsible for the outflow of sucrose from the cells into the phloem exosomes, participates in many remarkable physiological and biochemical processes (Yuan and Wang, 2013; Chen, 2014; Chen et al., 2015; Patil et al., 2015). Members of the SWEET family of plants typically have seven TMHs. By predicting TMH domains, we found that the number of TMH in *PmSWEET* genes ranged from 2 to 8. Among them, six *PmSWEET* genes (*PmSWEET1*, *2*, *4*, *7*, *9*, *15*) contained seven TMH structures, four *PmSWEET* genes (*PmSWEET5*, *6*, *12*, *16*) included five TMH structures, three *PmSWEET* genes (*PmSWEET8*, *10*, *13*) contained six TMH structures. There are also four genes (*PmSWEET14*, *11*, *17*, *3*) that each contains two, three, four, and eight TMHs. Fewer than seven TMHs in the eukaryotic SWEET family were also found in wheat and walnut (Gao et al., 2018; Jiang et al., 2020). To further validate the accuracy of the SWEET protein, we sent the protein sequence to NCBI-CDD and SMART to predict their conserved domains and found that all of them contained MtN3_slv domain of the SWEET family. The genes containing two, three, four TMH had one

MtN3_slv domain, and the genes containing six, seven and eight TMH included two MtN3_slv domains. It's worth noting that the genes containing five TMH most included two MtN3_slv domains, but only one has one MtN3_slv domain. Genetic missing or amplification and the emergence of certain SWEETs with only two, three, four, five or six TMHs means that SWEETs replication and fusion might be taking place in the *P. mume* genome.

According to the phylogenetic evolutionary relationship of *AtSWEET* and *OsSWEET*, *PmSWEETs* were classified into four Clades. The ratios of four clades vary from species to species, indicating that these species have different expansion rates. For example, in *A. thaliana* and *R. chinensis*, Clades II and III were dominated; in *O. sativa*, *P. communis* and *M. domestica*, Clades I and III were dominated; in *P. yedoensis*, Clades III was dominated (Table S3). In total 230 SWEET genes, Clade III has more members than other clades, suggesting that the Clade III may expand during evolution. In *P. mume*, Clades I, II and III have the same number of SWEET genes, Clades IV has only two SWEET genes, indicating high conservation in the SWEET family in the process of evolution. Previous studies have found that *SWEETs* containing similar conserved motif cluster together in the phylogeny trees (Jia et al., 2017; Miao et al., 2017). Our results also support previous findings that most closely related genes in the family have similar motif components, which suggested that *PmSWEETs* with similar motifs have similar functions. The diversity of gene structure and conservative divergence in protein motif plays a vital role in the evolution of the *SWEETs* family (Xu et al., 2012). The gene members of each clade have some distinctive conserved motif, suggesting functional diversity of the SWEET genes in *P. mume*.

Gene duplication, including tandem and segmental duplication events, are the origins 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 *SWEETs* 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. In this study, PlantCARE online software was used to analyze the components of the promoter interval. There are various light-responsive elements, hormone response elements, stress reaction elements, and circadian control elements. All *PmSWEETs* contain at least one light-responsive and anaerobic induction cis-element, suggesting that light and anaerobic might have an essential role in *PmSWEETs* regulation. Moreover, 13 *PmSWEETs* contained one or more methyl jasmonate (MeJA) responsive and drought-inducibility cis-element, indicating that these *PmSWEETs* also play considerable roles in response to 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 the 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 researches 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). 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, indicating that it may degenerate or lose its function during evolution. Each *PmSWEETs* in different organs had its specific expression pattern (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, which indicates that the gene is only expressed in specific tissues.

Furthermore, the expression of *PmSWEET* genes from the same Clade varied considerably, while the expression of *PmSWEET* genes from different Clades may be similar. *PmSWEET1*, *9* (Clade III), *PmSWEET12*, *17* (Clade I) and *PmSWEET*6 (Clade II) were heavily expressed in fruit, indicating these genes may regulate sugar allocation during fruit ripening. *PmSWEET13*, *16* (Clade II), *PmSWEET14*, *15* (Clade III) and *PmSWEET*3 (Clade I) were heavily 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 heavily expressed in the stem, suggesting the potential roles of these genes in long-distance sugar transport. This study also showed that, compared with other tissues, most *PmSWEETs* were expressed in flower buds and fruit tissues at different stages of the Endo-dormancy period, and these genes were expressed differently during flower development (Figure 7A, 7B); this indicates that *PmSWEET* was closely involved in the development of reproduction, and many *PmSWEETs* play particular roles in different stages of development.

Meanwhile, this condition was consistent with rice, *Arabidopsis* and soybean, which were also relatively higher expressed in reproductive tissues than other tissues (Yuan et al., 2014; Patil et al., 2015). *PmSWEETs* also have different expressions during the dormancy release on flower buds (from November to February). Thus, we speculate that these *PmSWEETs* participated in the cold reaction at low temperatures to protect the flower bud. Besides, some *PmSWEETs* were expressed more at colder temperatures in the spring (3.2~5.3 °C) and around -5 °C in the winter (Figure 7C), indicating that these two temperatures can trigger their cold stress response and increase the *PmSWEETs* expression to reduce stress injury.

