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) (#92897)

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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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SWEET (sugars will eventually be exported transporters) transporter is a novel type of sugar transporter that play crucial roles in plant growth and development as well as stress responses. Cranberry (*Vaccinium macrocarpon* Ait) is a small berry with rich nutrition and high economic benefits, but little is known about SWEET gene family function in this small fruit tree. In this research, 13 *Vm*SWEET genes belonging to four subfamilies were identified from cranberry genome for the first time. In the conserved domains, seven phosphorylation sites and four amino acid residues which were deemed crucial for the binding function were observed. Majority of *VmSWEETs* in each subfamily shared similar gene structures and conserved motifs, showing that the *VmSWEET* genes were very conservative during evolution. Chromosomal localization and synteny analysis showed that *VmSWEET* genes were unevenly distributed in eight chromosomes and two pairs of them have collinearity. Promoter regions analysis uncovered four major categories of plant hormone, light response, growth and development, as well as stress responses. Tissue-specific analysis showed *VmSWEET1* was highly expressed in flower, *VmSWEET5* was highly expressed in uprights and runners stem, *VmSWEET7* was highly expressed in both types of leaves. In fruit, the expression level of *VmSWEET5* and *VmSWEET11* were the highest among all members and down-regulated with the development of the fruit. While *VmSWEET10* expressed higher in color transition and maturity stages than in early development stages. In addition, qRT-PCR results displayed that *VmSWEET2* with the highest expression level significantly was up-regulated under drought, salinity, salt-alkali, and aluminum stress suggesting its essential role in mediating plant responses to various environmental stresses. Overall, these results provided new insights into the characteristics and the evolution of *VmSWEET* genes, and the important candidate

VmSWEET genes involved in the growth and development as well as abiotic stress responses in cranberry can be explored for promoting molecular breeding to improve fruit quality and abiotic stress resistance.

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Abstract: SWEET (sugars will eventually be exported transporters) transporter is a novel type of sugar transporter that play crucial roles in plant growth and development as well as stress responses. Cranberry (*Vaccinium macrocarpon* Ait) is a small berry with rich nutrition and high economic benefits, but little is known about SWEET gene family function in this small fruit tree. In this research, 13 *Vm*SWEET genes belonging to four subfamilies were identified from cranberry genome for the first time. In the conserved domains, seven phosphorylation sites and four amino acid residues which were deemed crucial for the binding function were observed. Majority of *VmSWEETs* in each subfamily shared similar gene structures and conserved motifs, showing that the *VmSWEET* genes were very conservative during evolution. Chromosomal localization and synteny analysis showed that *VmSWEET* genes were unevenly distributed in eight chromosomes and two pairs of them have collinearity. Promoter regions analysis uncovered four major categories of plant hormone, light response, growth and development, as well as stress responses. Tissue-specific analysis showed *VmSWEET1* was highly expressed in flower, *VmSWEET5* was highly expressed in uprights and runners stem, *VmSWEET7* was highly expressed in both types of leaves. In fruit, the expression level of *VmSWEET5* and *VmSWEET11* were the highest among all members and down-regulated with the development of the fruit. While *VmSWEET10* expressed higher in color transition and maturity stages than in early

development stages. In addition, qRT-PCR results displayed that *VmSWEET2* with the highest expression level significantly up-regulated under drought, salinity, salt-alkali, and aluminum stress suggesting its essential role in mediating plant responses to various environmental stresses. Overall, these results provide new insights into the characteristics and the evolution of *VmSWEET* genes, and the important candidate *VmSWEET* genes involved in the growth and development as well as abiotic stress responses in cranberry can be explored for promoting molecular breeding to improve 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 nearly all morphological and physiological processes in plants. Apart from serving 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, and light, to modulate growth and development in response to changing environmental conditions (Mishra et al., 2022). It is generally known that sugars serve as the primary photoassimilate and are synthesized in source leaves before being transported long distances to sink tissues, such as flowers, stems, tubers, swollen tap roots, fruits, or seeds, via the phloem for various life activities (Sonnewald and Fernie, 2018). Phloem loading in source leaves and unloading in sink tissues involve a combination of the symplastic, apoplastic, and/or polymer trapping pathways. The symplastic and polymer trapping pathways are passive processes, correlated with source activity and sugar gradients. In contrast, apoplastic pathway is energetically active, sugars translocation require the assistance of sugar transporters (Li et al., 2020). In higher plant, three key sugar transporters families play a crucial role in phloem loading and unloading, namely the monosaccharide transporter-like (MST) gene family, sucrose transporters (SUT/SUC), and sugars will eventually be exported transporter (SWEET) proteins (Doidy et al., 2012). MSTs and SUTs contain 12 transmembrane domains (TMDs) and require energy to complete the transmembrane transport of sugars. However, SWEET protein

harboring 7 TMDs is a novel sugar transporter, which can transport sugars in bi-directions and promote sugars diffusion along a concentration gradient independent 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 crops, horticultural crops, legume crops, oil crops, fiber crop, and other plant species, such as wheat (Gao et al., 2018), sorghum (Mizuno et al., 2016), soybean (Patil et al., 2015); oilseed rape (Jian et al., 2016), cotton (Li et al., 2018), apple (Wei et al., 2014), strawberry (Liu et al., 2019), jujube (Yang et al., 2022), tomato (Feng et al., 2015), cucumber (Li et al., 2017), cabbage (Zhang et al., 2019), daylily (Huang et al., 2022), saccharum (Hu et al., 2018) and so on. Phylogenetically, plant SWEETs are divided into four clades (clades I, II, III and IV) based on the functional characterization of SWEET genes in Arabidopsis. Clade I, II, and IV prefer to transport monosaccharides, whereas clade III predominantly transport 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 most members in other clades are situated on the plasma membrane, with some exceptions found on Golgi membrane and chloroplast (Breia et al., 2021).

Since the discovery of SWEET gene researchers have been devoted to exploring their physiological functions in plants. Initially, a two-step mechanism of SWEET-mediated phloem loading was clearly elucidated. In Arabidopsis, *AtSWEET11* and *12* were found to facilitate the release of sucrose from parenchyma cells to the apoplast (Chen et al., 2012). Subsequently, sucrose is accumulated in the companion cell (CC) by an energy-dependent AtSUC2 H^+/sucrose symporter and finally transported to the sieve element (SE) through plasmodesmata (Stadler and Sauer, 1996; Gottwald et al., 2000). Recent studies have revealed that SWEET-mediated phloem loading is regulated by sugar signals. In Chinese jujube, *ZjSWEET2.2* transcription was activated because its promoter *cis*-elements was bound with the low sugar signals, while its expression decreased and photosynthetic rate reduced by high sugar signals (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 were reported

constantly (Eom et al., 2015; Wen et al., 2022; Grantam et al., 2022). In Arabidopsis, *Brassica rapa* and tobacco, SWEET9 was identified to transport sucrose from nectary parenchyma to extracellular space to reward pollinators, and mutant lines failed in nectar secretion (Lin, et al., 2014). In maize and rice, SWEET play a role in further transfer of sugars imported from the maternal phloem. Mutants of *ZmSWEET4c*, *OsSWEET4, OsSWEET11*, and *OsSWEET11;15* significantly decreased the sucrose concentration in the embryo, accumulated starch in the pericarp, and exhibited functional deficiency of 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 pineapple callus and tomato exhibited enhanced sugar content (Lin et al., 2022). In tomato, elimination of *SlSWEET15* function resulted in a significant reduction in the average size and weight of fruits, accompanied by severe impairments in seed filling and embryo development (Ko et al., 2021). Above results indicated SWEET mediate unloading step of sucrose in sink organs to improve the yield and quality of important economic crops. Transcription factors are pivotal regulatory proteins that modulate the transcriptional rate of target genes by selectively binding to *cis*-acting elements of promoter upon activation or deactivation of upstream signaling cascades (Riaño-Pachón et al., 2007). Some studies have reported DNA binding with one finger (DOF) transcription factors and WRKY transcription factor can bind the promoter regions of SWEET. For example, OsDOF11 directly binds the promoter regions of *OsSWEET11* and *OsSWEET14* to transport sucrose via apoplastic loading (Wu et al., 2018). *PuWRKY31* with high histone acetylation level directly binds to *PuSWEET15* promoter then actives sucrose transporter transcription, resulting in high levels of sucrose in pear fruits (Li et al., 2020).

