Genome-wide Identification and Expression Analyses of SWEET Gene Family Reveal Potential Roles in Plant Development, Fruit Ripening and Abiotic Stress Responses in Cranberry (*Vaccinium macrocarpon* Ait)

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The SWEET (sugars will eventually be exported transporter) family is a novel class of sugar transporters that play a crucial role in plant growth, development, and responses to stress. Cranberry (*Vaccinium macrocarpon* Ait.) is a nutritious berry with economic importance, but little is known about *SWEET* gene family functions in this small fruit. In this research, 13 *VmSWEET* genes belonging to four clades were identified in the cranberry genome for the first time. In the conserved domains, we observed seven phosphorylation sites and four amino acid residues that might be crucial for the binding function. The majority of *VmSWEET* genes in each clade shared similar gene structures and conserved motifs, showing that the *VmSWEET* genes were highly conserved during evolution. Chromosomal localization and duplication analyses showed that *VmSWEET* genes were unevenly distributed in eight chromosomes and two pairs of them displayed synteny. A total of 79 *cis*-acting elements were predicted in the promoter regions of *VmSWEETs* including elements responsive to plant hormones, light, growth and development and stress responses. qRT-PCR analysis showed that *VmSWEET10.1* was highly expressed in flowers, *VmSWEET16* was highly expressed in upright and runner stems, and *VmSWEET3* was highly expressed in the leaves of both types of stems. In fruit, the expression of *VmSWEET14* and *VmSWEET16* was highest of all members during the young fruit stage and were downregulated as fruit matured. The expression of *VmSWEET4* was higher during later developmental stages than earlier developmental stages. Furthermore, qRT-PCR results revealed a significant up-regulation of *VmSWEET10.2*, under osmotic, saline, salt-alkali, and aluminum stress conditions, suggesting it has a crucial role in mediating plant responses to various environmental stresses. Overall, these results provide new insights into the characteristics and evolution of *VmSWEET* genes. Moreover, the candidate

VmSWEET genes involved in the growth, development and abiotic stress responses can be used for molecular breeding to improve cranberry fruit quality and abiotic stress resistance.

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Abstract: The SWEET (sugars will eventually be exported transporter) family is a novel class of sugar transporters that play a crucial role in plant growth, development, and responses to stress. Cranberry (*Vaccinium macrocarpon* Ait.) is a nutritious berry with economic importance, but little is known about *SWEET* gene family functions in this small fruit. In this research, 13 *VmSWEET* genes belonging to four clades were identified in the cranberry genome for the first time. In the conserved domains, we observed seven phosphorylation sites and four amino acid residues that might be crucial for the binding function. The majority of *VmSWEET* genes in each clade shared similar gene structures and conserved motifs, showing that the *VmSWEET* genes were highly conserved during evolution. Chromosomal localization and duplication analyses showed that *VmSWEET* genes were unevenly distributed in eight chromosomes and two pairs of them displayed synteny. A total of 79 *cis*-acting elements were predicted in the promoter regions of *VmSWEETs* including elements responsive to plant hormones, light, growth and development and stress responses. qRT-PCR analysis showed that *VmSWEET10.1* was highly expressed in flowers, *VmSWEET16* was highly expressed in upright and runner stems, and *VmSWEET3* was highly expressed in the leaves of both types of stems. In fruit, the expression of *VmSWEET14* and *VmSWEET16* was highest of all members during the young fruit stage and were downregulated as fruit matured. The expression of *VmSWEET4* was higher during later developmental stages than earlier developmental stages. Furthermore, qRT-PCR results revealed

a significant up-regulation of *VmSWEET10.2*, under osmotic, saline, salt-alkali, and aluminum stress conditions, suggesting it has a crucial role in mediating plant responses to various environmental stresses. Overall, these results provide new insights into the characteristics and evolution of *VmSWEET* genes. Moreover, the candidate *VmSWEET* genes involved in the growth, development and abiotic stress responses can be used for molecular breeding to improve cranberry fruit quality and abiotic stress resistance.

Keywords: cranberry; SWEET; bioinformatics analysis; expression analysis; growth and development; abiotic stress

INTRODUCTION

Sugars are important molecules that regulate a wide range of morphological and physiological processes in plants. Apart from their functions as energy sources, osmoregulators, storage molecules, and structural components, sugars also act as signaling molecules that interact with diverse plant signaling pathways including hormones, stress responses, and light perception mechanisms. Consequently, sugars modulate growth and development in response to dynamic environmental conditions (Mishra et al., 2022). As the primary photoassimilate, sugars are synthesized in leaves before being transported via the phloem to sink tissues, such as flowers, stems, tubers, swollen tap roots, fruits, and seeds (Sonnewald and Fernie, 2018). Phloem loading in source leaves and unloading in sink tissues involves a combination of the symplastic, apoplastic, and/or polymer trapping pathways. The symplastic and polymer trapping pathways are passive processes that are correlated with source activity and sugar gradients. In contrast, the apoplastic pathway is characterized by active energy consumption, which necessitates the involvement of sugar transporters for efficient translocation of sugars (De Schepper et al., 2013). In higher plants, three sugar transporter families play a crucial role in phloem loading and unloading: the monosaccharide transporter-like (MST) gene family, the sucrose transporters (SUT/SUC), and the sugars will eventually be exported transporters (SWEET) (Doidy et al., 2012). The MSTs and SUTs contain 12 transmembrane domains (TMDs) and require energy to complete the transmembrane transport of sugars. However, SWEETs have seven TMDs and act

as uniporters that facilitate sugar translocation along a concentration gradient independently of the proton gradient and pH (Chen et al., 2010; Chen et al., 2014; Yuan et al., 2013; Julius et al., 2017). To date, SWEET genes have been identified in grain, horticultural, legume, oil and fiber crops and other plant species, such as wheat (Gao et al., 2018), soybean (Patil et al., 2015); oilseed rape (Jian et al., 2016), cotton (Li et al., 2018), apple (Wei et al., 2014), jujube (Yang et al., 2023), tomato (Feng et al., 2015), cabbage (Zhang et al., 2019a), daylily (Huang et al., 2022), and saccharum (Hu et al., 2018). Phylogenetically, plant SWEETs are divided into four clades (Clades I, II, III and IV) based on the functional characterization of SWEET genes in Arabidopsis (Chen et al., 2010). Clades I, II, and IV tend to transport monosaccharides, and Clade III predominantly transports sucrose (Le Hir et al., 2015). Additionally, the Clade IV members are typically localized to the tonoplast (Chardon et al., 2013; Klemens et al., 2013), while members of other clades are situated primarily on the plasma membrane and sometimes on the Golgi membrane and chloroplast (Breia et al., 2021).

Passive unloading of sucrose from the mesophyll into the apoplast and its subsequent active loading into the phloem have been described by Giaquinta in the late 1970s (Giaquinta, 1977), but the mechanism underlying this unloading process remained elusive until the discovery of *SWEET* genes. In Arabidopsis, *AtSWEET11* and *AtSWEET12* were found to encode proteins that facilitate the release of sucrose from parenchyma cells to the apoplast, and the *atsweet11;12* double mutation suppressed phloem transport, which led to the accumulation of starch in the leaves (Chen et al., 2012). Recent studies have revealed that SWEET-mediated phloem loading in leaves is regulated by sugar signals. In Chinese jujube, the transcription of *ZjSWEET2.2* was activated by a low sugar signal, as its promoter *cis*-elements were bound to it, while expression decreased and the photosynthetic rate was reduced by a high sugar signal (Geng et al., 2020). In addition, multiple physiological functions of SWEET transporter including nectar secretion, pollen nutrition, grain filling, fruit ripening, shoot branching and bud outgrowth have been reported (Eom et al., 2015; Wen et al., 2022; Grantam et al., 2022). In Arabidopsis, *Brassica rapa* and tobacco, the *SWEET9* gene was identified in the transport of sucrose from nectary

parenchyma to the extracellular space for rewarding pollinators, and *atsweet9* mutant lines failed in nectar secretion (Lin, et al., 2014). In maize and rice, *SWEET* genes play a role in the transfer of sugars imported from the maternal phloem. Notably, mutants *zmsweet4c*, *ossweet4* and *ossweet11*, *as well as the* *ossweet11;15* double mutants, exhibited significantly decreased sucrose concentration in the embryo, accumulated starch in the pericarp, and experienced functional deficiency in seed filling (Sosso et al., 2015; Ma et al., 2017; Yang et al., 2018). In pineapple, *AcSWEET11* was strongly expressed in ripening fruit, overexpression of *AcSWEET11* in the pineapple callus and in tomato enhanced sugar content (Lin et al., 2022). In tomato, function elimination of the *SlSWEET15* gene resulted in a significant reduction in the average size and weight of fruits and was accompanied by severe impairments in seed filling and embryo development (Ko et al., 2021). The above results indicate that SWEET proteins mediate the unloading of sucrose in sink organs that affect the yield and quality of important economic crops.

Sugar transport and partitioning not only affect plant growth and development, but also respond to abiotic and biotic stress. As SWEETs facilitate the efflux of sugars, they are highly susceptible to being hijacked by pathogens, making them central players in plant-pathogen interactions. In Arabidopsis, the root tonoplast *AtSWEET2* was induced during *Pythium irregulare* infection, which led to enhanced cytosolic sugar accumulation in the vacuole. Overexpression of *AtSWEET2* enhanced plant resistance to *P. irregulare* by limiting sugar availability to the pathogen (Breia et al., 2021). However, the opposite behavior was observed in grapes. Overexpression of *VvSWEET4* improved resistance to *P. irregulare*, while high sugar accumulation in hairy roots provided better support for increased energy demand during pathogen infection (Meteier et al., 2019). Thus, it is difficult to define roles for SWEET transporters in plant-pathogen interactions, because the metabolic signatures and regulatory nodes that determine susceptibility or resistance responses remain poorly understood. Previous studies on the response of SWEET transporters to abiotic stress focused on drought, cold, and salinity. In Fen Jiao [*M. acuminata* AAB group] which exhibits high tolerance to abiotic stresses resistant, some *MaSWEETs* exhibited increased expression in response to cold, drought, salinity,

and fungal disease (Miao et al., 2017). In tea (*Camellia sinensis*), the tonoplast sugar transporter gene *CsSWEET16* was downregulated under cold stress. Overexpression of *CsSWEET16* in Arabidopsis resulted in enhanced cold tolerance, which was accompanied by glucose accumulation in the vacuole and reduced levels of fructose (Wang et al., 2018). Although our understanding of SWEET functions is increasing, their roles in sugar transport, distribution, metabolism, and signaling require further study.

Cranberry (*Vaccinium macrocarpon* Ait.), a diploid plant (2n = 2x = 24), is a woody perennial belonging to the Ericaceae family *Vaccinium* genus (Kron et al., 2002). It is endemic to North America and can also be found in the Changbai Mountains of northeastern China. Like other members of its botanical family, such as blueberry, bilberry, and lingonberry, cranberry is uniquely adapted to life in cool and moist peat bogs; it thrives in acidic, nutrient poor soils (Fajardo et al., 2012). This small but economically important berry fruit offers immense potential for global development due to its versatility. The growing importance of cranberries has created a demand for enhanced productivity and superior quality. However, during commercial cultivation, cranberries frequently encounter abiotic stress including extreme temperatures (i.e. frost damage and heat stress) and water availability (both drought and flooding) due to disparities between cultivation the environment and their native habitat (Neyhart et al., 2022). Plant SWEET transporters have been demonstrated to play important roles in growth, development, and plant-environment interactions in many species, but systematic studies on *SWEET* genes in cranberry have not been reported. In this study, we conducted a genome-wide analysis of *SWEET* genes in cranberry and analyzed their phylogenetic relationship, gene structure, motif distribution, chromosomal localization, and *cis*-regulatory elements. In addition, spatiotemporal expression, and abiotic stress responses were carried out using qRT-PCR. This study provides valuable insights into the roles of *VmSWEET* genes in cranberry growth, development, and stress responses.