The qRT-PCR analysis suggested that six of 17 *PmSWEET* genes (*PmSWEET5*, *6*, *9*, *11*, *15*, *16*) were non-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 8). *PmSWEET13* was up-regulated in 'Zaolve' and 'Songchun' with the extension of cold treatment time, which suggested that it was susceptible to low temperatures and participated in cold-response. The expression of *PmSWEET2* from two cultivars declined after chilling stress, which was similar to the homologue gene *BoSWEET16a* and *BoSWEET17* of *Brassica oleracea* var. *capitate* (Zhang et al., 2019) and the orthologous from *Arabidopsis AtSWEET16* and *AtSWEET17* genes. Notably, *AtSWEET16* and *AtSWEET17* overexpressed plants showed significant freeze resistance, which may be connected with the accumulation of sugar in the leaves under low-temperature stress (Klemens et al., 2013). The phylogenetic analysis suggested that *PmSWEET7* was clustered with *AtSWEET16* and *AtSWEET17* into Clade IV (Chardon et al., 2013; Klemens et al., 2013; Guo et al., 2014), and the expression of *PmSWEET7* from two cultivars declined after chilling stress, which we speculated this gene might have similar functions in mediating cold tolerance. Moreover, the *PmSWEET14* was clustered with *AtSWEET11* and *AtSWEET12* into Clade III in the phylogenetic tree. The expression of *PmSWEET14* from two cultivars is up-regulated after chilling stress, which may be related to cold stress (Le Hir et al., 2015; Durand et al., 2016). The expression profiles of the remaining six genes in two cultivars showed different patterns based on cold treatment. Interestingly, the expression of *PmSWEET4* and *PmSWEET10* from 'Songchun' declined rapidly after chilling stress, which indicated these genes might significantly be inhibited by cold stress, and the results were consistent with *Arabidopsis* (Le Hir et al., 2015; Durand et al., 2016) and *Camellia sinensis* (Yue et al., 2015). *PmSWEET1* was rapidly increased at 48 h in 'Zaolve' and was increased at 1 h under 4 °C treatments, and then decreased with the treatment time increase in 'Songchun', which was similar to the orthologous gene *AtSWEET15* in *Arabidopsis* (Seo et al., 2011). Furthermore, *PmSWEET*12 was rapidly up-regulated at 72 h in 'Zaolve', up-regulated at 1 h and then down-regulated with the increase of treatment time in 'Songchun', which was similar to its orthologous genes *CsSWEET1* in *Camellia sinensis*. The discrepancy in the tissue expression pattern between *PmSWEET1, 4, 10, 12, 17* is potentially due to the species differences between 'Songchun' and 'Zaolve'. In addition, the expression levels of five *PmSWEETs* (*PmSWEET2*, *4*, *7*, *8*, *10*) in 'Songchun' and three *PmSWEETs* (*PmSWEET2*, *7*, *8*) in 'Zaolve' decreased with the increasing of treatment times (Figure 8A and 8B), which suggested these genes might be negatively regulated by cold stress 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 the prolongation of treatment times (Figure 8A), which suggested these genes might be positively regulated by cold stress responses and increased cold sensitivity.

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 in cold stress conditions were analyzed by qRT-PCR assay. Our results could provide important information for further research on the biological functions of the *PmSWEETs*.

Funding

This work was supported by Forestry and Grassland Science and Technology Innovation Youth Top Talent Project of China (No. 2020132608), the National Key Research and Development Program of China (2018YFD1000401), and the National Natural Science Foundation of China (No. 31870689).

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 | Phylogenetic trees of *Arabidopsis thaliana*, *Prunus mume* and Rice
Supplementary Figure 2 | 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 | The specific number of genes in the Clades used in the present study
Supplementary Table 4 | Duplication events between *P. mume* and *A. thaliana*, *P. armeniaca* and *P. salicina*
Supplementary Table 5 |The data of cis-acting element in *PmSWEET*spromoters
Supplementary Table 6 |Expression profiles of 17 *PmSWEET* genes in five different tissues (root, stem, leaf, bud and fruit) (RPKM)
Supplementary Table 7 |Expression profiles of *PmSWEET* genes during the process of flower bud dormancy release (RPKM)
Supplementary Table 8 |Expression profiles of 17 *PmSWEET* genes in different regions and seasons (FPKM)

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

Ayre, B.G. (2011). Membrane-transport systems for sucrose in relation to whole-plant carbon partitioning. *Mol. Plant* 4(3)**,** 377-394. doi: 10.1093/mp/ssr014