Sugar transport and partitioning not only affect plant growth and development, but also respond to abiotic and biotic stress. As SWEET transporters facilitate the efflux of sugars, they are highly susceptible to hijacking by pathogens, making them central players in plant-pathogen interaction (Breia et al., 2021). In Arabidopsis, the root tonoplast *AtSWEET2* was strongly induced during *Pythium* infection, leading to enhanced cytosolic sugar accumulation in the

vacuole. Overexpression of *AtSWEET2* enhanced plant resistance to *Pythium* infection by limiting sugar availability to the pathogen (Chen et al., 2015). However, the opposite behavior has been observed in grape, overexpression of *VvSWEET4* also improved the resistance to *P. irregulare* infection, while high sugar accumulation in hairy roots provided better support to the increased energy demand during pathogen infection (Meteier et al., 2019). So it is difficult to define the roles for SWEET transporters in plant-pathogen interactions, because we still know little about the metabolic signatures and regulatory nodes that decide the susceptibility or resistance responses. Likewise, previous studies on SWEET transporters response to abiotic stresses only focused on drought, cold, and salinity. The *MaSWEETs* in the highly resistant banana cultivar FJ exhibited increased expression levels in response to cold, drought, salt, and fungal disease stresses (Miao et al., 2017). In tea plants, the tonoplast sugar transporter *CsSWEET16* was downregulated under cold stress. Overexpression of *CsSWEET16* in Arabidopsis plants resulted in increased cold tolerance, which was accompanied by glucose accumulation in the vacuole and reduced fructose levels (Wang et al., 2018a). Although more and more researches about SWEET are reported, evidence is still scarce and fragmented to elucidate the function about the transport, distribution, metabolism, and signaling of sugars.

Cranberry (*Vaccinium macrocarpon* Ait.) is a diploid (2n = 2x = 24), woody perennial in the family Ericaceae genus *Oxycoccus* (Kron et al., 2002), which is endemic to North America, and also can be found in the Changbai Mountain area northeast of China. Like other members of this family, such as blueberry, bilberry, and lingonberry, it is uniquely adapted to life in cool and moist peat bog and can thrive in acidic, nutrient poor soils (Fajardo et al., 2012). Cranberry, a small but economically important berry fruit, holds significant potential for global development. Its versatility enables consumption in various forms, including fresh or processed as dried fruit, juice, jam, and other derivatives, positioning it as a superior food choice that encompasses a harmonious amalgamation of flavor, nutritional value, and advantageous health properties. The growing importance of cranberry and its products has created a demand for high yield and quality. However, during commercial cultivation, cranberries frequently encounter various

abiotic stresses due to the difference between cultivation environment and their original environment. The SWEET transporter have been demonstrated to play important roles in plant 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 the genome-wide analysis of SWEET genes in cranberry, named *VmSWEETs*, and analyzed their phylogenetic relationships, gene structure, motif distribution, chromosomal localization, and *cis*-regulatory elements. What's more, spatiotemporal expression and abiotic stress response expression was carried out by qRT-PCR. This study will provide valuable insights for future research on the roles of *VmSWEET* genes in cranberry growth, development, and stress responses.

MATERIALS AND METHODS

Plant Materials

A typical cultivar 'Bain 11' planted in the small berry germplasm resource garden of Jilin Agricultural University was used as plant material to detect the expression of *VmSWEET* genes in cranberry tissues and fruits at different development stages (Figure 1). Root, uprights stem, leaf of uprights stem, runners stem, leaf of runners stem, and flower were collected at flowering. Young fruit (young fruit stage), white fruit (expansion stage), pink fruit (color turning stage) and red fruit (maturity stage) were collected at 10, 30, 60, 80 days after full bloom respectively. Tissue cultured seedling of 'Bain13' was used to detect the expression pattern under different abiotic stresses. Drought treatment (20% PEG 8000), salt treatment (200 mM NaCl), saline-alkaline treatment (30 mM Na_2CO_3 and 30 mM $NaHCO_3$) and $AlCl_3$ treatment (5mM $AlCl_3$) were applied by placing the root-induced plantlets in containers with different solutions. At various time points during the different stress treatments (0, 3, 6, 9, 12 and 24 h), leaf samples were collected. All fresh plant samples were collected with three independent replicates and immediately frozen in liquid nitrogen, then stored at -80 °C.

Identification SWEET Gene Family in Cranberry

Cranberry SWEET gene family was identified by protein Blast of the 17 Arabidopsis SWEET proteins against *Vaccinium macrocarpon* genome database (https://www.ncbi.nlm.nih.gov/genome/?term=cranberry). The CDS sequences of *VmSWEET*

genes were showed in supplementary file S1. The NCBI CDD (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and PFAM (http://pfam.sanger.ac.uk/) website 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, and theoretical isoelectric point pI were analyzed on 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 transmembrane (TM) structures prediction. The exon/intron structures and conserved protein motifs were analyzed by TBtools Software. Promoter *Cis*-acting regulatory elements of target genes were predicted by submitting 2 kb upstream sequenceto the PlantCARE web site (http://bioinformatics.psb.ugent.be/webtools/plantcare/html). The promoter sequences of *VmSWEET* genes were showed in supplementary 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 ClustalX 1.83 and MEGA7.0 sofware with bootstrap values for 1000 replicates. Then the phylogenetic tree was beautified by ITOL v6 (https://itol.embl.de/). SWEET amino acid sequences from Arabidopsis, rice and grape were downloaded from the NCBI (https://www.ncbi.nlm.nih.gov/) (supplementary file S3). The VmSWEET protein sequences alignment was performed using the ClustalX 1.83 and phosphorylation sites were predicted by NetPhos 3.1 (https://services.healthtech.dtu.dk/services/NetPhos-3.1/). The GENEDOC 3.20 software was used to highlight conserved or similar amino acid sequences.

Chromosomal Distribution and Gene Synteny Analysis

MapChart was used to construct the chromosomal distribution map of *VmSWEET* genes, as well as MCScanX and CRCOS were used to analysis gene synteny.

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

Total RNA was isolated by a modified CTAB method. The integrity and concentration of RNA were assessed using electrophoresis on 1.2% agarose gels and NanoPhotometer® spectrophotometer (IMPLEN P330), respectively. A 1 μg sample of the extracted RNA was reverse transcribed into cDNA using a *TransScript*® Uni One-Step gDNA Removal and cDNA Synthesis SuperMix (TransGen Biotechnology). qRT-PCR was performed on 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 measured 20 μL and refered to the instruction manual of *PerfectStart*® Green qPCR SuperMix (TransGen Biotechnology). 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; 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 triplicate. Primers were designed by Premier BLAST tool (http://blast.ncbi.nlm.nih.gov/). The *VmSAND* gene was considered the optimal internal reference gene for analyzing various cranberry organs and abiotic stress treatment (Chen et al., 2019). It was utilized as a control to standardize the expression of *VmSWEETs*. The designed qRT-PCR primers were shown in supplementary file S4. The raw data of Ct was shown in supplementary file S5. Relative quantitative analysis of 13 target genes in different cranberry tissues and fruit development stages were calculated using the $2^{-\Delta Ct}$ method, and column charts were obtained by SigamaPlot 10.0. Expression profiles of *VmSWEETs* under abiotic stress were calculated using the $2^{-\Delta\Delta Ct}$ method, then the expression level was log2 transformed and normalized to obtain a heatmap by TBtools. The completed MIQE checklist was shown in supplementary file S6.

RESULTS

Genome-wide Identification and Analysis of *VmSWEET* Genes

Thought homologous alignment and conservative domain verification, a total of 13 genes

encoding SWEET protein were identified and renamed as *VmSWEET1* to *VmSWEET13*. The physical and chemical details of *VmSWEETs* were summarized in Table 1. The coding domain sequences length (CDS) of *VmSWEETs* ranged from 519 bp to 1065 bp, corresponding to amino acid number ranging from 196 to 354. The molecular weight (MWs) of the 13 proteins ranged from 21.38 to 40.24 KD, and the theoretical isoelectric point (PI) ranged from 6.24 to 9.61. The instability index ranged from 30.52 to 51.71, suggesting that 62% VmSWEETs were hydrophobic protein. Aliphatic index of almost all proteins was greater than 100, and the grand average of hydropathicity (GRAVY) values varied from 0.205 to 1.002, indicating that they were all hydrophobic properties. Subcellular localization prediction revealed that VmSWEET4 and VmSWEET11 localized in tonoplast membrane, VmSWEET6 may be localized in endoplasmic reticulum, and the other 10 VmSWEETs located in the plasma membrane.