MATERIALS AND METHODS

Plant Materials

The cultivar Bain 11, in the small berry germplasm resource garden of Jilin Agricultural University (43°48′05″N, 125°24′15″E), was used to detect the expression of *VmSWEET* genes in cranberry tissues and fruit at different stages of development (Figure 1). The average annual precipitation in this area is 600–700 mm, with an average temperature of 4.6 ℃ and an annual frost-free period lasting 140–150 d. In order to improve the garden soil for optimal cranberry growth, it was mixed with sand, peatmoss, perlite, and sulfur powder. The pH of the improved soil was 5.0, which is conducive to the successful cultivation of cranberries. Roots, upright stems, leaves of upright stem, runner stems, leaves of runner stem, and flowers were collected during the flowering period. Fruits at young stage (S1), expansion stage (S2), color turning stage (S3), and maturity stage (S4) were collected 10, 30, 60, and 80 d after full bloom, respectively. Tissue and fruit samples were randomly obtained from three plants and replicated three times.

Plantlets of Bain11 were used to detect expression patterns under abiotic stress. An osmotic treatment (20% PEG 8000), saline treatment (200 mM NaCl), saline-alkaline treatment (30 mM Na_2CO_3 and 30 mM $NaHCO_3$), and aluminum treatment (5mM $AlCl_3$) were administered by immersing the roots of plantlets in containers with different solutions. Tender stem apexes were collected 0, 3, 6, 9, 12, and 24 h during the different stress treatments. Samples were collected from three containers every time and replicated thrice. All samples were immediately frozen in liquid nitrogen and stored at -80 °C.

Identification *SWEET* Gene Family in Cranberry

Members of the cranberry *SWEET* gene family were identified by protein Blast of the 17 Arabidopsis SWEET proteins against the *V. macrocarpon* genome database (https://www.ncbi.nlm.nih.gov/genome/?term=cranberry). The coding domain sequences (CDS) of *VmSWEET* genes are shown in File S1. The NCBI CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and PFAM (http://pfam.sanger.ac.uk/) websites were used to search for the conserved domains of the candidate members.

Protein Domain, Conserved Motifs, Gene Structure and Promoter *cis*-regulatory Elements Analysis

The number of amino acids, molecular weights (MWs), and theoretical isoelectric points (PIs) were analyzed using the ExPASy website (http://web.expasy.org/potparam/). Subcellular localization of VmSWEETs was predicted using WoLFPSORT (https://www.genscript.com/wolf-psort.html). A more recent and better transmembrane predictor TMHMM 2.0 (https://services.healthtech.dtu.dk/services/TMHMM-2.0/) was used for prediction of TMDs. The conserved motifs of SWEETs were predicted using online MEME tools (https://meme-suite.org/meme/tools/meme). The MEME parameter settings were as follows: the number of motifs was 10 and the range of motifs varied from 5–50. The exon/intron structures were analyzed using TBtools software (South China Agricultural University, Guangzhou, China, https://github.com/CJ-Chen/TBtools/releases) (Chen et al., 2020). Promoter *cis*-acting regulatory elements were predicted by submitting 2 kb upstream sequences of the translation start site of *VmSWEET* genes to the PlantCARE web site (http://bioinformatics.psb.ugent.be/webtools/plantcare/html). The promoter sequences and *cis*-acting elements of *VmSWEET* genes are shown in File S2.

Phylogenetic Analyses and Multiple Sequence Alignment

The amino acid sequences of 17 *Arabidopsis thaliana SWEET* genes, 21 *Oryza sativa SWEET* genes, 14 *Vitis vinifera SWEET* genes and 13 *Vaccinium macrocarpon SWEET* genes were used to construct an unrooted phylogenetic tree using MEGA 7.0 sofware (Mega Limited, Auckland, New Zealand, www.megasoftware.net) (Kumar et al., 2016) by the maximum likelihood method with bootstrap values for 1000 replicates. Then the phylogenetic tree was beautified by ITOL v6 (https://itol.embl.de/). The amino acid sequences of SWEET proteins from Arabidopsis, rice and grape are shown in File S3. Alignment of SWEET protein sequences was performed using the ClustalX software (Trinity College Dublin, Ireland, UK) and phosphorylation sites were predicted by NetPhos 3.1 (https://services.healthtech.dtu.dk/services/NetPhos-3.1/). The GENEDOC 3.20 software (http://nrbsc.org/gfx/genedoc) was used to highlight conserved or similar amino acid sequences.

Chromosomal Distribution and Gene Synteny Analysis

MapChart (http://www.joinmap.nl) (Voorrips, 2002) was used to construct the chromosomal distribution map of *VmSWEET* genes, and MCScanX (https://github.com/wyp1125/MCScanX) was used to analyze gene synteny. First, potential homologous gene pairs in cranberry were identified by local all-vs-all BLASTP algorithm-based searches ($E < 1e^{-10}$). Then, syntenic chains were identified by MCScanX using homologous pairs as input (Tang et al., 2008). Duplications in the SWEET gene family were identified by downstream analysis tools in the MCScanX package. Finally, synteny analysis was beautified by CIRCOS.

Quantitative RT-PCR (qRT-PCR) for SWEET Genes

Total RNA was isolated by a modified CTAB method (Li et al., 2019). The integrity and concentration of RNA were assessed using electrophoresis on 1.2% agarose gels and an IMPLEN P330 spectrophotometer (IMPLEN, Munich, Germany), respectively. A 1 μg sample of the extracted RNA was reverse transcribed into cDNA using a cDNA Synthesis SuperMix (TransGen Biotechnology, Beijing, China). qRT-PCR was performed with an ABI StepOne Plus Real-Time Quantitative PCR System (Applied Biosystems, Foster City, CA, USA) following the MIQE guidelines (the Minimum Information for Publication of Quantitative Real-Time PCR Experiments). The reaction system totaled 20 μL, following the Green qPCR SuperMix manufacturer instructions (TransGen Biotechnology, Beijing, China). SYBR Green I fluorescent dye as the detection signal was used for detection of *VmSWEETs*. The reaction procedure was as follows: denaturation at 94 °C for 30 s; denaturation at 94 °C for 5 s; annealing at 60 °C for 30 s; and then 94 °C for 10 s, 60 °C for 60 s, and 94 °C for 15 s to generate the melting curve. All experiments were run in three biological replicates. Primers were designed with the Premier-BLAST tool (http://blast.ncbi.nlm.nih.gov/). The *VmSAND* gene was considered the optimal internal reference gene for analyzing various cranberry tissues and abiotic stress treatments (Li et al., 2019). It was utilized as a control to standardize the expression of *VmSWEETs*. The designed qRT-PCR primers are shown in File S4. The raw data for Ct values are shown in File S5. Relative quantitative analysis of 13 target genes in different cranberry tissues and fruit development stages was performed using the $2^{-\Delta Ct}$ method, and column charts were obtained

using SigmaPlot 10.0 (Systat Software, Inc., Melbourne). Expression profiles of *VmSWEETs* under abiotic stress were calculated using the $2^{-\Delta\Delta Ct}$ method, and the expression levels were $\log_2$ transformed and normalized to obtain a heatmap by TBtools (South China Agricultural University, Guangzhou, China, https://github.com/CJ-Chen/TBtools/releases) (Chen et al., 2020). The completed MIQE checklist is shown in File S6. Statistical analysis was carried out by One-way ANOVA and LSD-test using the SPSS software (IBM Corporation, USA). A p value of less than 0.05 ($p < 0.05$) was considered statistically significant.

RESULTS

Genome-wide Identification and Analysis of *VmSWEET* Genes

Through homologous alignment and conservative domain verification, a total of 13 genes encoding SWEET proteins were identified. *VmSWEETs* were named based on their phylogenetic grouping into the four SWEET clades (Figure 2), according to Doidy's taxonomic framework (Doidy et al., 2019). The physical and chemical details of *VmSWEET* genes are summarized in Table 1. The CDS lengths of *VmSWEETs* varied from 519–1065 bp, corresponding to amino acid numbers ranging from 196–354. The MWs of the 13 proteins ranged from 21.38–40.24 KD, and the PIs spanned from 6.24–9.61. The instability index ranged from 30.52–51.71, suggesting that 62% of VmSWEETs were hydrophobic. The aliphatic index of nearly all proteins exceeded 100, while the grand average of hydropathicity (GRAVY) values varied from 0.205–1.002, indicating their inherent hydrophobic properties. The transmembrane domains prediction using TMHMM suggested that VmSWEETs exhibit 5–7 TMDs. Subcellular localization prediction using WoLFPSORT revealed that VmSWEET1.1 and VmSWEET2.2 may be localized in tonoplast membrane, VmSWEET1.2 was likely to be localized in endoplasmic reticulum, and the other 10 VmSWEETs were primarily located in the plasma membrane.

Phylogenetic Analysis of putative VmSWEET proteins

To investigate the phylogenetic relationships among *SWEET* genes in cranberry and other plant species, a phylogenetic tree was constructed by aligning the predicted amino acids of 13 VmSWEET sequences, 17 AtSWEET sequences, 21 OsSWEET sequences, and 15 VvSWEET

sequences. The 66 proteins were clustered into four different clades (Figure 2). Clade I contained 5 VmSWEETs (VmSWEET1.1, 1.2, 2.1, 2.2, and 3), 3 AtSWEETs (AtSWEET1–3), 6 OsSWEETs (OsSWEET1a, 1b, 2a, 2b, 3a, and 3b) and 2 VvSWEETs (VvSWEET1 and 2). Two VmSWEETs (VmSWEET4 and 5), 5 AtSWEETs (AtSWEET4–8), 9 OsSWEETs (OsSWEET7a–7e, 6a, 6b, 4, and 5) and 4 VvSWEETs (VvSWEET4, 5a, 5b, and 7) belonged to Clade II. Five VmSWEETs (VmSWEET10.1, 10.2, 12, 13, and 14), 7 AtSWEETs (9–15), 5 OsSWEETs (OsSWEET11–15) and 5 VvSWEETs (VvSWEET9, 10, 11, 12, and 15) were included in Clade III. Clade IV had the the fewest members and contained 1 VmSWEET (VmSWEET16), 1 OsSWEETs (OsSWEET16), 2 AtSWEETs (AtSWEET16 and 17), and 3 VvSWEETs (VvSWEET17a, 17b, and 17d).

Multiple Sequence Alignment, Conserved Domain and Gene Structure Analysis of VmSWEETs

The result of multiple sequence alignment is presented in File S7. The amino acid sequence identity among the 13 VmSWEETs ranged from 18–70%. The majority of VmSWEET members contained two MtN3/saliva domains, also known as PQ-loop-repeat, which consist of 3 + 1 + 3 transmembrane helices. Four S, two Y sites, and one T phosphorylation site were predicted in the two conserved MtN3/saliva regions and were indicated by the red triangles. Additionally, to search for the key amino acid sites for VmSWEETs binding to sugars, we found a very conserved asparagine pair (N77 and N197), which were located in the binding pocket of OsSWEET2b in rice, presented at equivalent VmSWEET positions. Furthermore, S54 on THB1 and W76 on THB2 have been confirmed to play analogous role in AtSWEET1 (Tao et al., 2015). In all VmSWEETs, W was present at the equivalent position of W176, except for in VmSWEET2.1, 13, and 14, where it was replaced with aromatic residue F or Y. Similarly, at the corresponding S54 position, it was replaced with F, W, L, Y, or C.

The conserved motifs were predicted to provide more insights into the characteristics of *VmSWEET* genes. As shown in Figure 3b, a total of 9 different conserved motifs were identified. Detailed information for each motif is provided in File S8. Motif 2, motif 4, and motif 5 were

observed in all 13 VmSWEET proteins. Motif 2 and motif 4 belong to the first conserved MtN3/saliva domain, while motif 5 belongs to the second conserved MtN3/saliva domain. These findings suggest that the three conserved motifs may be essential for cranberry SWEET protein function. The conserved motifs within the N-terminus of most VmSWEET proteins exhibited the same order (motif 8, motif 4, and motif 2), except when motif 8 was absent in VmSWEET2.1, VmSWEET4, VmSWEET5, and VmSWEET16. Significantly, motif 7 was exclusively present in VmSWEET16 in Clade IV, suggesting a specific function. Motif 1 was not present in VmSWEET2.2, VmSWEET5, VmSWEET13, and VmSWEET14, but an additional transmembrane-domain structure appeared at the same position (Figure 3c).