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

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

564 Chong, J., Piron, M.C., Meyer, S., Merdinoglu, D., Bertsch, C., and Mestre, P. (2014). The
565 SWEET family of sugar transporters in grapevine: *VvSWEET4* is involved in the
566 interaction with Botrytis cinerea. *J. Exp. Bot.* 65(22), 6589-6601. doi: 10.1093/jxb/eru375
567 Daccord, N., Celton, J.M., Linsmith, G., Becker, C., Choisne, N., Schijlen, E., et al. (2017).
568 High-quality de novo assembly of the apple genome and methylome dynamics of early
569 fruit development. *Nat. Genet.* 49(7), 1099-1106. doi: 10.1038/ng.3886
570 Durand, M., Porcheron, B., Hennion, N., Maurousset, L., Lemoine, R., and Pourtau, N. (2016).
571 Water deficit enhances C export to the roots in *Arabidopsis thaliana* plants with
572 contribution of sucrose transporters in both shoot and roots. *Plant Physiol.* 170(3), 1460-
573 1479. doi: 10.1104/pp.15.01926
574 Eom, J.S., Chen, L.Q., Sosso, D., Julius, B.T., Lin, I.W., Qu, X.Q., et al. (2015). SWEETs,
575 transporters for intracellular and intercellular sugar translocation. *Curr. Opin. Plant Biol.*
576 25, 53-62. doi: 10.1016/j.pbi.2015.04.005
577 Feng, L., and Frommer, W.B. (2015). Structure and function of SemiSWEET and SWEET sugar
578 transporters. *Trends Biochem. Sci.* 40(8)**,** 480-486. doi: 10.1016/j.tibs.2015.05.005.
579 Feng, C.Y., Han, J.X., Han, X.X., and Jiang, J. (2015). Genome-wide identification, phylogeny,
580 and expression analysis of the SWEET gene family in tomato. *Gene* 573(2), 261-272. doi:
581 10.1016/j.gene.2015.07.055
582 Finn, R.D., Clements, J., Arndt, W., Miller, B.L., Wheeler, T.J., Schreiber, F., et al. (2015).
583 HMMER web server: 2015 update. *Nucleic Acids Res.* 43(W1), W30-W38. doi: 10.1093/
584 nar/gkv397
585 Ganko, E.W., Meyers, B.C., and Vision, T.J. (2007). Divergence in expression between
586 duplicated genes in *Arabidopsis*. *Mol. Biol. Evol.* 24(10), 2298-2309. doi:
587 10.1093/molbev/msm158
588 Gao, Y., Wang, Z.Y., Kumar, V., Xu, X.F., Yuan, P., Zhu, X.F., et al. (2018). Genome-wide
589 identification of the SWEET gene family in wheat. *Gene* 642, 284-292. doi:
590 10.1016/j.gene.2017.11.044
591 Gasteiger, E. (2005). Protein identification and analysis tools on the ExPASy server. *The*
592 *proteomics protocols handbook*. Humana Press, pp. 571-607
593 Ge, Y.X., Angenent, G.C., Wittich, P.E., Peters, J., Franken, J., Busscher, M., et al. (2000).
594 NEC1, a novel gene, highly expressed in nectary tissue of *Petunia hybrida*. *Plant J.* 24(6),
595 725-734. doi: 10.1046/j.1365-313x.2000.00926.x
596 Guo, W.J., Nagy, R., Chen, H.Y., Pfrunder, S., Yu, Y.C., Santelia, D., et al. (2014). *SWEET17*, a
597 facilitative transporter, mediates fructose transport across the tonoplast of
598 *Arabidopsis*roots and leaves. *Plant Physiol.* 164(2), 777-789. doi: 10.1104/pp.113.232751
599 Jia, B., Zhu, X.F., Pu, Z.J., Duan, Y.X., Hao, L.J., Zhang, J., et al. (2017). Integrative view of the
600 diversity and evolution of SWEET and SemiSWEET sugar transporters. *Front. Plant Sci.*
601 8, 2178. doi: 10.3389/fpls.2017.02178
602 Jian, H., Lu, K., Yang, B., Wang, T., Zhang, L., Zhang, A., et al. (2016). Genome-wide analysis
603 and expression profiling of the SUC and SWEET gene families of sucrose transporters in

Oilseed Rape (*Brassica napus* L.). *Front. Plant Sci.* 7, 1464. doi: 10.3389/fpls.2016.01464

Jiang, F., Zhang, J., Wang, S., Yang, L., Yingfeng, L., Gao, S., et al. (2019). The apricot (*Prunus armeniaca* L.) genome elucidates Rosaceae evolution and beta-carotenoid synthesis. *Hortic. Res.* 6, 128. doi: 10.1038/s41438-019-0215-6

Jiang, S., Balan, B., Assis, R.d.A.B., Sagawa, C.H.D., Wan, X., Han, S., et al. (2020). Genome-wide profiling and phylogenetic analysis of the SWEET sugar transporter gene family in walnut and their lack of responsiveness to *Xanthomonas arboricola* pv. juglandis infection. *Int. J. Mol. Sci.* 21(4), 1251. doi: 10.3390/ijms21041251

Katoh, K., and Standley, D.M. (2013). MAFFT multiple sequence alignment software version 7: improvements in performance and usability. *Mol. Biol. Evol.* 30(4), 772-780. doi: 10.1093/molbev/mst010

Klemens, P.A., Patzke, K., Deitmer, J., Spinner, L., Le Hir, R., Bellini, C., et al. (2013). Overexpression of the vacuolar sugar carrier *AtSWEET16* modifies germination, growth, and stress tolerance in *Arabidopsis*. *Plant Physiol.* 163(3), 1338-1352. doi: 10.1104/pp.113.224972

Le Hir, R., Spinner, L., Klemens, P.A.W., Chakraborti, D., de Marco, F., Vilaine, F., et al. (2015). Disruption of the sugar transporters *AtSWEET11* and *AtSWEET12*affects vascular development and freezing tolerance in *Arabidopsis*. *Mol. Plant* 8(11), 1687-1690. doi: 10.1016/j.molp.2015.08.007

Lemoine, R. (2000). Sucrose transporters in plants: update on function and structure. *BBA. Bioenergetics* 1465(1-2), 246-262. doi: 10.1016/s0005-2736(00)00142-5

Lemoine, R., La Camera, S., Atanassova, R., Dedaldechamp, F., Allario, T., Pourtau, N., et al. (2013). Source-to-sink transport of sugar and regulation by environmental factors. *Front. Plant Sci.* 4, 272. doi: 10.3389/fpls.2013.00272