Phylogenetic Analysis of *VmSWEET* Genes

To study the phylogenetic relationships among *SWEET* genes in cranberry and other plant species, phylogenetic tree was constructed by aligning 13 VmSWEET sequences, 17 AtSWEET sequences, 21 OsSWEET sequences and 15 VvSWEET sequences. Apparently, 66 proteins were clustered into four different clades (Figure 2). In detail, clade I contained 5 VmSWEETs (VmSWEET4, 6, 7, 8, 13), 3 AtSWEETs (AtSWEET1-3), 6 OsSWEETs (OsSWEET1a,1b,2a,2b,3a,3b) and 2 VvSWEETs (VvSWEET1, 2); 2 VmSWEETs (VmSWEET10, 12), 5 AtSWEETs (AtSWEET4-8) 9 OsSWEETs (OsSWEET7a-7e,6a,6b,4,5) and 4 VvSWEETs (VvSWEET4, VvSWEET5a, 5b, 7) belonged to class II; Five VmSWEETs (VmSWEET1, 2, 3, 9, 11), 7 AtSWEETs (9–15), 5 OsSWEETs (OsSWEET11-15) and 5 VvSWEETs (VvSWEET9, 10, 11, 12, 15) were included in clade III. Clade IV was the subfamily with the fewest members, containing 1 VmSWEET (VmSWEET5), 1 OsSWEETs (OsSWEET16), 2 AtSWEETs (AtSWEET16, 17) and 3 *VvSWEETs* (VvSWEET17a, 17b, 17d).

Multiple Sequence Alignment, Conserved Domain and Gene Structure Analysis of *VmSWEET* Genes

Multiple sequence alignment was shown in Figure 3 The majority of VmSWEET members

contained two MtN3/saliva domains, however VmSWEET11 and VmSWEET13 had only one complete MtN3/saliva domain. Four Ser and two Tyr sites, as well as one Thr phosphorylation site, were predicted in the two conserved MtN3/saliva regions and indicated by the red triangles. Additionally, in order to search for the key amino acid sites for VmSWEET binding to sugars, we found a very conserved asparagine pair (Asn77 and Asn197) located in the binding pocket of OsSWEET2b in rice also presented at equivalent positions of VmSWEETs. What's more, Ser54 on THB1 and Trp176 on THB2 have been confirmed to play the same role in AtSWEET1. In all VmSWEETs, Trp was present at the equivalent position of Trp176, except for VmSWEET8, 9, and 11, where it was replaced with the aromatic residues Phe or Tyr. Similarly, at the corresponding position of Ser54, it was replaced with Phe, Trp, Leu, Tyr, or Cys.

The conserved motifs were predicted to gain more insights into the characteristic of *VmSWEET* genes. As shown in Figure 4, a total of 9 different conserved motifs were identified, named Motif1 to Motif9. Motif2, Motif4, and Motif5 existed on all of the 13 VmSWEET proteins, suggesting that the three conserved motifs were essential for cranberry SWEET proteins. Significantly, Motif7 was unique to VmSWEET5 in Clade IV indicating its specific functions. The conserved motifs within the N-terminus of all VmSWEET proteins exhibited remarkable similarity. Motif1 was missing in *VmSWEET9*, *VmSWEET11*, *VmSWEET12* and *VmSWEET13*, but an additional transmembrane-domain structure appeared at the same position. To further investigate the structural differences of *VmSWEET* genes, the arrangement patterns of introns and exons were determined by the TBtools software. As shown in Figure 5, the number of exons in 13 *VmSWEET* genes ranged from 4 to 9, accordingly the number of introns changed from 3 to 8. The number of introns in Clade I and Clade III changed significantly, *VmSWEET* genes in Clade I varied from 4 to 8, and in Clade III varied from 3 to 5. But gene pairs in the sister branch exhibited similar structural features, such as *VmSWEET4* and *VmSWEET6*, *VmSWEET8* and *VmSWEET13*, *VmSWEET1* and *VmSWEET2*, *VmSWEET3* and *VmSWEET11*, with comparable intron and exon numbers and CDS lengths. Additionally, *VmSWEETs* in Clade II and Clade IV exhibited a same exon count of 4.

Chromosomal Localization and Collinearity Analysis of *VmSWEETs*

Chromosomal localization and collinearity analysis were conducted to study the repetitive events of the SWEET gene family. As shown in Figure 6, 13 *VmSWEET* genes were unevenly distributed over the majority of cranberry chromosomes, except for chromosomes 7, 8, 10 and 11. The greatest number of genes was mapped to chromosome 5, *VmSWEET2* and *VmSWEET3* are closely positioned. Chromosomes 1 and 4 contained two *VmSWEET* genes respectively. Notably, the distance between *VmSWEET1* and *VmSWEET9* on Chromosome 4 was found to be remarkably short. Whereas chromosomes 2, 3, 6, 9 and 12 each contained just one VmSWEET gene. Additionally, collinearity relationships were analyzed to investigate potential evolutionary mechanisms of *VmSWEET* gene family. The result showed collinearity existed in *VmSWEET11* located at chromosome 1 and *VmSWEET1* located at chromosome 4, as well as *VmSWEET10* located at chromosome 3 and *VmSWEET12* located at chromosome 5, indicating two pairs of segmental duplicated events in the evolution of cranberry (Figure 7).

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

In order to investigate the potential regulatory factors of *VmSWEET* genes, promoter *cis*-regulatory elements were predicted by PlantCARE. The results as follow (Figure 8), a total of 87 *cis*-acting elements were identified in the promoter regions of cranberry *SWEET* genes. Besides the necessary components for normal transcriptional activity, such as CAAT and TATA elements, the rest were mainly 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 was the least, containing G-box/GT1-motif (light responsive element) and circadian (circadian rhythm regulatory cis-acting elements).

Expression Profile of *VmSWEET* Genes in Different Tissues and Fruit Development Stages of Cranberry

To investigate the functions of *VmSWEET* genes in cranberry growth and fruit ripening, spatiotemporal expression pattern of 13 *VmSWEET* genes was determined by qRT-PCR. As illustrated in Figure 9, *VmSWEET1*, *VmSWEET3*, *VmSWEET10*, *VmSWEET11* and *VmSWEET12* exhibited similar pattern, with intense expression in flower compared with other tissues. Notably, *VmSWEET1* displayed the highest expression level among the 5 genes. Four *VmSWEET* genes (*VmSWEET5*, *VmSWEET8*, *VmSWEET9* and *VmSWEET13*) have exceedingly high expression in the uprights and runners stem, all except *VmSWEET9* being significantly more highly expressed in the runners stem. In particular, the expression levels of *VmSWEET5* in uprights and runners stem were 6~23 folds and 10~42 folds more than other tissues respectively. *VmSWEET2* and *VmSWEET7* exhibited similar expression pattern, showing higher expression levels in uprights and runners leaf compared to other organs. *VmSWEET6* showed the highest relative expression not only in runners leaf but also in flowers, albeit lower than *VmSWEET1* and *VmSWEET7* which specifically expressed in flower and runners leaf. No *VmSWEET* exhibited specific expression in the roots, although *VmSWEET5* displayed the highest expression level in root, its value was extremely low.

The expression level and pattern of *VmSWEET* genes was different at four distinct ripening stages: young fruits (S1), developing fruits (S2), color changing fruits (S3) and ripe fruits (S4) (Figure 10). *VmSWEET2*, *VmSWEET4*, *VmSWEET6*, *VmSWEET9*, *VmSWEET11* and *VmSWEET12* showed similar expression profile, characterized by an initial upregulation followed by a subsequent downregulation during fruit development. It was noteworthy that *VmSWEET11* expression was the hightest one in all gene members, and it reached peak at fruit expansion stage then decreased by 78.43% and 94.90% at the color-changing and ripening stages,

328 respectively. In contrast, *VmSWEET1*, *VmSWEET3*, and *VmSWEET10* presented an opposite
329 trend to the previous 6 *VmSWEETs*. Among them, *VmSWEET1* and *VmSWEET3* exhibited
330 relatively weak expression in the whole development period, *VmSWEET10* gradually increased
331 with the development of fruit and reached a high level at the fruit color transition and maturity
332 periods. Additionally, the expression of *VmSWEET5*, *VmSWEET7*, *VmSWEET8*, and
333 *VmSWEET13* gradually declined during fruit development. *VmSWEET5* exhibited the highest
334 expression level, but decreased by 46.96%, 91.94%, and 94.45% during the developing, color
335 changing and ripe fruits, respectively.