To further investigate the structural differences in *VmSWEET* genes, the arrangement of introns and exons was determined. As shown in File S9, the number of exons in the 13 *VmSWEET* genes ranged from 4–9, and the number of introns changed from 3–8. The number of introns in Clade I versus Clade III differed significantly, *VmSWEET* genes varied from 4–8 in Clade I and from 3–5 in Clade III. However, gene pairs in the sister branch exhibited similar structural features, such as *VmSWEET1.1* and *VmSWEET1.2*, *VmSWEET2.1* and *VmSWEET2.2*, *VmSWEET10.1* and *VmSWEET10.2*, and *VmSWEET12* and *VmSWEET14*, with comparable intron and exon numbers and CDS lengths. Additionally, *VmSWEETs* in Clades II and IV exhibited the same exon count of 4.

Chromosomal Localization and Duplication Analysis of *VmSWEETs*

Gene duplication events were the main drivers of *SWEET* gene family expansion and these included segmental and tandem duplications. Tandem replication occurs within regions of chromosome recombination and results in the formation of gene family members that are typically closely arranged on the same chromosome, thereby constituting a gene cluster with homologous sequences and similar functionalities. However, genes arising from segmental duplication are widely separated and sometimes located on distinct chromosomes. Chromosomal localization and synteny analysis were conducted to study the repetitive events in the *SWEET* gene family. As shown in File S10, 13 *VmSWEET* genes were unevenly distributed over the cranberry chromosomes, except for chromosomes 7, 8, 10, and 11. Chromosome 5 exhibited the

highest number of genes mapped, which highlights the proximity of *VmSWEET10.2* and *VmSWEET12*. Chromosomes 1 and 4 contained two *VmSWEET* genes each. Notably, the distance between *VmSWEET10.1* and *VmSWEET13* on chromosome 4 was remarkably short. Chromosomes 2, 3, 6, 9, and 12 each contained just one *VmSWEET* gene. Additionally, synteny relationships were analyzed to investigate potential evolutionary mechanisms in the *VmSWEET* gene family. The result showed synteny existed in *VmSWEET14* located on chromosome 1 and *VmSWEET10.1* located on chromosome 4, as well as *VmSWEET4* located on chromosome 3 and *VmSWEET5* located on chromosome 5, indicating two pairs of segmental duplicated events in the evolution of cranberry (Figure 4).

Promoter *cis*-acting Elements Analysis of *VmSWEET* Genes

To investigate the potential regulatory factors in *VmSWEET* genes, promoter *cis*-regulatory elements were predicted using PlantCARE. A total of 79 *cis*-acting elements were identified in the promoter regions of cranberry *SWEET* genes (Figure 5). Besides the necessary components for normal transcriptional activity, such as CAAT and TATA elements, the rest were related to plant hormone, light responsive, growth and development, and stress responses. The growth and development responsive elements included meristem expression (CAT-box), HD-Zip1/HD-Zip3 (differentiation of palisade mesophyll cells), MSA-like (cycle regulation), and RY-element (seed-specific regulation). The stress responsive elements included ARE (anaerobic induction responsive element), MBS/MYC (drought stress responsive element), LTR (low-temperature responsive element), WUN-motif (wound-responsive element), and MYB/TC-rich repeats (defense and stress responsive elements). The hormone responsive elements included TCA-element/AuxRR-core (salicylic acid responsive element), TGA-element (auxin responsive element), ABRE (abscisic acid responsive element), TGACG-motif/CGTCA-motif (methyl-jasmonate responsive element), GARE-motif/P-box/TATC-box (gibberellin responsive element), and ERE (ethylene responsive element). The number of light responsive elements were the least common and included G-box/GT1-motif (light responsive element) and circadian (circadian rhythm regulatory cis-acting elements).

Expression Profiles of *VmSWEET* Genes in Different Tissues and Fruit Development

Stages of Cranberry

Spatiotemporal expression patterns of 13 *VmSWEET* genes were determined by qRT-PCR to investigate the functions of *VmSWEET* genes in cranberry growth and fruit ripening. As illustrated in Figure 6, *VmSWEET4*, *VmSWEET5*, *VmSWEET10.1*, *VmSWEET12,* and *VmSWEET14* demonstrated significantly higher expression levels in flowers compared to roots, stems, and leaves. It was noteworthy that *VmSWEET10.1* displayed the highest expression among these 5 genes. The expression of 4 *VmSWEET* genes (*VmSWEET2.1*, *VmSWEET2.2*, *VmSWEET13*, and *VmSWEET16*) was predominantly observed in the upright and runner stems. Specifically, the expression of *VmSWEET16* in upright and runner stems was 6–23 fold and 10–42 fold higher than other tissues respectively. Although *VmSWEET13* exhibited higher expression in upright stems compared to runner stems, no statistically significant difference was observed between the two types of stems. *VmSWEET3* and *VmSWEET10.2* exhibited similar expression patterns, with significantly higher expression in upright and runner leaves compared to other tissues. *VmSWEET1.2* had the highest relative expression, not only in runner leaves but also in flowers. However, its expression was lower than that of *VmSWEET3* and *VmSWEET10.1*, which were specifically expressed in flowers and runner leaves. No *VmSWEET* exhibited specific expression in the roots. *VmSWEET16* had the highest expression among all members in the roots, but the level of expression remained extremely low.

The expression patterns of *VmSWEET* genes were different at the four distinct stages (Figure 7). *VmSWEET1.1*, *VmSWEET1.2*, *VmSWEET5*, *VmSWEET10.2*, *VmSWEET13*, and *VmSWEET14* exhibited similar expression profiles, which were characterized by an initial upregulation (S1–S2) followed by a subsequent downregulation during fruit development (S3–S4), with a peak value during S2 that was significantly higher than in other stages. It was notable that the expression of *VmSWEET14* was the highest among all members. Compared with the fruit at S2, significant decreases in *VmSWEET14* expression of 78.43% and 94.90% occurred at S3 and S4, respectively. In contrast, *VmSWEET4*, *VmSWEET10.1*, and *VmSWEET12* had “high-low-high” patterns of expression. Among them, *VmSWEET10.1* and *VmSWEET12* exhibited weak

expression throughout S2. *VmSWEET4* exhibited a significant decrease in expression from S1–S2, followed by a significant increase upon S4, but no significant differences were observed between the late stages. Additionally, the expression of *VmSWEET2.1*, *VmSWEET2.2*, *VmSWEET3*, and *VmSWEET16* gradually declined during fruit development. Compared with the fruit at S1, *VmSWEET16*, with the highest expression level among the four genes, experienced significant reductions of 46.96%, 91.94%, and 94.45% in the fruit at S2, S3 and S4, respectively.

Expression Profile of *VmSWEET* Genes in Response to Abiotic Stress

In vitro cranberry plantlets received abiotic stress treatments (osmotic, saline, saline-alkaline and aluminum) to investigate the differential expression patterns of *VmSWEET* genes (Figure 8). Under osmotic conditions, the most prominent finding was that *VmSWEET10.2* and *VmSWEET14* exhibited the highest expression levels in the *SWEET* gene family, while displaying contrasting trends. *VmSWEET10.2* increased sharply within the first 9 h and subsequently decreased, and significant differences were observed among different treatments, except for 12 h and 24 h. Conversely, the expression of *VmSWEET14*, which was initially low, decreased within the first 6 h and subsequently significantly upregulated until reaching peak value at 24 h. Notably, peak expression was 14.5-fold higher than that of the control. Other genes exhibited relatively low expression and little fluctuation. For instance, the expression levels of *VmSWEET1.2*, *VmSWEET2.1*, *VmSWEET10.1*, and *VmSWEET12* were downregulated, whereas *VmSWEET5, VmSWEET2.2*, and *VmSWEET13* were upregulated. Under the saline stress, *VmSWEET10.2* and *VmSWEET14* exhibited high expression with an upward trend over time. However, their response times differed. A significant upregulation of *VmSWEET10.2* was observed within the first 3 h, after which there was no significant difference in expression at 6, 9, and 12 h compared with that at 3 h. However, the expression of *VmSWEET14* did not exhibit a significant increase until 24 h, at which time it was 13.5 times higher than that of the control. During the saline-alkaline treatment, *VmSWEET10.2* consistently exhibited the highest expression among all genes and displayed significant upregulation, with expression progressively increasing 5.9, 6.6, 8.2, 10.1, and 11.0-fold over time. Furthermore, significant

differences were observed between different treatments. In response to aluminum stress, *VmSWEET10.2* exhibited slightly increased expression within the first 9 h, and then peaked at 12 h with an expression level 20-fold higher than that of the control. Subsequently, a significant decrease was observed. Other genes were expressed at low levels and were continuously downregulated under aluminum stress, and these genes included *VmSWEET1.2*, *VmSWEET2.1*, *VmSWEET2.2*, *VmSWEET4*, *VmSWEET5, VmSWEET12*, and *VmSWEET16.*

DISCUSSION

Characters and Function of SWEET Family Genes in Cranberry

The SWEET gene family is widely present in plants, animals, fungi, and bacteria, and these transporters mediate bidirectional cross-membrane movement of sugars through an alternating access mechanism to regulate various life activities (Eom et al., 2015; Latorraca et al., 2017). The *SWEET* gene family has been extensively characterized in many plant species due to the popularity of high-throughput techniques, such as genomic and transcriptome sequencing. In general, plant genome ananlyses have revealed an approximate presence of 20 *SWEET* paralogs (Anjali et al., 2020). In this study, 13 *SWEET* genes were identified in cranberry via a comprehensive genome-wide investigation. The number of *VmSWEETs* is comparable to the number in tea (13) (Wang et al., 2018) and blueberry (23) (Unpublished), but less than the number in soybean (52) (Patil et al., 2015), oilseed rape (68) (Jian et al., 2016), or wheat (108) (Gautam et al., 2019). Variation in the scale of gene family among different species can be attributed to gene duplication events, which play a vital role in the evolution of gene families by providing the basic materials necessary for the emergence of new genes and enabling the acquisition of novel functions (Yin et al., 2013). In rice, the monosaccharide transporter gene family is significantly large (with 65 genes), and two subfamilies have expanded greatly via tandem duplications (Johnson and Thomas, 2007). Recent studies have indicated that the *SWEET* gene family expanded throughout its evolution (Patil et al., 2015). A tandem duplication event is defined as the presence of two or more genes within a chromosomal region of 200 kb (Holub, 2001), so we speculate that *VmSWEET* gene duplication involved two tandem duplications on

chromosomes 4 and 5 (*VmSWEET10.1/13* and *VmSWEET10.2/12*). Descendant genes, as a result of segmental duplications, are far apart and even located on different chromosomes. According to synteny analysis, *VmSWEET* gene duplication involved two segmental duplications on chromosomes1, 4, 3, and 5 (*VmSWEET4/VmSWEET5* and *VmSWEET10.1/VmSWEET14*), which is less than large-scale gene duplication events reported in soybeans (Schmutz, et al., 2010). Segmental duplication is prevalent in plants due to their diploidized polyploid nature, which results in the retention of multiple duplicated chromosomal blocks within genomes (Cannon et al., 2004). Cranberry is a diploid plant and is reported to have gone through a severe genetic bottleneck, possibly during the Pleistocene (Bruederle, et al.,1996; Stewart and Nilsen, 1995). In contrast to other higher plants that have undergone genome duplication, such as wheat, oilseed rape, and soybeans, the lack of a diploidized polyploid may be the primary reason for the limited number of *VmSWEETs* in cranberry. Furthermore, the gene pairs originating from gene duplications were found to belong to Clades II and III, suggesting that tandem duplication and segmental duplication have played a significant role in the expansion of Clades II and III in cranberry.