Lescot, M., Dehais, P., Thijs, G., Marchal, K., Moreau, Y., Van de Peer, Y., et al. (2002). PlantCARE, a database of plant cis-acting regulatory elements and a portal to tools for in silico analysis of promoter sequences. *Nucleic Acids Res.* 30(1), 325-327. doi: 10.1093/nar/30.1.325

Letunic, I., and Bork, P. (2019). Interactive Tree Of Life (iTOL) v4: recent updates and new developments. *Nucleic Acids Res.* 47(W1), W256-W259. doi: 10.1093/nar/gkz239

Li, H., Li, X., Xuan, Y., Jiang, J., Wei, Y., and Piao, Z. (2018). Genome wide identification and expression profiling of SWEET genes family reveals its role during plasmodiophora brassicae-induced formation of clubroot in *Brassica rapa*. *Front. Plant Sci.* 9, 207. doi: 10.3389/fpls.2018.00207

Li, J., Qin, M., Qiao, X., Cheng, Y., Li, X., Zhang, H., et al. (2017). A new insight into the evolution and functional divergence of SWEET transporters in Chinese white pear (*Pyrus bretschneideri*). *Plant Cell Physiol.* 58(4), 839-850. doi: 10.1093/pcp/pcx025

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, H.T., Lyu, W.Y., Tian, S.H., Zou, X.H., Zhang, L.Q., Gao, Q.H., et al. (2019). The SWEET family genes in strawberry: Identification and expression profiling during fruit development. *SAfr. J. Bot.* 125, 176-187. doi: 10.1016/j.sajb.2019.07.002

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

Rolland, F., Baena-Gonzalez, E., and Sheen, J. (2006). Sugar sensing and signaling in plants: Conserved and novel mechanisms. *Annu. Rev. Plant Biol.* 57, 675-709. doi: 10.1146/annurev.arplant.57.032905.105441

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

719 transporter for monosaccharides. *J. Biol. Chem.* 285(2), 1138-1146. doi:
720 10.1074/jbc.M109.054288
721 Yao, T., Xie, R., Zhou, Y., Hu, J., Gao, Y., and Zhou, C. (2021). Genome-wide identification of
722 SWEET gene family and its response to abiotic stresses in valencia sweet orange. *Plant*
723 *Mol. Biol. Rep*. doi: 10.1007/s11105-020-01268-1
724 Yuan, M., and Wang, S. (2013). Rice MtN3/Saliva/SWEET family genes and their homologs in
725 cellular organisms. *Mol. Plant* 6(3), 665-674. doi: 10.1093/mp/sst035
726 Yuan, M., Zhao, J., Huang, R., Li, X., Xiao, J., and Wang, S. (2014). Rice MtN3/saliva/SWEET
727 gene family: Evolution, expression profiling, and sugar transport. *J. Integr. Plant Biol.*
728 56(6), 559-570. doi: 10.1111/jipb.12173
729 Yue, C., Cao, H.L., Wang, L., Zhou, Y.H., Huang, Y.T., Hao, X.Y., et al. (2015). Effects of cold
730 acclimation on sugar metabolism and sugar-related gene expression in tea plant during the
731 winter season. *Plant Mol. Biol.* 88(6), 591-608. doi: 10.1007/s11103-015-0345-7
732 Zhang, Q., Chen, W., Sun, L., Zhao, F., Huang, B., Yang, W., et al. (2012). The genome of
733 *Prunus mume*. *Nat. Commun.* 3, 1318. doi: 10.1038/ncomms2290
734 Zhang, W., Wang, S., Yu, F., Tang, J., Shan, X., Bao, K., et al. (2019). Genome-wide
735 characterization and expression profiling of SWEET genes in cabbage (*Brassica*
736 *oleracea*var. *capitata* L.) reveal their roles in chilling and clubroot disease responses.
737 *BMC Genomics* 20(1), 93. doi: 10.1186/s12864-019-5454-2
738 Zhang, Z., Zhuo, X., Zhao, K., Zheng, T., Han, Y., Yuan, C., et al. (2018). Transcriptome profiles
739 reveal the crucial roles of hormone and sugar in the bud dormancy of *Prunus mume*. *Sci.*
740 *Rep.* 8(1), 5090. doi: 10.1038/s41598-018-23108-9
741 Zhao, L., Yao, J., Chen, W., Yan, L.I., Youjun, L., Guo, Y., et al. (2018). A genome-wide
742 analysis of SWEET gene family in cotton and their expressions under different stresses. *J.*
743 *Cotton Res.* 1(2),1–15. doi: 10.1186/s42397-018-0007-9
744

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*.