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

337 In *Vitro* cranberry plantlets were treated with different abiotic stress treatment (drought,
338 salinity, salt-alkali and aluminum) to study the differential expression pattern of *VmSWEET*
339 genes. Under drought conditions, the most evident result was that *VmSWEET2* and *VmSWEET11*
340 with high expression showed opposite tendency. *VmSWEET2* showed a significant increase in
341 the first 9 hours followed by a decrease, while *VmSWEET11* exhibited significantly down-
342 regulated in the first 6 hours and then up-regulated, ultimately reaching an expression level 14.5
343 times higher than that of the control after 24 hours of treatment. Other members exhibited
344 relatively low expression and slight fluctuations, *VmSWEET1*, *VmSWEET3*, *VmSWEET6*, and
345 *VmSWEET8* were down-regulated expression, *VmSWEET9 VmSWEET12* and *VmSWEET13* were
346 up-regulated expression (Figure 11A). During the salinity stress treatment, *VmSWEET2* and
347 *VmSWEET11* exhibited highly expression and increased over time. However, their response time
348 to stress was different, *VmSWEET2* showing significant up-regulation within the first 3 hours and
349 *VmSWEET11* showing significant up-regulation at 24 hours (Figure 11B). During the salt-alkali
350 stress treatment, *VmSWEET2* still was the highest expression gene and exhibited an up-regulated
351 expression profile, with expression value increasing by 5.9-, 6.6-, 8.2-, 10.1-, and 11.0-folds over
352 time (Figure 11C). To obtain insight into the underlying functional role of *VmSWEET* genes in
353 the response to aluminum stress, the expression pattern of the *VmSWEET* members was analyzed
354 for the first time. The conspicuous gene *VmSWEET2* exhibited slightly increased expression

within the first 9 hours, followed by a sharp surge to 20-fold higher levels compared to the control group after 12 hours of stress. Subsequently, a slight decrease was observed. The majority of the rest genes with low expression were continuously down-regulated under aluminum stress, such as *VmSWEET3*, *VmSWEET5*, *VmSWEET6*, *VmSWEET8*, *VmSWEET10*, *VmSWEET12* and *VmSWEET13* (Figure 11D). The above results under various abiotic stresses suggested that *VmSWEET* genes acted as an important regulator of plant responses.

DISCUSSION

Characters and Function of SWEET Family Genes in Cranberry

SWEET transporters widely present in plants, animals, fungi, and prokaryotic bacteria, and they 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). At present, SWEET gene family has been characterized in many plant species due to the popularity of high-throughput techniques. In general, plant genome contains approximately 20 SWEET paralogs (Anjali et al., 2020). Thirteen SWEET genes were discovered in cranberry via a comprehensive genome-wide investigation in this study. The number of *VmSWEETs* is comparable to that of tea (13) (Wang et al., 2018), grapes (17) (Chong et al., 2014), pears (18) (Li et al., 2017), but less than soybean (52) (Patil et al., 2015), oilseed rape (68) (Jian et al., 2016), and wheat (108) (Gautam et al., 2019). The significant differences of SWEET gene family scale between different species can be attributed to gene duplication events. Research indicates that the expansion of the SWEET gene family has occurred throughout the course of evolution (Patil et al., 2015). Gene duplication events play a vital role in the evolution of gene families as they furnish the basic materials necessary for the emergence of new genes, thereby enabling the emergence of novel functions (Yin et al., 2019). Segmental duplication, a common form of gene duplication, is prevalent in plants due to their diploidized polyploid nature, which results in the retention of multiple duplicated chromosomal blocks within their genomes (Cannon et al., 2004). Based on the amount of *VmSWEET* gene family, we could speculate that small-scale gene duplication event occurred in the evolution of cranberry, which was also verified by the collinearity analysis.

The synteny analysis revealed only two pairs of collinear *VmSWEET* gene, a much smaller number than in soybeans which has been demonstrated occurrence of large-scale gene duplication events (Schmutz, et al., 2010). Furthermore, the gene pairs *VmSWEET1/VmSWEET11* and *VmSWEET10/VmSWEET12* belonged to the same subfamily, suggesting that segmental replication events of *VmSWEET* genes occurred within this subgroup (Figure 4).

Based on the evolutionary relationships inferred from phylogenetic analysis, the *VmSWEET* genes were categorized into four distinct clades (Figure 2). SWEET transporter in different clade exhibits selective preferences for monosaccharides or disaccharides. The Clade I and II subfamilies specifically transport hexose, the Clade III subfamily display preferential transport activity for sucrose over glucose, and the Clade IV subfamily exhibit specific transport of fructose (Eom et al., 2015). According to subcellular localization, we speculated that tonoplast-localized *VmSWEET4* and *VmSWEET13* mediated transmembrane transport of hexose, such as glucose and fructose. *VmSWEET7, VmSWEET8, VmSWEET10* and *VmSWEET12* mediate hexose across plasma membrane. *VmSWEET1, VmSWEET2, VmSWEET3, VmSWEET9* and *VmSWEET11* located on the plasma membrane efflux sucrose from cytosol into the apoplast. Additionally, *VmSWEET5* in Clade IV may be control the flux of fructose across the plasma membrane. This was different from the tonoplast localization of their homologs *AtSWEET16* and *AtSWEET17* (Klemens et al., 2013; Guo et al., 2013), the reason may be low accuracy of subcellular localization prediction by WoLF PSORT for membrane protein, secrete proteins, or proteins present on multiple cell organelles. So the precise subcellular localization and substrate specificity of *VmSWEETs* still need further research. To seek the reasons for substrate specificity of SWEET, crystal structure and bioinformatics analyses was conducted in bacterial SemiSWEETs. A fascinating find was that the size of pocket presented above the center of the transporter protein played a critical role in determining substrate specificity. A large substrate-binding pocket with spacious substrate-binding cavity may facilitate the transport of both disaccharides (such as sucrose) and monosaccharides (such as glucose and fructose), while

409 smaller sized pockets with restricted substrate-binding cavity can only hold monosaccharides
410 (Wang et al., 2014). In higher plants, a conserved asparagine pair (Asn77 and Asn197) surround
411 the binding pocket at the equivalent positions in OsSWEET2b, as well as Ser54 on THB1 and
412 Trp176 on THB2 have been implicated in the transportation capacity of AtSWEET1 (Tao et al.,
413 2015). In our study, the two Asn residues also conserved in of VmSWEETs. Although at
414 equivalent positions of Trp176, the majority of VmSWEETs contains Trp except that
415 VmSWEET8,9,11 were replaced with aromatic residue Phe or Tyr (Figure 3). However, this
416 substitution did not affect the transport activity of VmSWEETs, because the presence of one
417 aromatic residue in THB2, rather than THB1, was important for transport activity (Tao et al.,
418 2015).we think four amino acid residues of VmSWEET still can interact with sugar molecules
419 via H-bonding or aromatic ring stacking. Phosphorylation sites also were crucial for proteins and
420 their transportation and function. The latest research found the carboxy-cytosolic regions of
421 AtSWEET11 and 12 were rapidly phosphorylated by SnRK2 protein kinases upon drought,
422 which enhances the oligomerization and sucrose transport activity of SWEETs (Urooj Fatima et
423 al., 2022). In our study, four serine, two tyrosine sites and one threonine phosphorylation site
424 were observed in the VmSWEETs conserved domains (Figure 3). These phosphorylation sites
425 were probably related to signal recognition and transduction functions of *VmSWEETs*.

426 The plant SWEET gene family is highly conserved, with accurate functioning and stability
427 maintained by seven transmembrane domains (TMDs) and two MtN3/saliva domains (Chen et
428 al., 2010). The result of Multiple sequence alignment revealed that 11 VmSWEET proteins
429 (about 85%) containing two complete MtN3/saliva domains (Figure 3). The phenomenon of few
430 SWEET members harbored one or one and a half MtN3/saliva domains was also observed in
431 other species, such as walnut (Jiang et al., 2020), and watermelon (Xuan et al., 2021). Because
432 SWEET protein with two MtN3/saliva domains in eukaryotes was considered replication or
433 horizontal gene transfer from one MtN3/saliva domain of prokaryotes during evolution process
434 (Xuan et al., 2013), we hypothesized that the two abbreviated *VmSWEET* genes, *VmSWEET11*
435 and *VmSWEET13*, were generated through tandem and domain duplication events throughout the

course of evolution. Phylogenetic analyses also supported the results of gene structure analysis. There was little change in quantity of intron and exon within each subfamily except for *VmSWE*ET7 in Clade I. Especially the gene pairs in the sister branches were generally identified to have the same number of intron and exon, suggesting that molecular features of SWEET genes were relatively conserved during evolution. Generally, the gene with the highest number of introns is regarded as the ancestral homolog of those members with fewer introns, as intron loss occurs more rapidly than gain after segmental duplication (Nuruzzaman et al., 2010). Here, *VmSWEET7* with 8 introns was proposed the original homologs in cranberry SWEET gene family (Figure 5). Conserved motifs analysis revealed that all *VmSWEET* proteins contained Motif 2, Motif 4 and Motif 5, indicating that the three conserved motifs play a crucial role in maintaining the structure and functioning. Additionally gene members within the same subfamily harbored similar motif arrangement, while there were obvious differences in the motif composition of different subfamily. For instance, motif 7 was uniquely present in members of cluster IV and motif 8 was specifically present in members of cluster I and III, these specific motifs were not available in members of the remaining two clusters (Figure 4). These results were consistent with other plant systems, such as rice (Yuan et al., 2013), banan (Miao et al., 2017) and wheat (Gautam et al., 2019).