Based on the evolutionary relationships inferred from phylogenetic analysis, the *VmSWEET* genes were categorized into four distinct clades (Figure 2), that were determined by SWEET preferences for monosaccharides or disaccharides. Those in Clades I and II specifically transport hexose, Clade III members display preferentially transport sucrose over glucose, and Clade IV members specifically transport fructose (Eom et al., 2015). According to their putative subcellular localizations and assigned clades, we speculate that tonoplast-localized *VmSWEET1.1* and *VmSWEET2.2* mediated transmembrane transport of hexoses, such as glucose and fructose, while *VmSWEET2.1*, *VmSWEET3*, *VmSWEET4*, and *VmSWEET5* facilitated the transport of hexoses across the plasma membrane. *VmSWEET10.1*, *VmSWEET10.2*, *VmSWEET12*, *VmSWEET13*, and *VmSWEET14*, putatively located on the plasma membrane, may efflux sucrose from the cytosol into the apoplast. Additionally, *VmSWEET16* in Clade IV may control the flux of fructose across the plasma membrane. The precise subcellular

localization and substrate specificity of *VmSWEETs* requires further research. To understand substrate specificity of SWEETs, crystal structure and bioinformatic analyses were conducted in bacterial SemiSWEETs (Wang et al., 2014). A fascinating finding was that the size of the pocket presented above the center of the transporter protein played a critical role in determining substrate specificity. A larger substrate-binding pocket with a spacious substrate-binding cavity may facilitate the transport of disaccharides (such as sucrose) and monosaccharides (such as glucose and fructose), while smaller pockets with a restricted substrate-binding cavity can only hold monosaccharides (Wang et al., 2014). In higher plants, a conserved asparagine pair (N77 and N197) surrounds the binding pocket at the equivalent positions in OsSWEET2b, and S54 on THB1 and W176 on THB2 have also been implicated in the transportation capacity of AtSWEET1 (Tao et al., 2015). In our study, N77 was conserved in all VmSWEETs, while the conservation of N197 was observed in the majority of VmSWEETs, except for an E substitution at the equivalent positions of VmSWEET13 and VmSWEET14. In addition, most VmSWEETs contained W at the positions equivalent to W176, but exceptions were observed in VmSWEET2.1, 13, and 14, where W was substituted with aromatic residue F or Y (File S7). Nevertheless, this substitution may not affect the transport activity of VmSWEETs, because the presence of one aromatic residue in THB2, rather than THB1, was important for transport activity (Tao et al., 2015). We speculate that four amino acid residues of VmSWEET can still interact with sugar molecules via H-bonding or aromatic ring stacking. Phosphorylation sites were also crucial for proteins and their transportation and function. Lately, research has shown that the carboxy-cytosolic regions of AtSWEET11 and 12 were rapidly phosphorylated by SnRK2 protein kinases upon drought, which enhances the oligomerization and sucrose transport activity of SWEETs (Fatima et al., 2022). In our study, four S, two Y and one T phosphorylation site were observed in the conserved domains of VmSWEETs (File S7). These conserved phosphorylation sites were also identified within CsSWEETs of watermelon (Xuan et al., 2021). We suggest that the seven phosphorylation sites are probably related to signal recognition and transduction functions of *VmSWEETs* that enable responses to multiple types of stress.

The SWEET gene family in plants is highly conserved, with accurate functioning and stability maintained by seven TMDs and two MtN3/saliva domains (Chen et al., 2010). Conserved structural domains analysis revealed that 11 VmSWEET proteins (about 85%) contained two complete MtN3/saliva domains, while VmSWEET2.2, VmSWEET13, and VmSWEET14 only exhibited one MtN3/saliva domain with 5–6 TMDs (Figure 3c and Table 1). Despite possessing two MtN3/saliva domains, both VmSWEET5 and VmSWEET6 exhibited a discrepancy in the number of transmembrane helices predicted by TMHMM 2.0 Tool, with only six instead of the expected seven. Similar observations of SWEET members containing one or one and a half MtN3/saliva domains have been reported in other species, such as walnut (Jiang et al., 2020) and watermelon (Xuan et al., 2021). As a SWEET protein with two MtN3/saliva domains in eukaryotes was considered to be due to replication or horizontal gene transfer from the one MtN3/saliva domain of prokaryotes (Xuan et al., 2013), we hypothesized that there were incomplete *VmSWEET* genes, *VmSWEET2.2*, *VmSWEET13*, and *VmSWEET14* were generated through tandem and domain duplication events throughout the course of evolution. Phylogenetic analyses also supported the results of gene structure analysis. Minimal variation in the quantity of introns and exons was observed within each clade, except for *VmSWE*ET3 in Clade I. The gene pairs in the sister branches generally had the same number of introns and exons, suggesting that molecular features of *SWEET* genes were conserved during evolution. Introns serve as hallmark features of eukaryotic genes and contribute to genetic diversity through alternative splicing (Jeena et al., 2019). There is a difference in the number of introns between unicellular and multicellular organisms. For example, fungi or oomycetes have no or few introns, while plants contain 4–5 introns per gene (Hu et al., 2016). In cranberry, *VmSWEETs* contain 3–8 introns, which is similar to tomato (Feng et al., 2015) and watermelon (Xuan et al., 2021). Conserved motif analyses revealed that all VmSWEET proteins contained motif 2, motif 4 and motif 5, suggesting their crucial role in maintaining structure and functioning. Additionally, gene members within the same clade exhibited similar motif arrangement, while there were obvious differences in the motif composition among different clades. For instance, motif 7 was uniquely

present in members of Clade IV and motif 8 was specifically present in members of Clade I and III. These specific motifs were not available in members of the remaining two clades (Figure 3). These results were consistent with other plant systems, such as rice (Yuan et al., 2013), banana (Miao et al., 2017) and wheat (Gautam et al., 2019).

Gene expression and functional divergence of SWEETs in Cranberry

The expression profile of a gene is closely related to its function. Previous studies revealed the importance SWEETs in plant growth and development. In this study, the expression patterns of 13 *VmSWEET* genes were analyzed in roots, stems, leaves, flowers and different development stages of fruit to explore the potential function of *SWEET* genes in cranberry. The results revealed distinct expression patterns for *VmSWEET* genes in different tissues (Figure 6). Notably, *VmSWEET3* and *VmSWEET10.2* were highly expressed in leaves of upright and runner stems. *VvSWEET1*, the homolog of *VmSWEET3*, was previously shown to be expressed in young and mature leaves of grape (Chong et al., 2014), and to have an expression pattern in vegetative organs that was similar to *VmSWEET7*. *AtSWEET11* and *AtSWEET12,* which were clustered in Clade III with *VmSWEET2*, were highly expressed in leaves and played crucial roles in sugar efflux from mesophyll cells to the apoplast in Arabidopsis, while the *atsweet11;12* mutant line accumulated starch in leaves, and radio tracer efflux from petioles was reduced (Chen, et al., 2012). Because sucrose is the predominant photoassimilate that is transported in Clade III, it has been hypothesized that *VmSWEET10.2* plays a role in the phloem loading of photoassimilates in cranberry leaves. The *SWEET* genes expressed in flowers primarily participate in reproductive development and nectar secretion (Eom et al., 2015; Wen et al., 2022; 2022; Lin et al., 2014). *VmSWEET10.1* exhibited the highest transcriptional level in flowers. Phylogenetic analysis showed that *OsSWEET11*, *AtSWEET13,14*, and *VmSWEET10.1* belonged to the same clade. Among them, *OsSWEET11* has been reported to play a role in rice pollen development, *ossweet11* knockouts produced defective pollen grains and had a lower fertility rate (Chu et al., 2006; Yang et al., 2006; Yuan et al., 2009). Consistent results were reported in Arabidopsis, *AtSWEET13* and *AtSWEET14* were expressed in the anther wall, which facilitated sucrose efflux

into locules to support pollen development and maturation. Consequently, an *atsweet13;14* mutant displayed decreased viability and germination of pollen (Sun et al., 2013; Wang et al., 2022). Therefore, *VmSWEET10.1* may play an important role in cranberry reproductive development. From source to sinks, the long-distance transportation of photosynthetic products in stems generally follows the symplastic route. However, when stems function as storage organs, SWEETs may be involved in unloading and storage of photosynthates in the stem. For example, *SsSWEET4a/4b* were mainly expressed in the stems of sugarcane and were forecasted to be involved in sugar transportation within the stalk (Hu et al., 2018). Although the stems of cranberry do not serve as storage sinks like those in sugarcane, the expression of *VmSWEET16* in upright stems and runner stems was higher than other tissues. To understand the role of SWEETs in plant stems, further functional validation is required. No *VmSWEET* genes were specifically expressed in the roots, because roots might not serve as an important storage sink during the sampling period.

Fruits are the most important storage organs in horticultural crops, their yield and quality are determined by the content of sugar. As a novel sugar transporter that function independently of energy or pH, SWEET proteins have attracted attention in the context of phloem unloading, transport and storage of sugars during fruit development. In jujube, the expression of *ZjSWEET11* and *ZjSWEET18* gradually increased during fruit development, peaking at complete maturity (Yang et al., 2023). In apple, there was a significant association between the expression of *MdSWEET2e,9b,15* and fruit sugar content. In particular, *MdSWEET15a* and *MdSWEET9b* accounted for a large proportion of phenotypic variation in sugar content (Zhen et al., 2018). In grape, *VvSWEET10* was strongly expressed in ripening fruit, and *VvSWEET10* overexpression in grapevine calli and tomatoes resulted in a significant increase in glucose, fructose and total sugar (Zhang et al., 2019b). In developing tomato fruits, *SlSWEET15* expression was notably elevated, while fruit sizes and weights were significantly reduced upon elimination of *SlSWEET15* (Ko et al., 2021). Together, the above results all indicate that *SWEET* genes exert a positive regulatory effect on fruit development and ripening. Conversely, silencing *SlSWEET7a* or *SlSWEET14* in

tomato increased plant height, fruit size, and sugar content (Zhang et al., 2021). In our study, expression of *VmSWEETs* changed dynamically during fruit development, with distinct sets of *VmSWEETs* being expressed in the young and mature fruits (Figure 7). For instance, *VmSWEET14* and *VmSWEET16* were highly expressed during the young fruit stage and expansion stage, respectively, whereas *VmSWEET4* was highly expressed during the color change and the maturity stage. We speculate that *VmSWEET4* positively regulates fruit development and ripening, while *VmSWEET14* and *VmSWEET16* may play roles that are similar to with *SlSWEET7a* and *SlSWEET14*. Suppressing the two genes could be a potential strategy for enhancing the sugar content of cranberry fruit.

Abiotic stress frequently impedes plant growth and development, and ultimately inhibits plant productivity and quality. Damage to sucrose phloem transport and source/sink relationships is an important factor (Lemoine et al., 2013). Plants have evolved sensory and response mechanisms to cope with various environmental stresses. Sugars serve as osmo-protectants and molecular switches, and their production and distribution are crucial physiological processes that are induced by various stresses (Saddhe et al; 2021). Previous studies found that SWEET proteins can regulate the redistribution of soluble sugars under abiotic stress, but expression patterns differed. For example, in bluegrass (Zhang et al; 2020), cotton (*GhSWEET5*, *20*, *49*, *and 50*) (Li et al., 2018), tea (*CsWEET1a*, *2a*, *2c*, *3a*, *7a*, *7b*, and *10*) (Jiang et al; 2021), and wheat (*TaSWEET14g-1A* and *16a-4A*) (Gautam et al., 2019) *SWEET* genes were induced by drought or osmotic stress, while *MtSWEET2a* and *MtSWEET3c* were downregulated in *Medicago truncatula* (Hu et al., 2019). In this research, the most noticeable result was that the expression of *VmSWEET10.2* was the highest of all the *VmSWEET*s, and it was upregulated under all abiotic stress treatments (Figure 8). The expression patterns in osmotic and saline treatments were consistent with those in the homologs *AtSWEET11*, *12*, *14*, *and 15*, which have been demonstrated to respond to a variety of abiotic stresses in Arabidopsis (Durand et al., 2016; Sellami et al., 2019; Seo et al., 2011). *AtSWEET11 and AtSWEET12* were upregulated and were responsible for the transport of sucrose from the leaves to the roots in water deficit plants

(Durand et al., 2016). *AtSWEET14* was upregulated in response to high salinity (Sellami et al., 2019), and *AtSWEET15* (also known as SAG29) was significantly upregulated during senescence and abiotic stresses that included cold, salinity, and drought (Seo et al; 2011). In our study, *VmSWEET10.2* also responded to saline-alkaline and aluminum treatments, suggesting its potential roles in regulating sucrose transport and distribution under abiotic stress. Previous research demonstrated that drought and salinity induced an ABA-responsive transcription factor *OsbZIP72* to bind directly to the promoters of *OsSWEET13* and *15*, thereby activating their transcription and increasing the sucrose content in leaves and roots (Mathan, et al., 2020). Thus, we predicted that *VmSWEET10.2* might harbor a site for the ABA-responsive transcription factor in its promoter region, similar to the homologues of *OsSWEET13* and *15*. This conjecture was consistent with the presence of ABRE (abscisic acid response element) by promoter analysis, but the regulatory mechanism of sugar homeostasis in cranberry under abiotic stress requires further exploration.