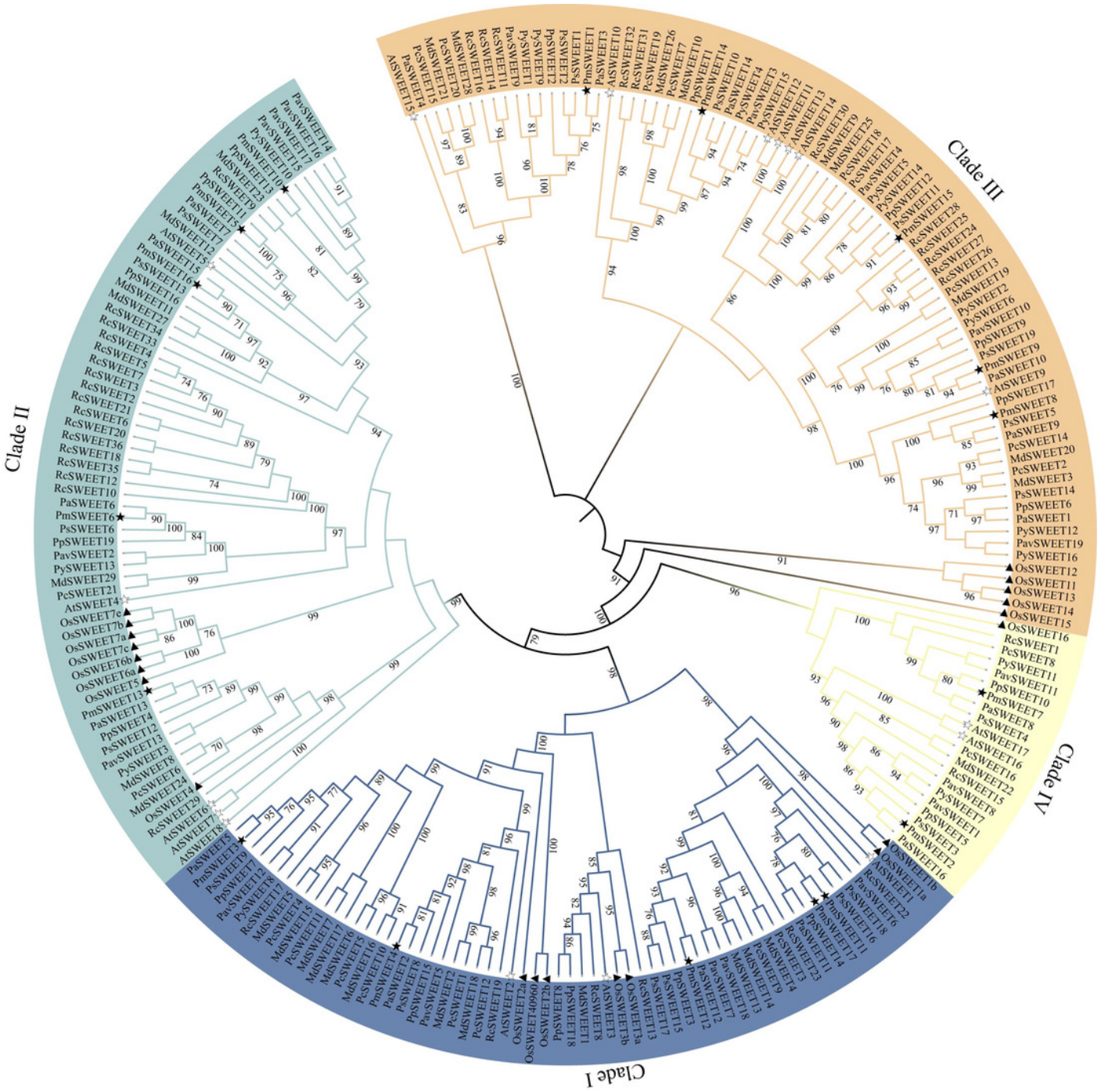
Clade I
Clade II
Clade III
Clade IV

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.

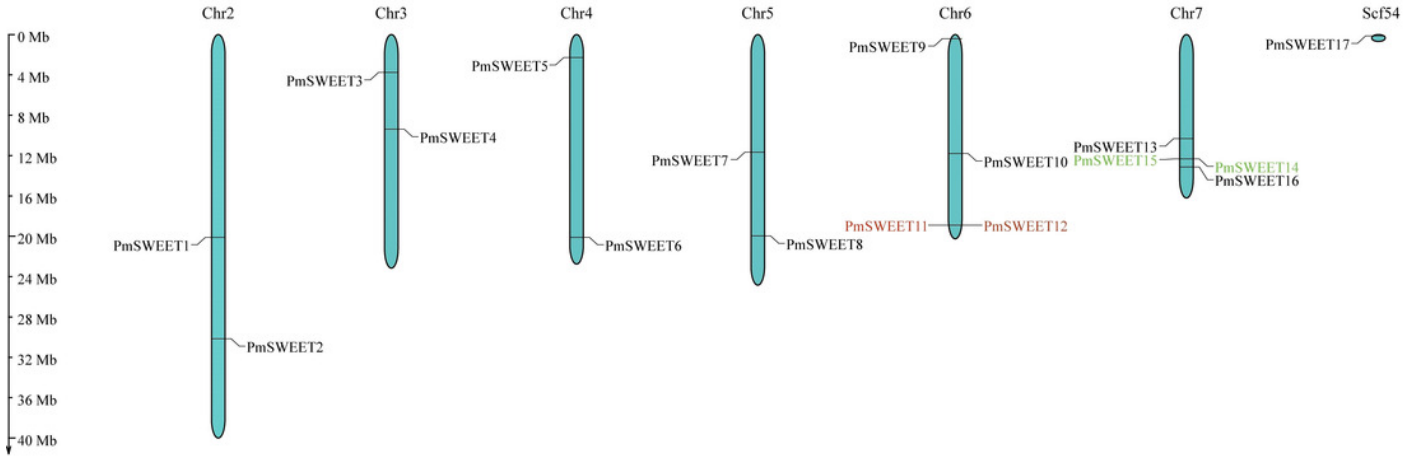

Figure 4

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

The red lines indicate segmented duplicated gene pairs.