Gene expression and functional divergence of SWEETs in creanberry

The expression profile of gene is closely related to its function. Many studies have revealed that SWEET plays an important role in plant growth and development. In this study, the expression pattern of 13 *VmSWEET* genes was analysed in roots, stems, leaves, flowers and different development stages of fruit to explore the potential function of SWEET genes in cranberry. The results demonstrated that each *VmSWEET* was expressed in various organs, albeit with distinct expression pattern (Figure 9). *VmSWEET2* and *VmSWEET7* were highly expressed in leaves of uprights stem and runners stem. *VvSWEET1*, the homolog of *VmSWEET7*, was mainly expressed in young and adult leaves of grape (Chong et al., 2014), showing similar expression patterns in vegetative organs 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*, *AtSWEET11;12* mutant line accumulated starch in leaves, and radio tracer efflux from petioles was reduced (Chen, et al., 2012). Due to sucrose being the predominant form of photoassimilates and transport substrate in Clade III, it is hypothesized that *VmSWEET2* played a role in the phloem loading of photoassimilates in cranberry leaves. SWEET genes expressed in flowers were mainly involved in reproductive development and nectar secretion. *VmSWEET1* with the highest transcriptional level was observed in flowers. Cluster analysis showed that the *OsSWEET11, AtSWEET13,14* and *VmSWEET1* belonged to the same subgroup. Among them, *OsSWEET11* have been reported to play a role in rice pollen development, knockout mutants of *OsSWEET11* produced defective pollen grains and a lower fertility rate in plants (Chu et al., 2006; Yang et al., 2006; Yuan et al., 2009). Consistent results were reported in *Arabidopsis*, *AtSWEET13* and *AtSWEET14* were found to be expressed in the anther wall, responsible for facilitating sucrose efflux into locules to support pollen development and maturation. The viability and germination of pollen from the double mutant *AtSWEET13,14* was observed to be reduced (Sun et al., 2013; Wang et al., 2022). Therefore, *VmSWEET1* may play important role in cranberry reproductive development. Form source to sinks, the long-distance transportation of photosynthetic products in stems generally follows the symplastic route. However, when stems function as storage organ, SWEET transport may be involved in unloading and storage of photosynthates in the stem. For example, *SsSWEET4a/4b* were mainly expressed in the stems of *Saccharum*, they were forecasted to involve in sugar transportation within the stalk (Hu et al., 2018). Although the stem of cranberry did not serve as storage sink like sugarcane, consistent results were also found in cranberry, the expression level of *VmSWEET5* in uprights stem and runners stem was higher than other tissues. In order to gain valuable insights into the role of SWEET transporter in plant stems and perfect the long-distance transportation mechanism, further functional validation research is required. No *VmSWEETT* genes specifically expressed in the roots, because the root might not serve as an important storage sink during the sampling period, or *SWEET* transcription in root was induced

by certain factors, such as cold stress, or osmotic stress.

Fruits are the most important storage organs in horticultural crops, their yield and quality were determined by the component and content of sugar. As a novel sugar transporter protein independently of energy or pH, SWEET proteins have attracted the attention of many researchers on phloem unloading, transport and storage of sugars during fruit development. In jujubes, the expression levels of *ZjSWEET11* and *ZjSWEET18* exhibited a gradually increase trend during fruit development, reaching a peak at the complete maturity stage (Yang et al., 2023). In apple, there is a significant association between the expression of *MdSWEET2e,9b,15* and fruit sugar content. Especially, *MdSWEET15a* and *MdSWEET9b* accounted for a relatively large proportion of phenotypic variation in sugar content (Zhen et al., 2018). In grape, *VvSWEET10* was strongly expressed in ripening fruit, *VvSWEET10* overexpression in grapevine calli and tomatoes resulted in a significant increase of glucose, fructose and total sugar (Zhang et al., 2019). In developing tomato fruits, the expression of *SlSWEET15* was notably elevated, while sizes and weights were significantly reduced upon elimination of *SlSWEET15* (Ko et al., 2021). The above results all indicated that SWEET gene positively regulated fruit development and ripening. Conversely, silencing *SlSWEET7a* or *SlSWEET14* of tomato led to increased plant height, fruit size and sugar content (Zhang et al., 2021). In the present study, expression of *VmSWEETs* was dynamically changed during fruit development, with distinct sets of *VmSWEETs* being expressed in the young and mature fruits (Figure 10). For instance, *VmSWEET5* and *VmSWEET11* were highly expressed during the young fruit and expansion stage respectively, whereas *VmSWEET10* was highly expressed during the color change and the maturity stage. We speculated that *VmSWEET10* positively regulates fruit development and ripening, while *VmSWEET5* and *VmSWEET11* might play similar roles with *SlSWEET7a* and *SlSWEET14*, suppressing the two genes could be a potential strategy for enhancing the sugar content of cranberry fruits.

Abiotic stresses frequently impede plants growth and development, ultimately inhibiting their productivity and quality. Interestingly, plants have evolved sensory and response mechanisms to cope with various environmental stresses. In plants, sugars serve as osmo-

protectants and molecular switches, and their production and distribution is a crucial physiological process that is induced by various stresses (Saddhe et al; 2021). Therefore, understanding the impact of abiotic stresses on plants and elucidating molecular mechanisms of sucrose transport is imperative to maintain sugar homeostasis for plants to adapt to stress. The previous studies have found that SWEET proteins can regulate the redistribution of soluble sugars under abiotic stress. In *Poa annuaLinn* (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 stress, and all showed the same up-regulation expression. However, there were opposite results. For example, the expression *MtSWEET2a* and *MtSWEET3c* were down-regulated in *Medicago truncatula* (Hu et al; 2019). In this research, the most noticeable result was that *VmSWEET*2 with the highest expression level was up-regulated under four abiotic stress treatments (Figure 11). The results in drought and salinity were consistent with its homologs *AtSWEET11-15*, which have been verified can respond to a variety of abiotic stresses in *Arabidopsis*. *AtSWEET11,12* up-regulated and were responsible for transport sucrose from the leaves to the roots under drought stress (Durand et al; 2016). *AtSWEET13* was down-regulated while *AtSWEET14* was up-regulated in response to high salinity (Sellami et al; 2019), *AtSWEET15* (SAG29) was significantly up-regulated during senescence and abiotic stresses including cold, salty, and drought treatments (Seo et al; 2011). However, whether SWEET transporters play a role in other stress except for cold, drought and salt stress in plants remains unknown. Our results showed that *VmSWEET2* was responded to salt-alkali and aluminum tolerance, it may regulate sucrose transport and distribution in response to the abiotic stresses relatively autonomous. Further research has found that drought and salinity stresses induced an ABA-responsive transcription factor *OsbZIP72* directly binds to the promoters of *OsSWEET13* and *15,* activating their transcription and increasing the sucrose content in leaf and root (Mathan, et al., 2020). So we guessed *VmSWEET2*, the homologue of *OsSWEET13* and *15,* also harbor a site for the ABA-responsive transcription factor in its promoter region. This conjecture was

544 consistent with the presence of ABRE (abscisic acid response element) by promoter analysis. But
545 the regulatory mechanism of sugar homeostasis in cranberry under abiotic stresses is still needed
546 further exploration.

547 According to the expression patterns of *VmSWEET* genes in different tissues and different
548 fruit development stages, a hypothetical model for SWEET involving in photosynthetic products
549 transport and distribution in cranberry were proposed. As shown in Figure 12, sucrose was
550 produced in uprights and runners leaves through photosynthesis, *VmSWEET7* participated in
551 phloem loading of photosynthetic products in the two types of leaves. Then sucrose was long-
552 distance transported from source to sink tissues by *VmSWEET5* in both uprights stem and
553 runners stem. *VmSWEET1* was likely to be implicated in pollen development in flower, which
554 was beneficial to pollination and fertilization. With sucrose unloading into the fruits,
555 *VmSWEET5* and *VmSWEET11* played an important role in early stage of fruit growth and
556 development, while *VmSWEET10* was responsible for the transport and accumulation of
557 monosaccharides (hexoses) during the veraison and mature stages to promote the fruit quality.
558 *VmSWEET2* maybe induced by abiotic stress to transport sucrose in root as a signaling molecule
559 to cope with different adversity constraints.