According to the expression patterns of *VmSWEET* genes in different tissues and at different fruit development stages, we propose a hypothetical model for SWEETs involved in the transport and distribution of photosynthetic products in cranberry. As illustrated in Figure 9, sucrose is produced in upright and runner leaves through photosynthesis, and *VmSWEET3* participates in phloem loading of photosynthetic products in both types of leaves. Then, long-distance transport of sucrose from source to sink tissues in upright stems and runner stems is facilitated by *VmSWEET16*. *VmSWEET10.1* is likely to be implicated in pollen development in flowers, which benefits pollination and fertilization. With sucrose unloading into the fruit, *VmSWEET14* and *VmSWEET16* play an important role in the early stage of fruit growth and development, while *VmSWEET4* is responsible for the transport and accumulation of monosaccharides (hexoses) during the S2 and S3. *VmSWEET10.2* may be induced by abiotic stress to transport sucrose in roots as a signaling molecule to cope with different constraints.

CONCLUSION

In recent years, numerous important advances in the study of SWEET transporters have

been reported, but several unresolved issues persist. For instance, it remains unclear whether the members of the *SWEET* gene family in plants function independently or collectively. Additionally, the relationship between structure and function requires further exploration. Questions remain regarding how SWEET proteins are regulated and whether they are regulated at the transcriptional or translational level. In this study, 13 *VmSWEET* genes distributed on eight chromosomes were identified in cranberry. Four conserved conserved amino acid residues and seven phosphorylation sites, which might be crucial for transport, were observed in the conserved domains. These genes were classified into four clades, similar homologous genes in the topology have similarly conserved motifs and gene structures. *Cis*-acting elements of *VmSWEET* promoters were related to plant hormone, light, growth, development, and stress responses. The expression of *VmSWEETs* varied across different tissues and fruit developmental stages. *VmSWEET3, VmSWEET16*, and *VmSWEET10.1* were specifically expressed in leaves, stems, and flowers, respectively. *VmSWEET4*, *VmSWEET14*, and *VmSWEET16* played crucial roles in fruit development and ripening. *VmSWEET1.02* was the key gene involved in the response of cranberry to abiotic stress during osmotic, saline, saline-alkaline and aluminum treatments. These results provide a foundation for future studies of *VmSWEET* gene function and provide a basis for improving yield, quality, and resistance in cranberry plants.

ADDITIONAL INFORMATION AND DECLARATIONS

Funding

This research was funded by grants from JiLin Provincial Natural Science Foundation of China (202101013697JC); National college student innovation training program (202310193041); JiLin Provincial Development and Reform Commission Project (2023C0354-4); JiLin Province Science and Technology Development Plan Project (20220208099RC).

Competing Interests

All authors have read and agreed to the published version of the manuscript.

Author Contributions

- Y.W., Y.L. and Z.W. designed the research.
- M.C. and J.L. performed the experiments.
- X.J. and J.L. prepared materials.
- L.C. analyzed the data and finished the manuscript.

Data Availability

The following information was supplied regarding data availability:

The raw data is available in the Supplementary Files.

Supplemental Information

Supplemental File 1 CDS sequenses of *VmSWEET* genes in cranberry

Supplemental File 2 Promoter sequenses and *cis*-acting elements of *VmSWEET* genes in cranberry

Supplemental File 3 The amino acid sequences used to phylogenetic analyses and multiple sequence alignment

Supplemental File 4 qRT-PCR primers of *VmSWEET* genes in cranberry

Supplemental File 5 The raw data of Ct value used for qRT-PCR

Supplemental File 6 MIQE checklist

Supplemental File 7 Multiple sequence alignment of *OsSWEET2b*, *AtSWEET1* and *VmSWEETs*

Supplemental File 8 Gene structure of cranberry *SWEET* gene family

Supplemental File 9 The different conserved motifs in cranberry

Supplemental File 9 Chromosome mapping of *SWEET* genes in cranberry

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Figure legends:

Figure 1. Different cranberry tissues and fruit at different stages of development.

Figure 2. Phylogenetic analysis of the *SWEET* gene family in four species. Different colors of the outer ring represent four different *SWEET* clades. Before the gene name, green triangles represent *Arabidopsis thaliana*, black boxes represent *Oryza sativa*, red dots represent *Vitis Vinifera*, blue stars represent *Vaccinium macrocarpon*. The evolutionary history was inferred using the neighbor joining method with 1,000 replicates.

Figure 3. Conserved motifs and conserved structural domains of the cranberry *SWEET* gene family. (a) The phylogenetic tree of *VmSWEETs*. (b) The conserved motifs of *VmSWEET* members. The colored squares correspond to nine different conserved motifs. (c) Conserved structural domains of *VmSWEET* genes. The green square represents MtN3-slv domain, yellow square represents PQ-loop domain, pink square represents transmembrane-domain, blue square represents low-complexity-region. X-axis represents the number of amino acids.

Figure 4. Duplication analysis of *SWEET* gene family in cranberry. Each box represents a scaffold, the number beside the box represents the position on chromosome. Gray lines indicate all synteny blocks in the cranberry genome, and the red lines indicate the duplication of *VmSWEET* gene pair.

Figure 5. Promoter *cis*-acting elements of *VmSWEETs*. *Cis*-elements related to stress responses: LTR, GC-motif, ARE, WUN-motif, CCGTCC motif, DRE, DRE 1, DRE core, MBS, TC-rich repeats, box s, and AP-1. *Cis*-elements related to growth and development: AT-rich sequence, AT-rich element, CCGTCC-box, AE-Box, AC II, AC I, RY-element, MBSI, E2Fb, CAT-box, circadian, HD-Zip 1, HD-Zip 3, MSA-like, CCAAT box, and O2-site. Light-responsive elements: MRE, Box-4, G-box, GT1-motif, GA-motif, ATCT-motif, LAMP-element, TCT-motif, chs-CMA2a, chs-CMA1a, chs-unit1 m1, I-box, GATA-motif, Gap-box, and TCCC-motif. *Cis*-elements related to hormone: GARE-motif, P-box, TATC-box, ABRE 4, ABRE 3a, ABRE 2, ABRE, F-box, AuxRR-core, TGA-element, CGTCA-motif, TGACG-motif, ERE, and TCA-element. Unknown elements: AT~TATA box, TATA, CTAG-motif, CARE, TCA, dOCT, as-1, A-Box, MYB recognition site, Myb-binding site, MYB, Myb, MYB-like sequence, MYC, Myc, STRE, AAGAA motif, box III, and box II. Core promoter: TATA-box and CAAT-box.

Figure 6. Expression analysis of *VmSWEET* genes in different tissues of cranberry. Rt, Roots; Ur, Upright stems; Rn, Runner stems; UrL, Leaves of upright stem; RnL, Leaves of runner stem; F, Flowers. Each value is the mean of three biological replicates, and the height of the vertical bar represents the standard deviation. Different lowercase letters represent the significant statistical difference between the different groups at $P < 0.05$. The same as bellow.

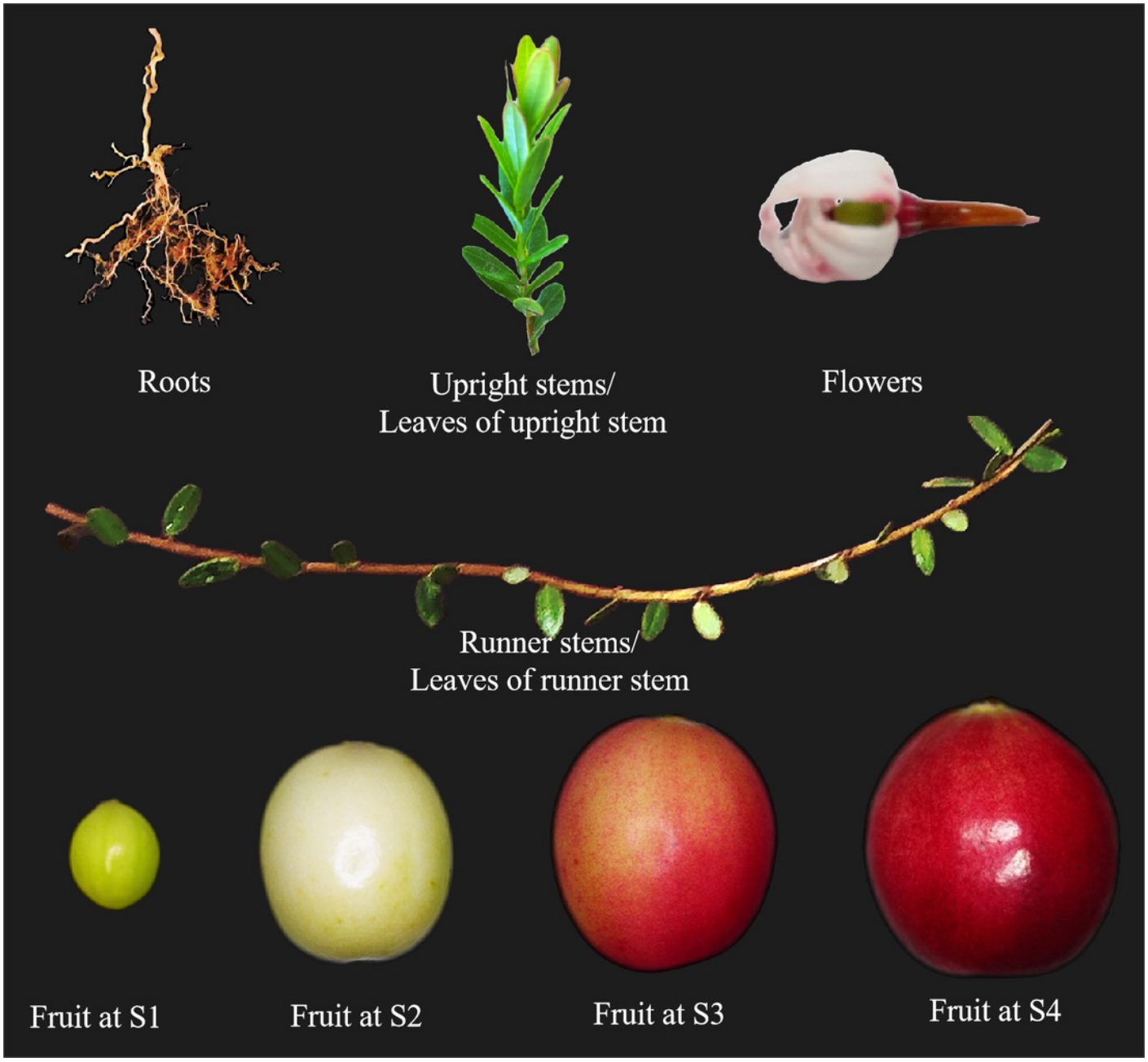
Figure 7. Expression analysis of *VmSWEET* genes in cranberry fruits at different developmental stages. The X-axis labels indicate cranberry fruits at different developmental stages. S1, Young fruit stage; S2, Fruit expansion stage; S3, Color turning stage; S4, Maturity stage.