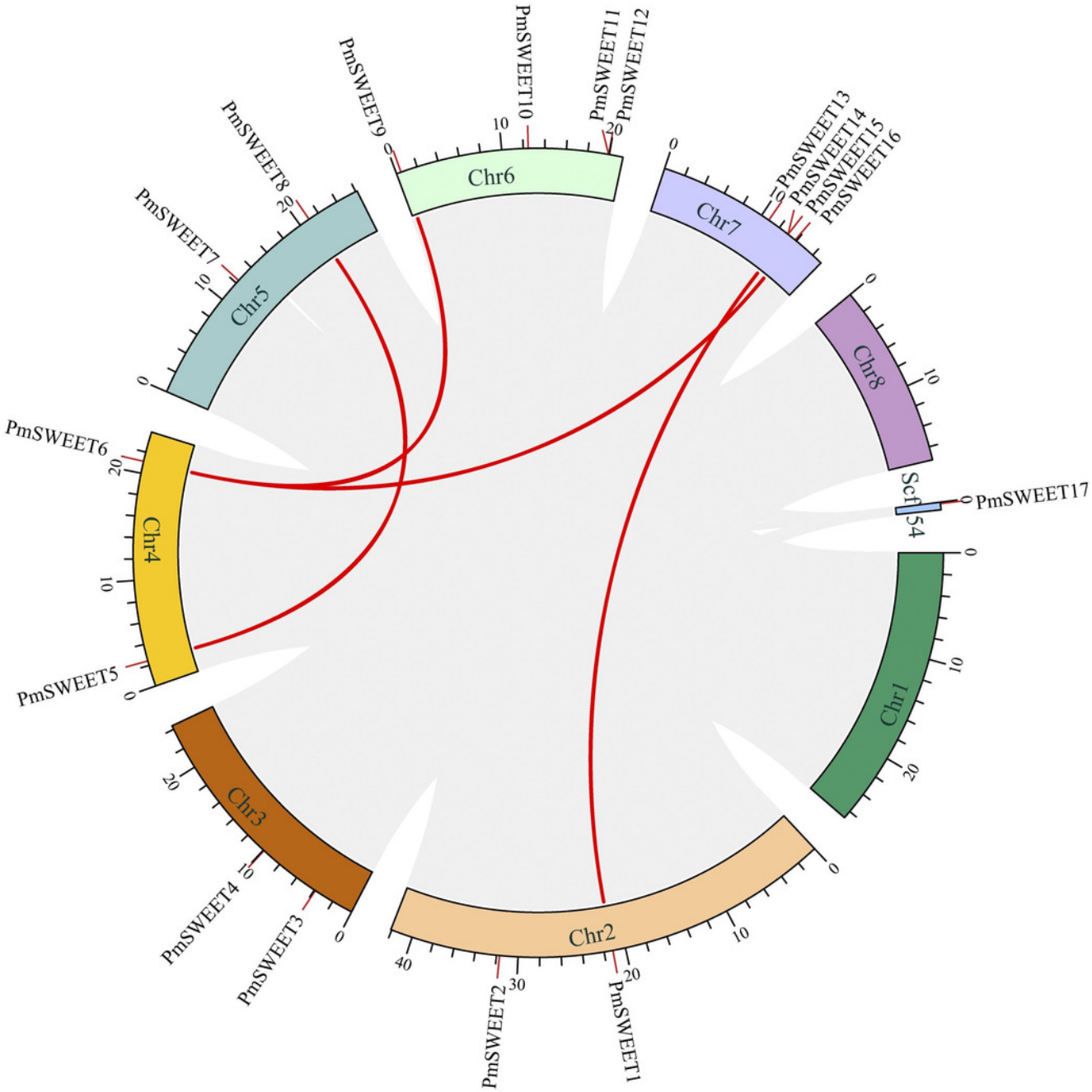

Figure 5

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.