560 **CONCLUSION**

561 In this study, 13 *VmSWEET* genes distributed on 8 chromosomes were identified in
562 cranberry. They divided into 4 Clades by phylogenetic analysis, and 4 conserved amino acid
563 residues and 7 phosphorylation sites, which were crucial for transport function, were observed in
564 conserved domains. The similar homologous genes in the topology have similarly conserved
565 motifs and gene structures. *Cis*-acting elements related to plant hormone, light responsive,
566 growth and development and stress responses were identified in promoter of *VmSWEETs*
567 sequences. The expression of *VmSWEETs* was tissue-specific and specific to fruit developmental
568 stage. *VmSWEET7, VmSWEET5*, and *VmSWEET1* were specifically expression in leaves, stems,
569 and flowers respectively. *VmSWEET5*, *VmSWEET11*, and *VmSWEET10* synergistically regulated
570 fruit development and ripening. *VmSWEET2* was the key gene involved in the response of

cranberry to abiotic stresses including drought, salinity, salt-alkali and aluminum conditions. Overall, these results provide a reference basis for future studies on *VmSWEET* genes function and explore their potential application to increase yield, improve quality, and enhance resistance in cranberry plants.

ADDITIONAL INFORMATION AND DECLARATIONS

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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.
- H.S. revised the manuscript.

Data Availability

The following information was supplied regarding data availability:

The raw data is available in the Supplementary Files.

Supplemental Information

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

Supplemental Information 2 Promoter sequenses of *VmSWEET* genes in cranberry

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

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

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

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791

Figure legends:

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

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, blue triangles represent *A. thaliana*, yellow triangles represent *O. sativa*, purple dots represent *V. vinifera*, red stars represent *V. macrocarpon.*

Figure 3. Multiple sequence alignment of the *VmSWEETs*. The sequences contained in the black boxes are conserved domains unique to *VmSWEETs* members. The position of the conserved serine (S), threonine (T), and tyrosine (Y) predicted to be the phosphorylation sites are indicated by the red triangles. A conserved asparagine pair Asparagine (N) in *OsSWEET2b* and a serine (S) as well as tryptophan (W) in *AtSWEET1* are indicated by the red arrows.

Figure 4. Conserved motifs and conserved structural domains of cranberry *SWEET* gene family.

Figure 5. Gene structure of cranberry *SWEET* gene family.

Figure 6. Chromosome mapping of *SWEET* genes in cranberry.

Figure 7. Collinearity analysis of *SWEET* gene family in cranberry. Gray lines indicate all synteny blocks in the cranberry genome, and the red lines indicate the duplication of *VmSWEET* gene pair.

Figure 8. Promoter *cis*-acting elements of *VmSWEETs*.

Figure 9. Expression analysis of *VmSWEET* genes in different tissues of cranberry. Rt, Root; U, Uprights stem; Rn, Runners stem; UrL, Uprights Leaf; RnL, Runners Leaf; F, Flower.

Figure 10. Expression analysis of *VmSWEET* genes in cranberry fruits at different developmental stages. S1, Young fruit stage; S2, Fruit expansion stage; S3, Colour turning stage; S4, Maturity stage.

Figure 11. Gene expression heatmap of the *VmSWEET* genes in the leaf under abiotic stress. A, Drought stress; B, Salinity stress; C, Salt-alkali stress; D, Aluminum stress.

Figure 12. Schematic model of gene expression and role 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 probably involved in phloem loading of sucrose in the leaf and unloading and accumulation in the flower, stem and fruit. The gene names under the tissue's name indicate that they are highly expressed in those tissues.