Figure 8. Gene expression heatmap of the *VmSWEET* genes in cranberry leaves under various abiotic stresses. The X-axis labels indicate the time points at which samples were collected (0, 3, 6, 9, 12, and 24 h) during the various stress treatments. Red and blue correspond to strong and weak expression of the *VmSWEE* genes, respectively.

Figure 9. Schematic model of preferential gene expression and proposed roles of *VmSWEET*s in different cranberry tissues and fruit development stages. This figure shows the representative genes highly expressed in each tissue and fruit development stage during the sugar accumulation stage, i.e. those likely implicated in the process of sucrose transportation from the leaf to other plant organs, such as the flower, stem, and fruit. The gene names under the tissue's name indicate that they are highly expressed in those tissues.

Figure 1

Different cranberry tissues and fruit at different stages of development.



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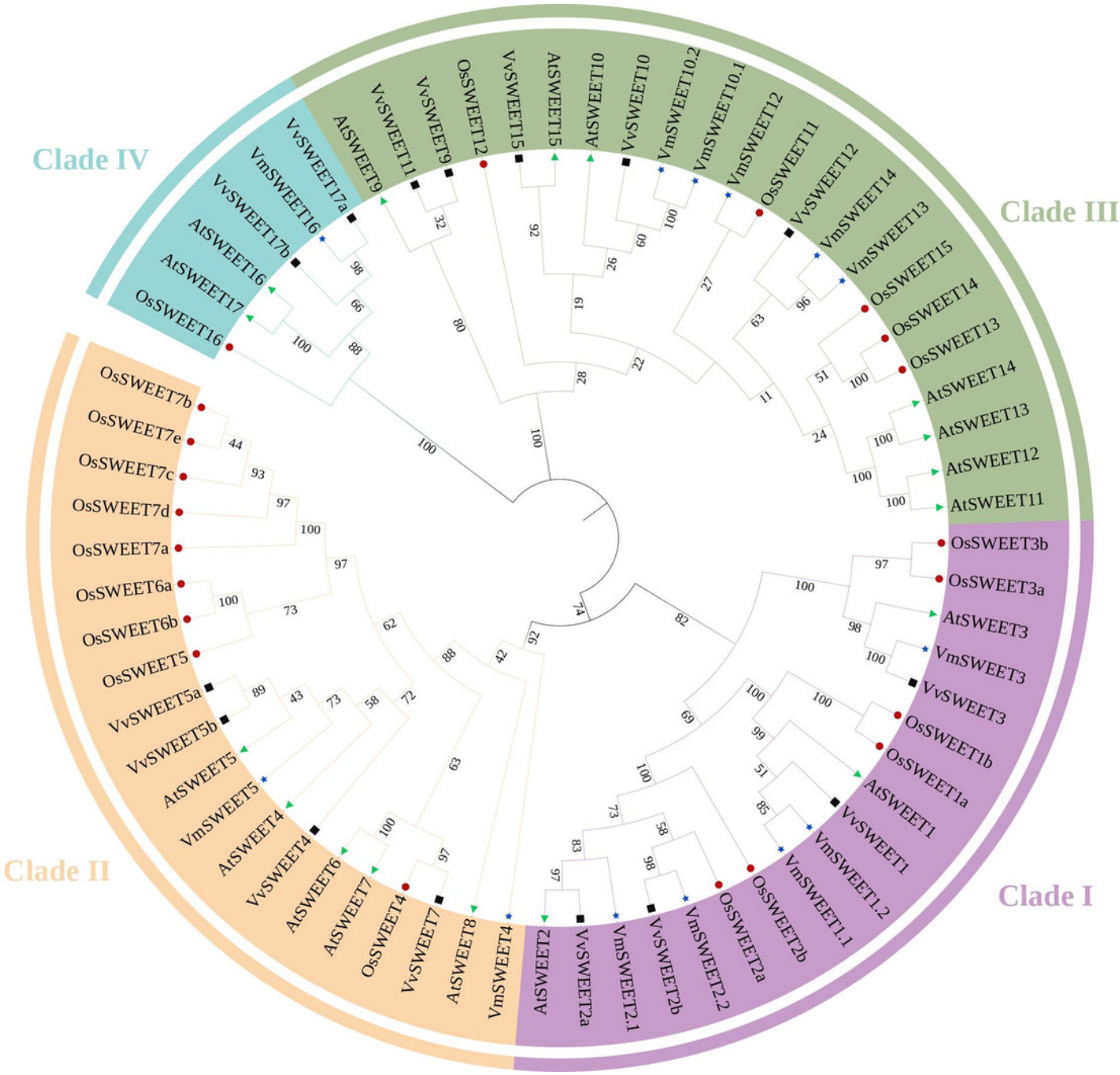
Clade IV
Clade III
Clade II
Clade I
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VmSWEET16
VvSWEET17b
AtSWEET16
AtSWEET17
OsSWEET16
AtSWEET9
VvSWEET11
VvSWEET9
OsSWEET12
VvSWEET15
AtSWEET15
AtSWEET10
VvSWEET10
VmSWEET10.2
VmSWEET10.1
VmSWEET12
OsSWEET11
VvSWEET12
VmSWEET14
VmSWEET13
OsSWEET15
OsSWEET14
OsSWEET13
AtSWEET14
AtSWEET13
AtSWEET12
AtSWEET11
OsSWEET3b
OsSWEET3a
AtSWEET3
VmSWEET3
VvSWEET3
OsSWEET1b
OsSWEET1a
AtSWEET1
VvSWEET1
VmSWEET1.2
VmSWEET1.1
OsSWEET2b
OsSWEET2a
VmSWEET2.2
VvSWEET2b
VmSWEET2.1
VvSWEET2a
AtSWEET2
VmSWEET4
AtSWEET8
VvSWEET7
OsSWEET4
AtSWEET7
AtSWEET6
VvSWEET4
AtSWEET4
VmSWEET5
AtSWEET5
VvSWEET5b
VvSWEET5a
OsSWEET5
OsSWEET6b
OsSWEET6a
OsSWEET7a
OsSWEET7d
OsSWEET7c
OsSWEET7e
OsSWEET7b

Figure 3

Conserved motifs and conserved structural domains of the cranberry *SWEET* gene family.

(a) The phylogenetic tree of *VmSWEETs* . (b) The conserved motifs of *VmSWEET* members. The colored squares correspond to nine different conserved motifs. (c) Conserved structural domains of *VmSWEET* genes. The green square represents MtN3-slv domain, yellow square represent s PQ-loop domain, pink square represents transmembrane- domain , blue square represents low-complexity-region. X-axis represents the number of amino acids.

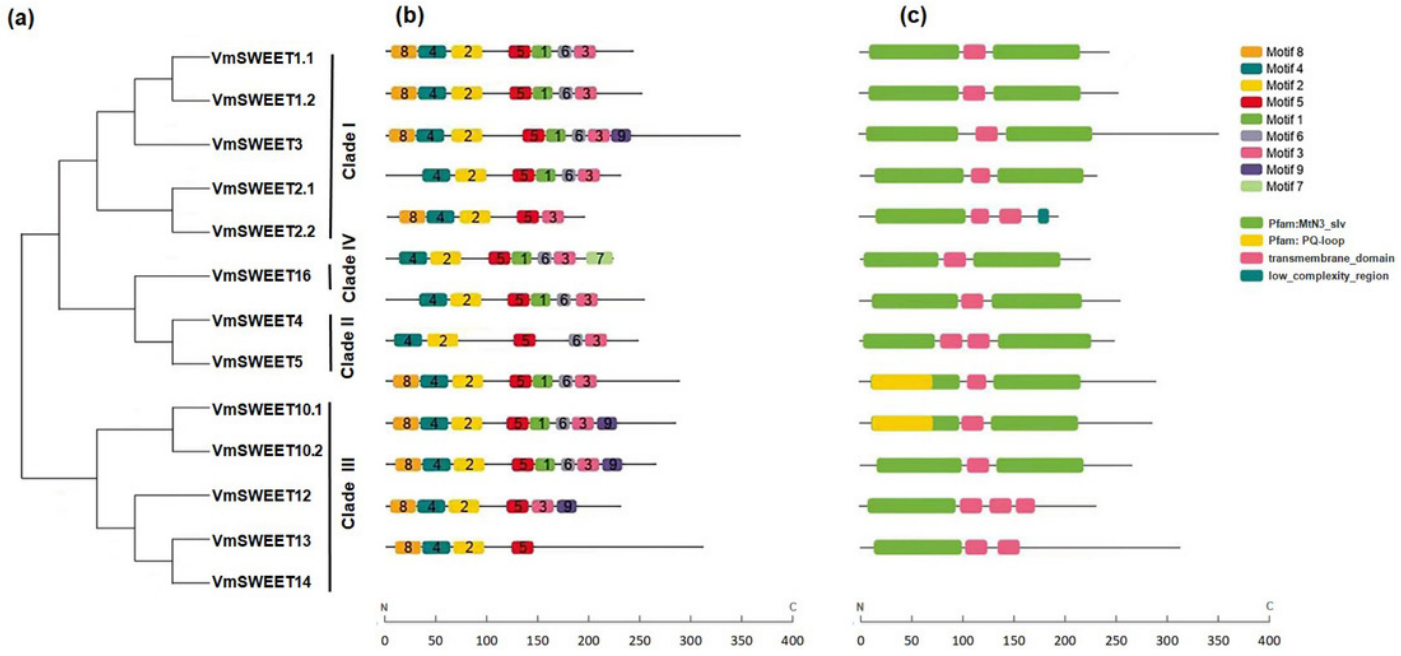

Figure 4

Duplication analysis of *SWEET* gene family in cranberry.

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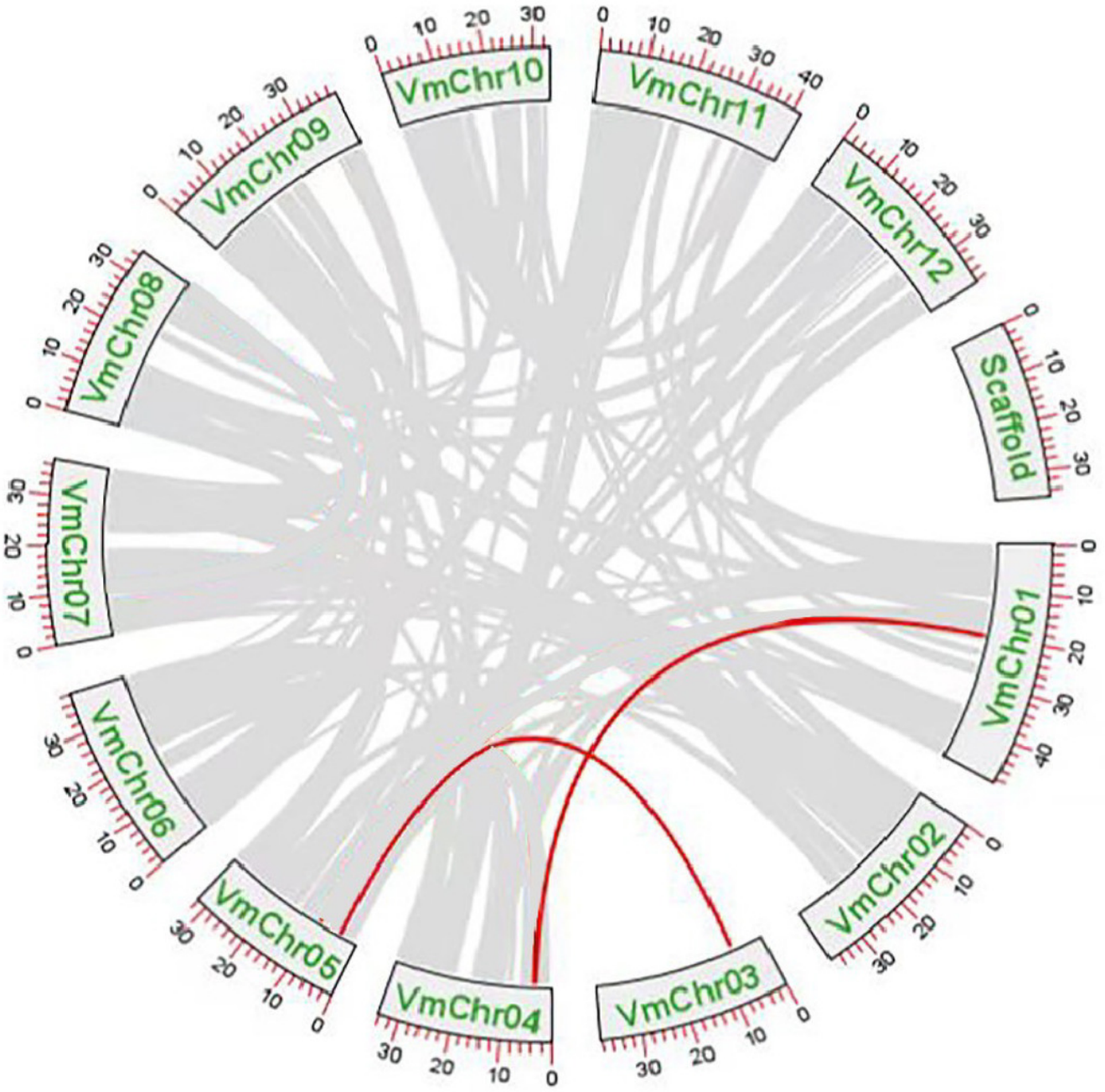
VmChr10
VmChr11
VmChr12
Scaffold
VmChr01
VmChr02
VmChr03
VmChr04
VmChr05
VmChr06
VmChr07
VmChr08
VmChr09