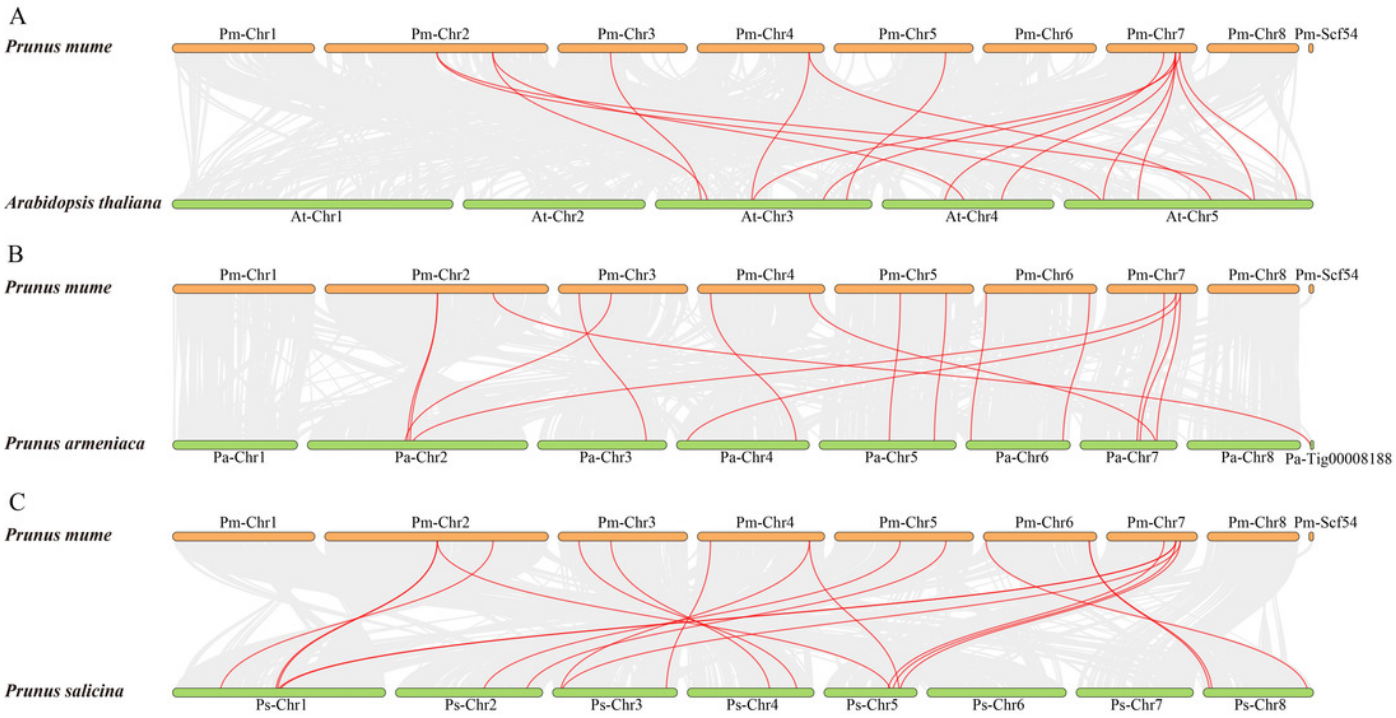

Figure 6

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.

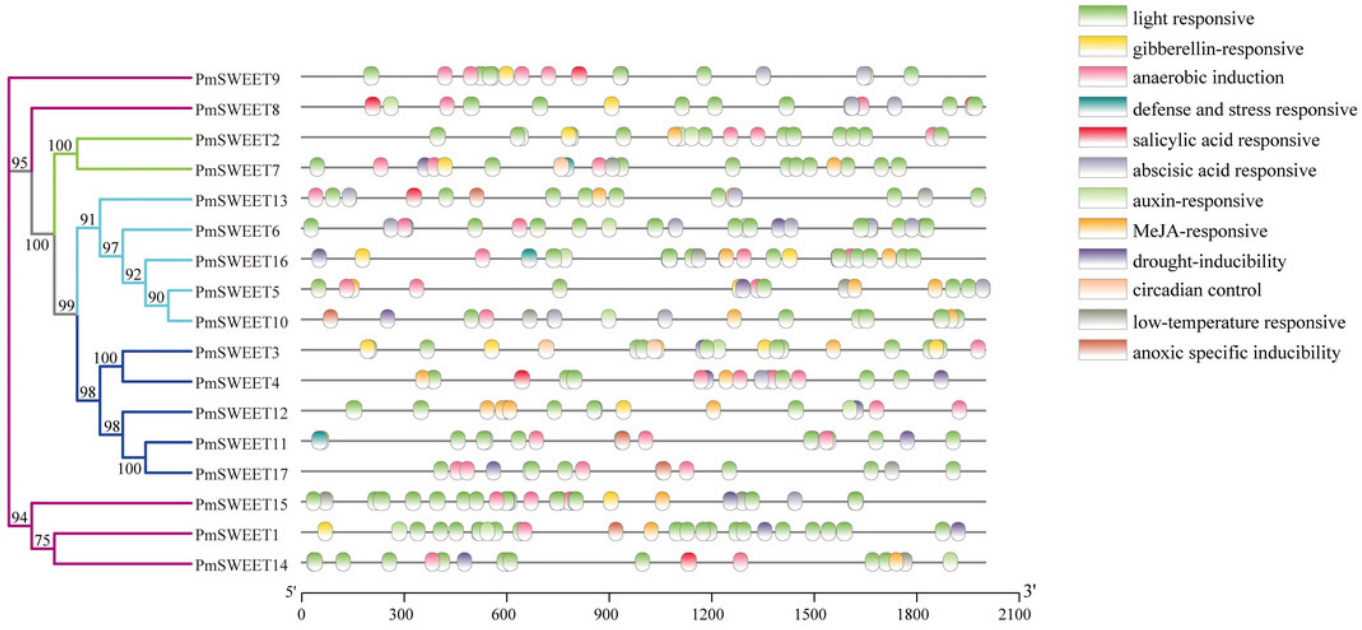

Figure 7

Figure 7. Expression profiles of *PmSWEET* genes under different conditions.

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. C: Expression profiles of *PmSWEETs* in stems of 'Songchun' in different seasons and regions. Spr, Spring; Aut, Autumn; Win, Winter. BJ, Beijing; CF, Chifeng; GZL, Gongzhuling.