824

Figure 1

Different tissues and fruit at different stages of cranberry

Different tissues and fruit at different stages of cranberry

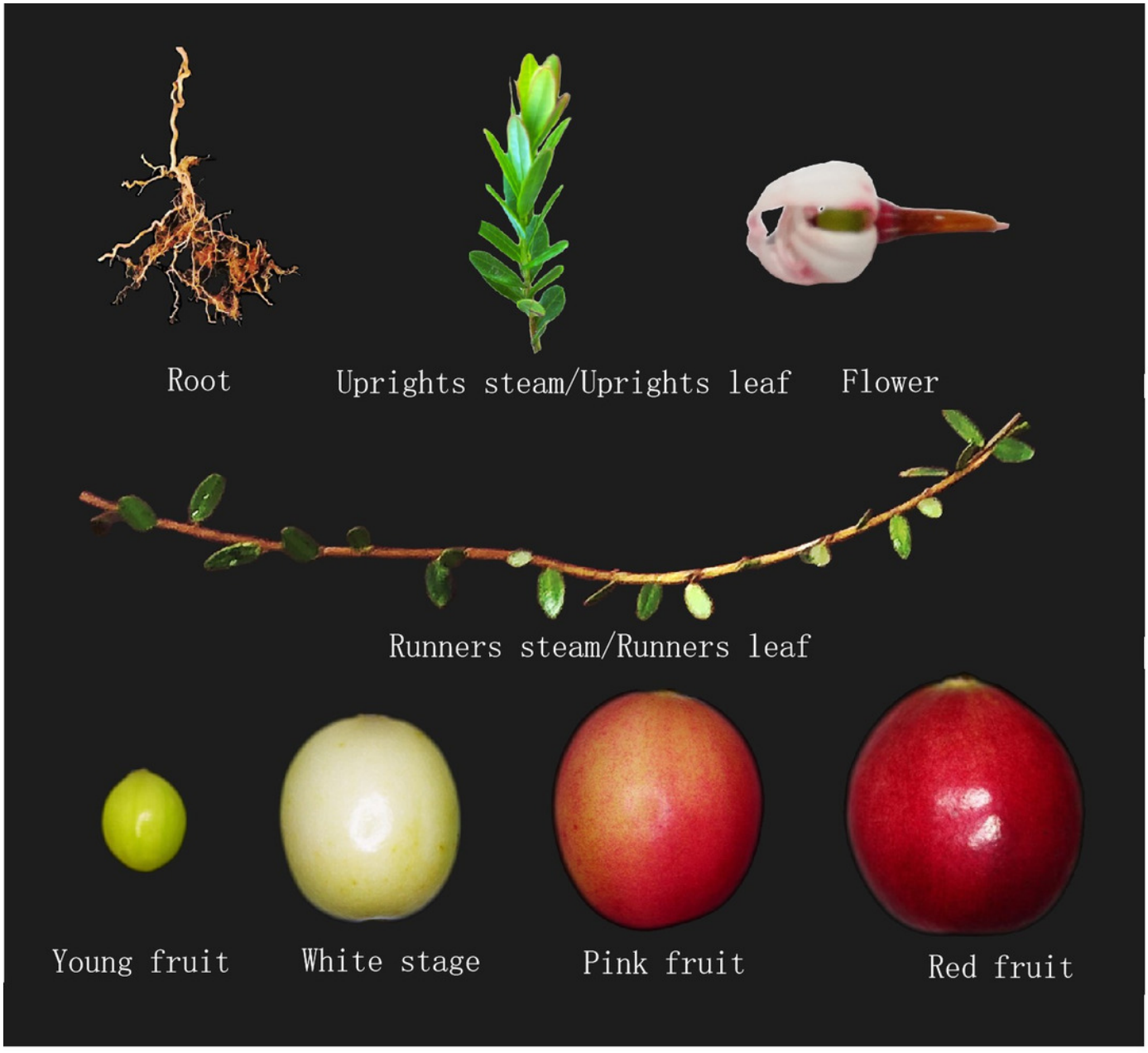

Figure 2

Phylogenetic analysis of the *SWEET* gene family in four species

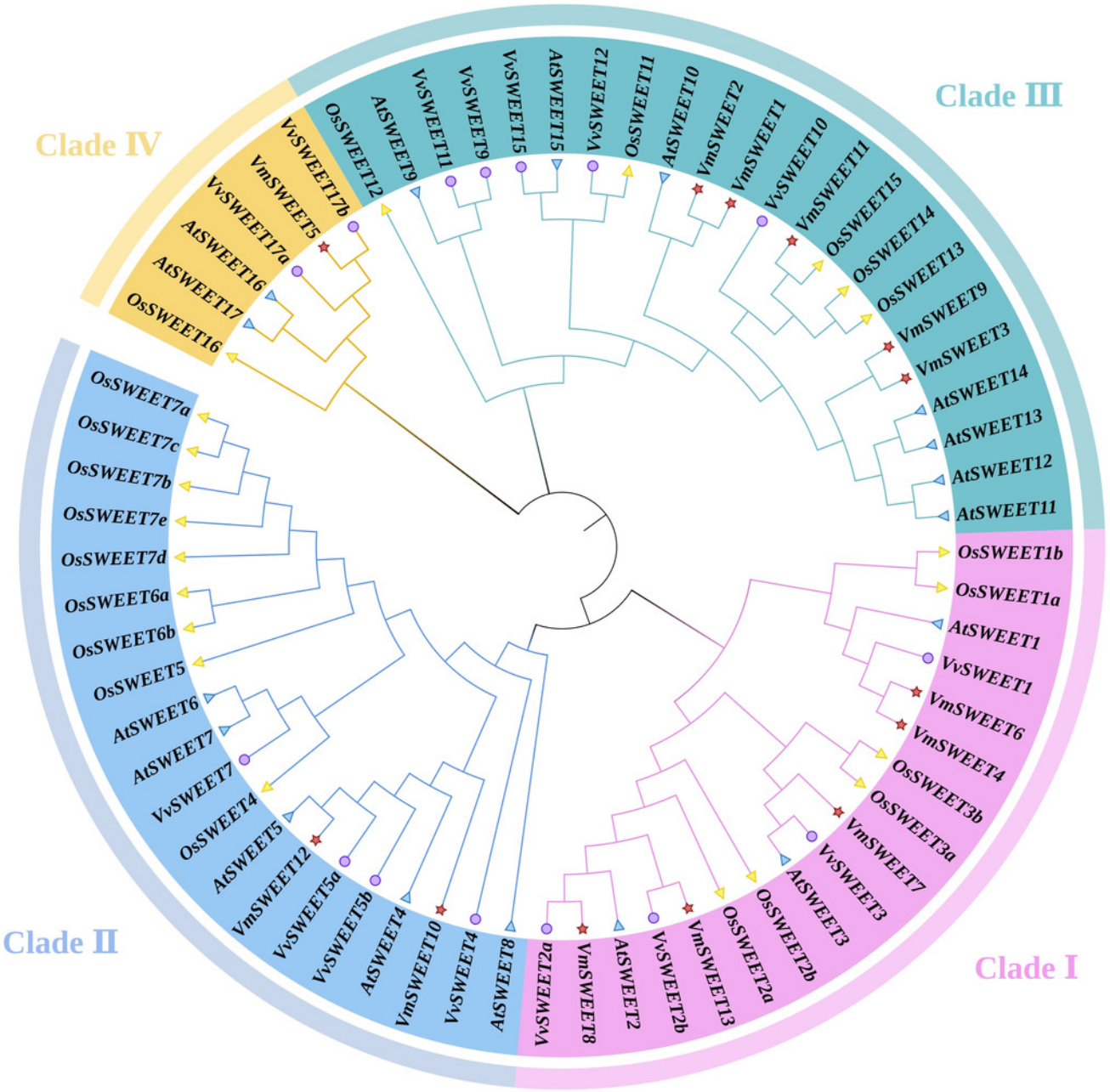

Figure 3

Multiple sequence alignment of the *VmSWEET*

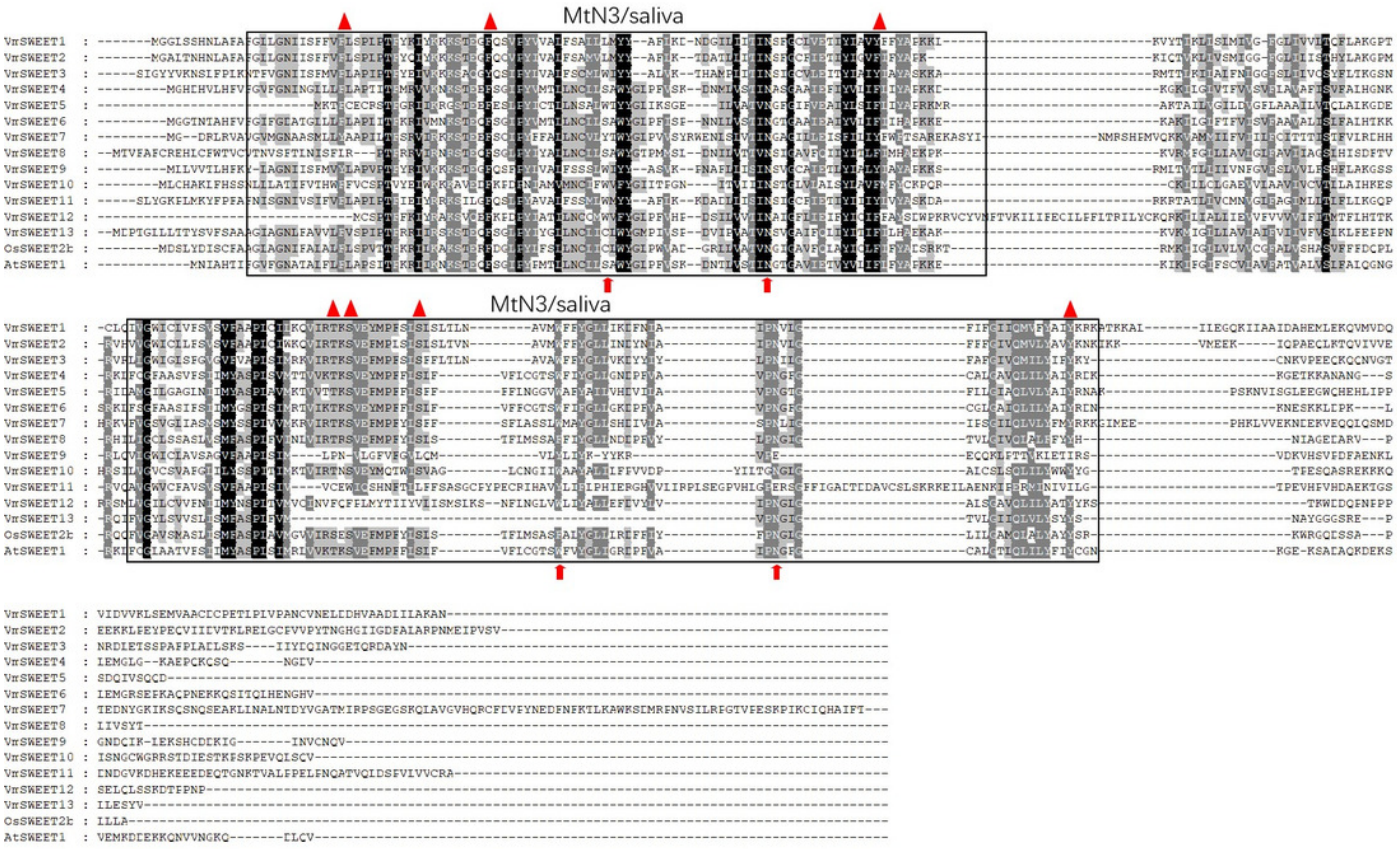

Figure 4

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