Figure 5

Promoter *cis*-acting elements of *VmSWEETs*.

Cis-elements related to stress responses: LTR, GC-motif, ARE, WUN-motif, CCGTCC motif, DRE, DRE 1, DRE core, MBS, TC-rich repeats, box s, and AP-1. *Cis*-elements related to growth and development: AT-rich sequence, AT-rich element, CCGTCC-box, AE-Box, AC II, AC I, RY-element, MBSI, E2Fb, CAT-box, circadian, HD-Zip 1, HD-Zip 3, MSA-like, CCAAT box, and O2-site. Light-responsive elements: MRE, Box-4, G-box, GT1-motif, GA-motif, ATCT-motif, LAMP-element, TCT-motif, chs-CMA2a, chs-CMA1a, chs-unit1 m1, I-box, GATA-motif, Gap-box, and TCCC-motif. *Cis*-elements related to hormone: GARE-motif, P-box, TATC-box, ABRE 4, ABRE 3a, ABRE 2, ABRE, F-box, AuxRR-core, TGA-element, CGTCA-motif, TGACG-motif, ERE, and TCA-element. Unknown elements: AT~TATA box, TATA, CTAG-motif, CARE, TCA, dOCT, as-1, A-Box, MYB recognition site, Myb-binding site, MYB, Myb, MYB-like sequence, MYC, Myc, STRE, AAGAA motif, box III, and box II. Core promoter: TATA-box and CAAT-box.

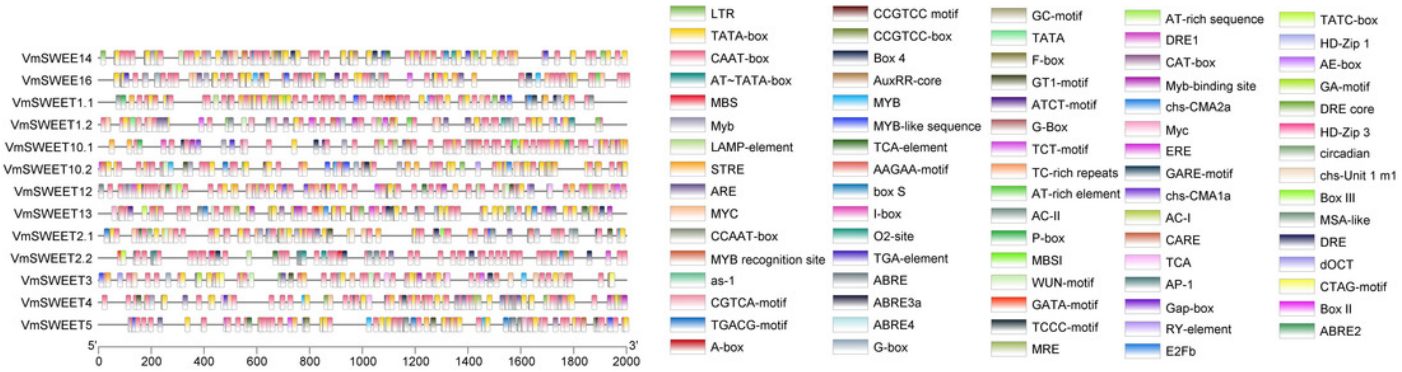

Figure 6

Expression analysis of *VmSWEET* genes in different tissues of cranberry.

Rt, Roots; Ur, Upright stems; Rn, Runner stem s; UrL, Leaves of upright stem ; RnL, Leaves of runner stem ; F, Flowers. Each value is the mean of three biological replicates, and the height of the vertical bar represents the standard deviation. Different lowercase letters represent the significant statistical difference between the different groups at $P < 0.05$. The same as bellow.

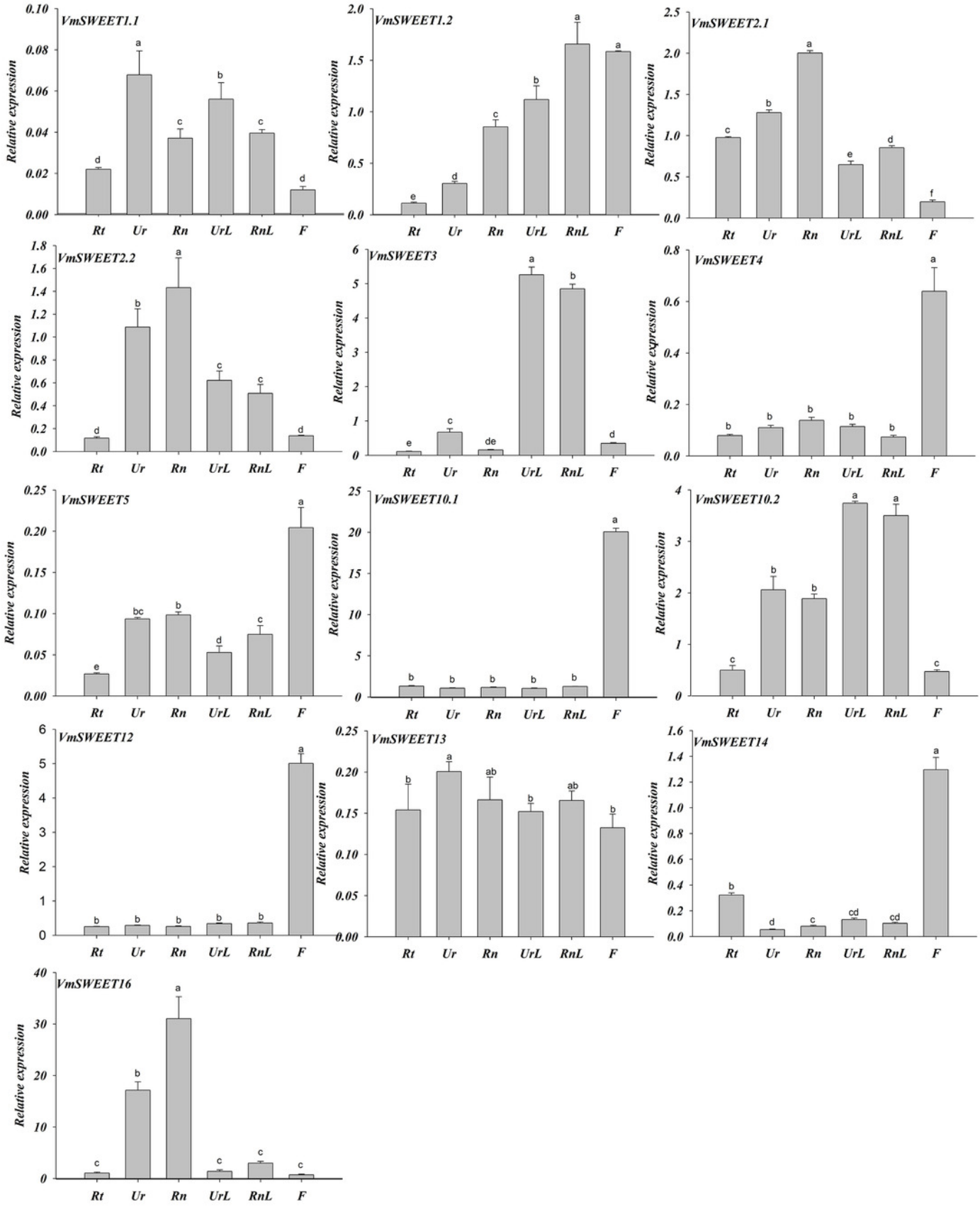
VmSWEET1.1
VmSWEET1.2
VmSWEET2.1
VmSWEET2.2
VmSWEET3
VmSWEET4
VmSWEET5
VmSWEET10.1
VmSWEET10.2
VmSWEET12
VmSWEET13
VmSWEET14
VmSWEET16
Relative expression
Rt
Ur
Rn
UrL
RnL
F

Figure 7

Expression analysis of *VmSWEET* genes in cranberry fruits at different developmental stages.

The X-axis labels indicate cranberry fruits at different developmental stages. S1, Young fruit stage; S2, Fruit expansion stage; S3, Color turning stage; S4, Maturity stage.