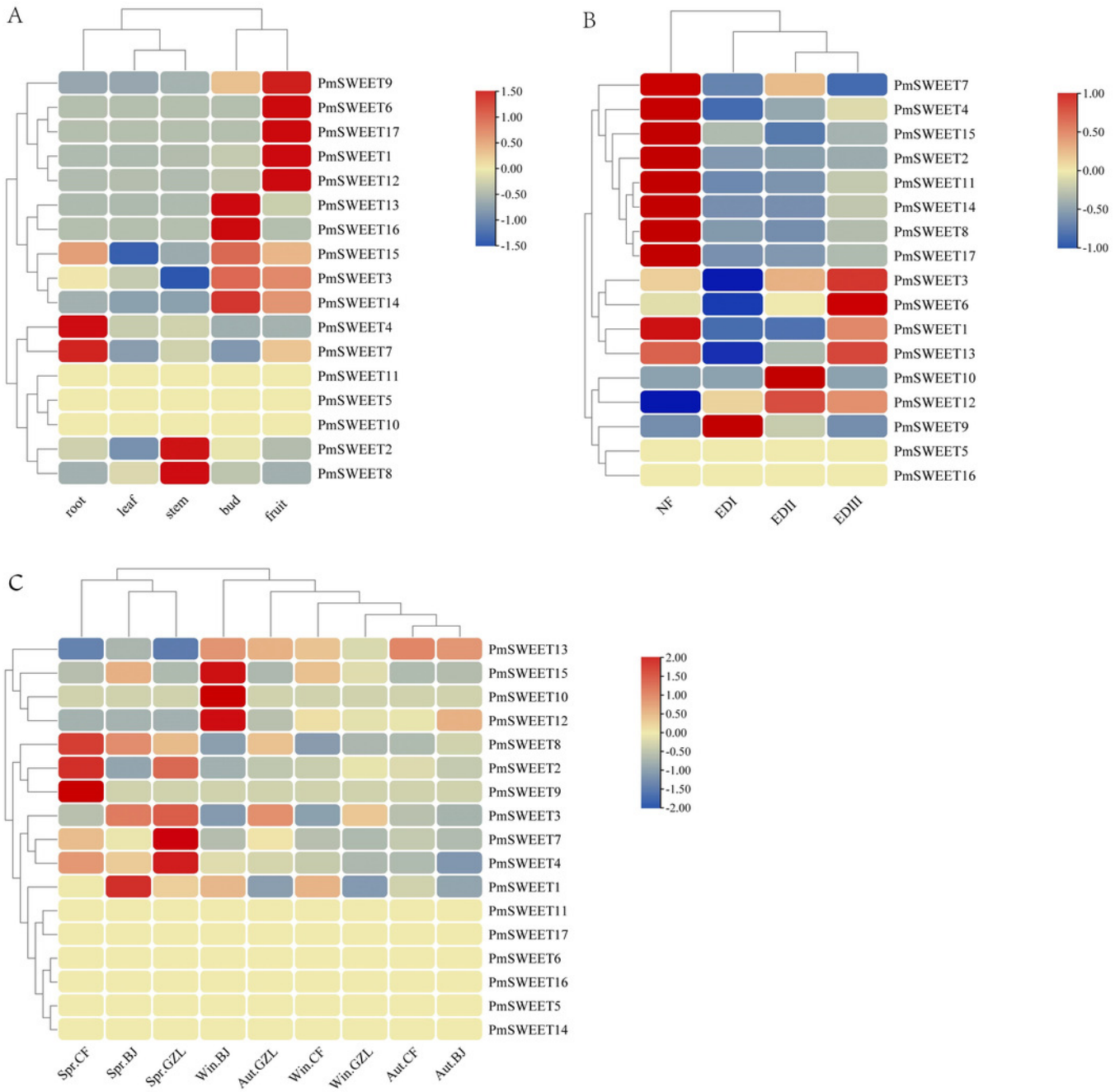
A
PmSWEET9
PmSWEET6
PmSWEET17
PmSWEET1
PmSWEET12
PmSWEET13
PmSWEET16
PmSWEET15
PmSWEET3
PmSWEET14
PmSWEET4
PmSWEET7
PmSWEET11
PmSWEET5
PmSWEET10
PmSWEET2
PmSWEET8
root
leaf
stem
bud
fruit
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
B
PmSWEET7
PmSWEET4
PmSWEET15
PmSWEET2
PmSWEET11
PmSWEET14
PmSWEET8
PmSWEET17
PmSWEET3
PmSWEET6
PmSWEET1
PmSWEET13
PmSWEET10
PmSWEET12
PmSWEET9
PmSWEET5
PmSWEET16
NF
EDI
EDII
EDIII
1.00
0.50
0.00
-0.50
-1.00
C
PmSWEET13
PmSWEET15
PmSWEET10
PmSWEET12
PmSWEET8
PmSWEET2
PmSWEET9
PmSWEET3
PmSWEET7
PmSWEET4
PmSWEET1
PmSWEET11
PmSWEET17
PmSWEET6
PmSWEET16
PmSWEET5
PmSWEET14
Spr.CF
Spr.BJ
Spr.GZL
Win.BJ
Aut.GZL
Win.CF
Win.GZL
Aut.CF
Aut.BJ
2.00
1.50
1.00
0.50
0.00
-0.50
-1.00
-1.50
-2.00

Figure 8

Figure 8. Expression analyses of 11 *PmSWEETs* in *P. mume* exposed to 4 °C for different times (0/1/4/6/12/24/48/72 h), where 0 h indicates control.

The relative quantification method ($2^{-\Delta\Delta Ct}$) was used to evaluate quantitative variation. Error bars represent percentage error for three replicates. A: 'Songchun' B: 'Zaolve'.