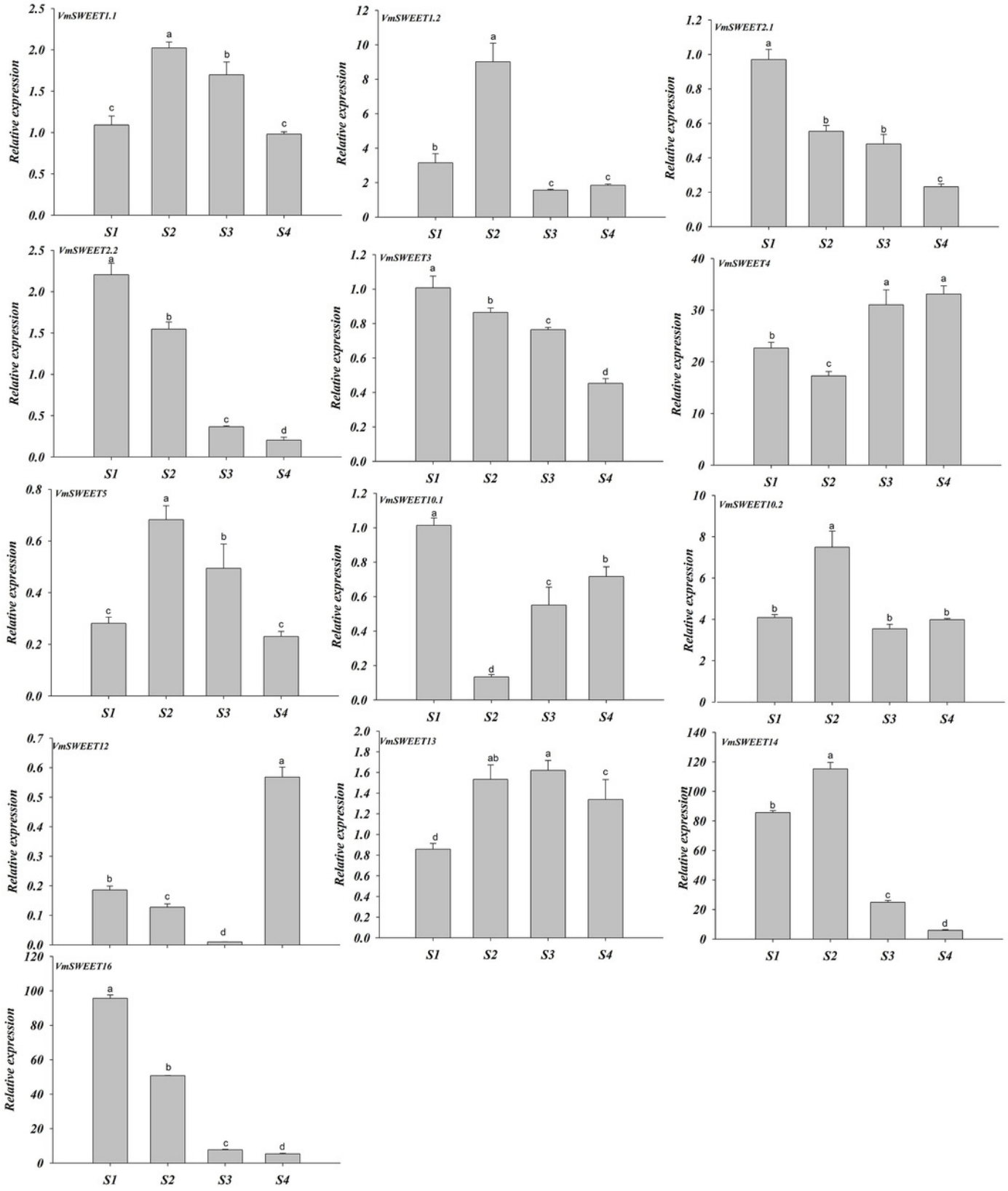
VmSWEET1.1
VmSWEET1.2
VmSWEET2.1
VmSWEET2.2
VmSWEET3
VmSWEET4
VmSWEET5
VmSWEET10.1
VmSWEET10.2
VmSWEET12
VmSWEET13
VmSWEET14
VmSWEET16
Relative expression
S1
S2
S3
S4

Figure 8

Gene expression heatmap of the *VmSWEET* genes in cranberry leaves under various abiotic stresses.

The X-axis labels indicate the time points at which samples were collected (0, 3, 6, 9, 12, and 24 h) during the various stress treatments. Red and blue correspond to strong and weak expression of the *VmSWEE* genes, respectively.

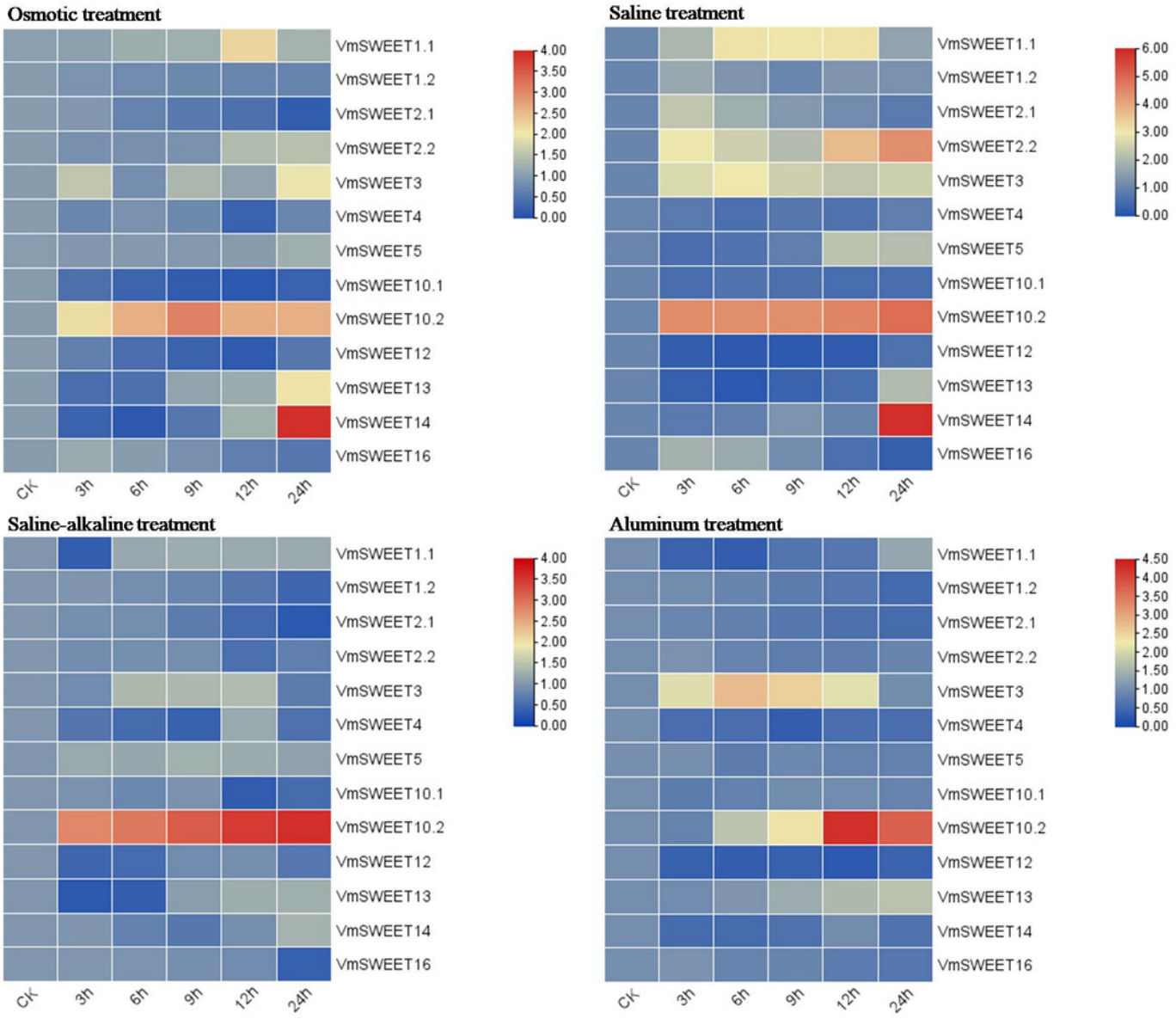

Figure 9

Schematic model of preferential gene expression and proposed roles of *VmSWEET*s in different cranberry tissues and fruit development stages.

This figure shows the representative genes highly expressed in each tissue and fruit development stage during the sugar accumulation stage, i.e. those likely implicated in the process of sucrose transportation from the leaf to other plant organs, such as the flower, stem, and fruit. The gene names under the tissue's name indicate that they are highly expressed in those tissues.

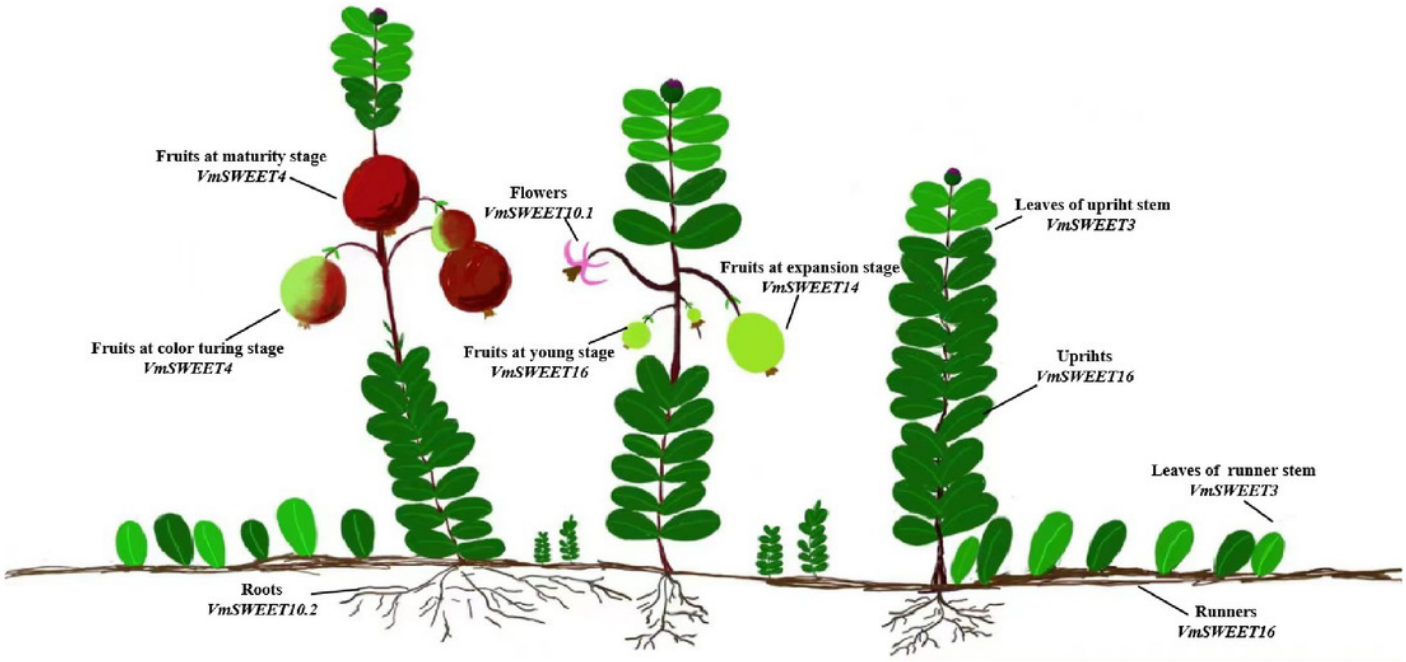

Table 1(on next page)

Physical and chemical properties of *SWEET* genes in cranberry

CDS: the length of coding domain sequences; MWs: the molecular weight; PI: theoretical isoelectric point; GRAVY: grand average of hydropathicity; TMDs: the number of transmembrane domains; PM: plasma membrane; ER: endoplasmic reticulum; TM: tonoplast membrane.

1 Table 1 Physical and chemical properties of *SWEET* genes in cranberry

Gene name	Gene ID	CDS/bp	Protein length/aa	MWs/KD	PI	Instability index	Aliphatic index	GRAVY	TMDs	Predicted location(s)
VmSWEET1.1	vmacro00890	741	246	26.91	9.2	30.53	106.95	0.628	7	TM
VmSWEET1.2	vmacro05470	768	255	28.11	9.61	30.52	107.49	0.507	6	ER
VmSWEET2.1	vmacro09417	702	233	26.25	8.87	47.52	120.86	0.861	7	PM
VmSWEET2.2	vmacro03987	591	196	21.38	9.1	33.31	134.69	1.002	5	TM
VmSWEET3	vmacro06571	1065	354	40.24	9.4	40.74	99.63	0.205	7	PM
VmSWEET4	vmacro18238	774	257	28.72	8.83	37.28	115.64	0.597	7	PM
VmSWEET5	vmacro19373	756	251	28.96	8.66	47.03	124.14	0.797	7	PM
VmSWEET10.1	vmacro16733	879	292	32.48	8.56	38.24	128.53	0.861	7	PM
VmSWEET10.2	vmacro19147	867	288	32.42	8.94	36.98	117.36	0.666	7	PM
VmSWEET12	vmacro19148	807	268	30.41	9.44	39.65	111.31	0.512	7	PM
VmSWEET13	vmacro16734	702	233	26.15	9.41	36.40	122.06	0.6	6	PM
VmSWEET14	vmacro01036	948	315	35.09	6.24	51.71	109.84	0.369	5	PM
VmSWEET16	vmacro08173	681	226	24.93	6.82	41.97	116.95	0.613	6	PM

2 CDS: the length of coding domain sequences; MWs: the molecular weight; PI: theoretical isoelectric point; GRAVY: grand average of hydropathicity;

3 TMDs: the number of transmembrane domains; PM: plasma membrane; ER: endoplasmic reticulum; TM: tonoplast membrane.

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