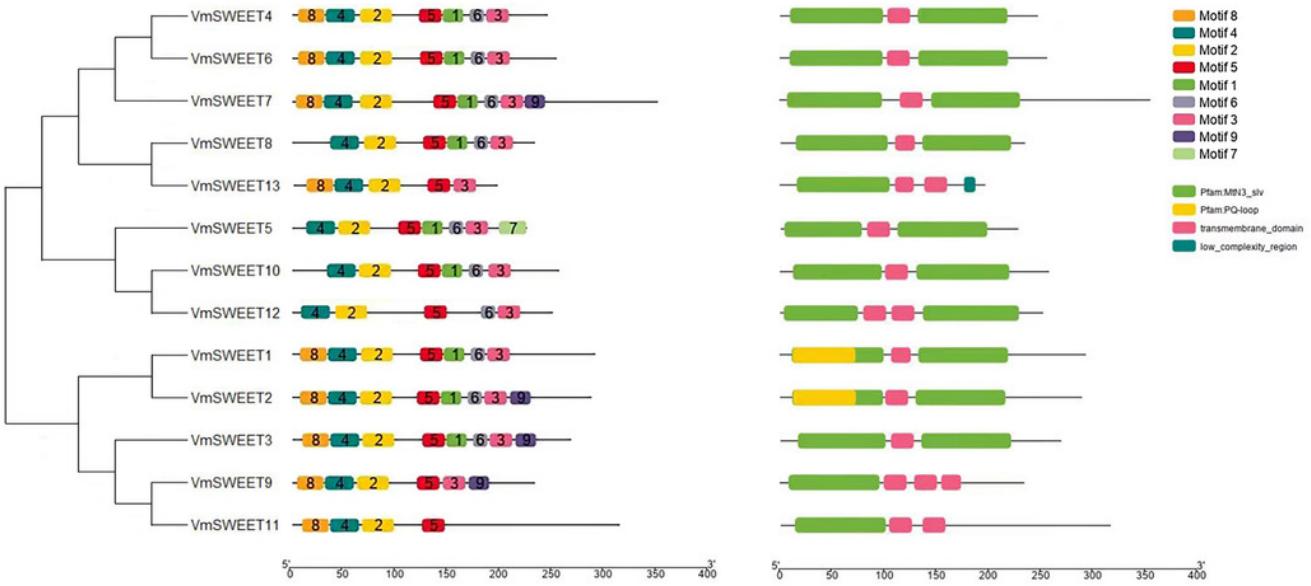

Figure 5

Gene structure of cranberry *SWEET* gene family

Figure 6

Chromosome mapping of *SWEET* genes in cranberry

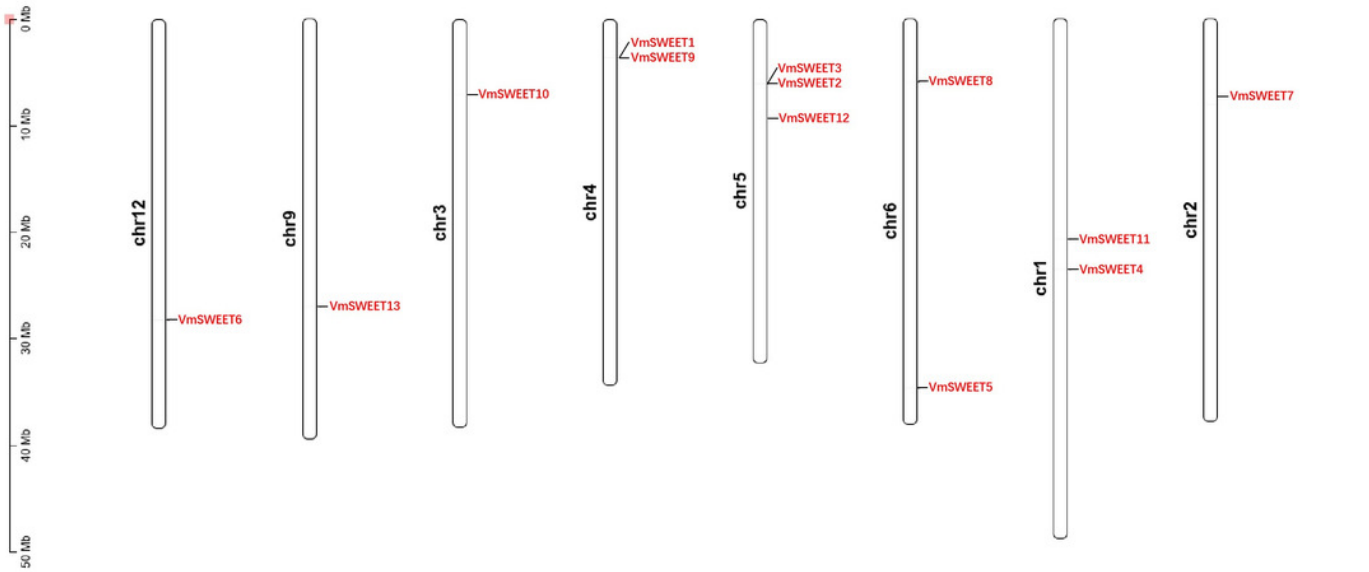

Figure 7

Collinearity analysis of *SWEET* gene family in cranberry

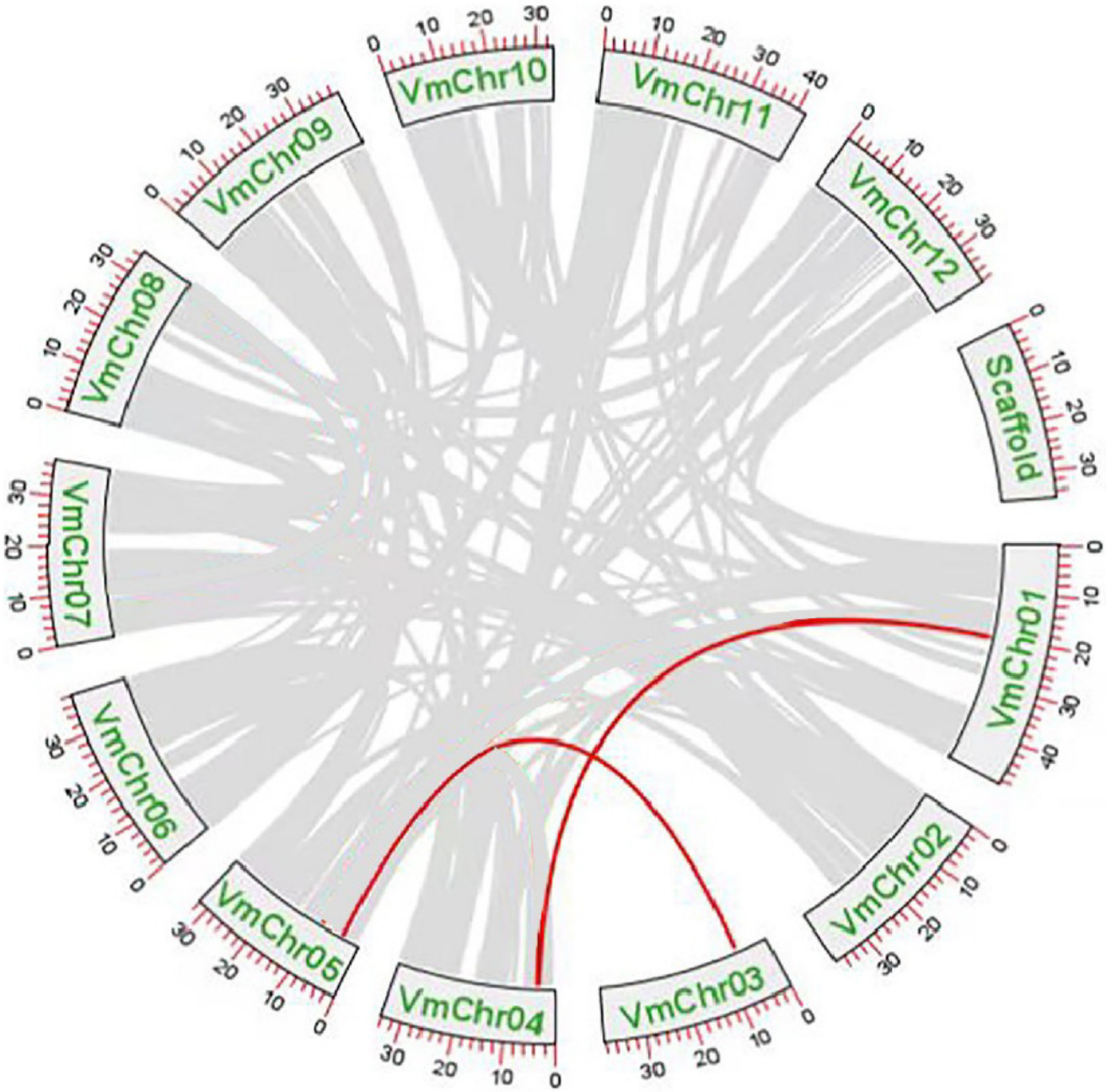

Figure 8

Promoter *cis*-acting elements of *VmSWEETs*

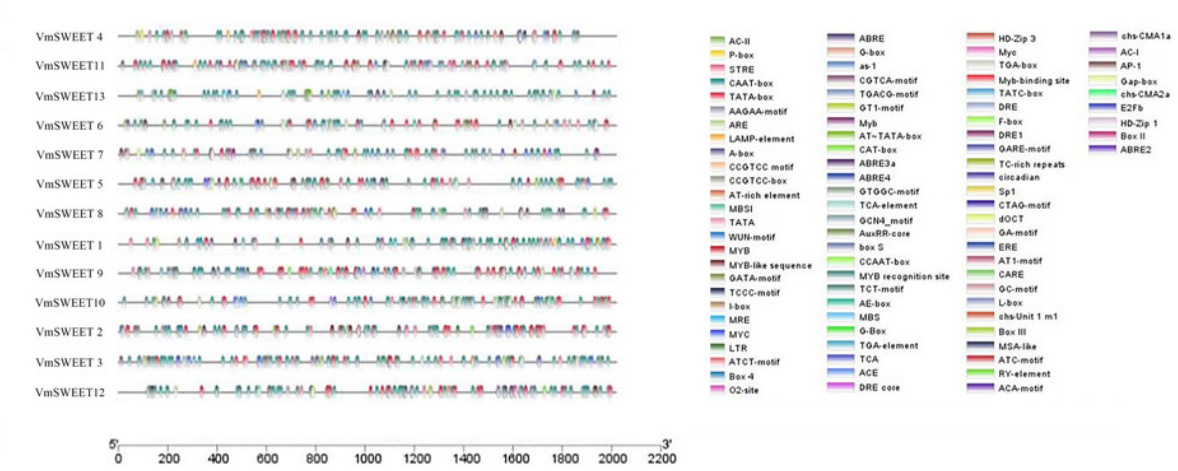

Figure 9

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

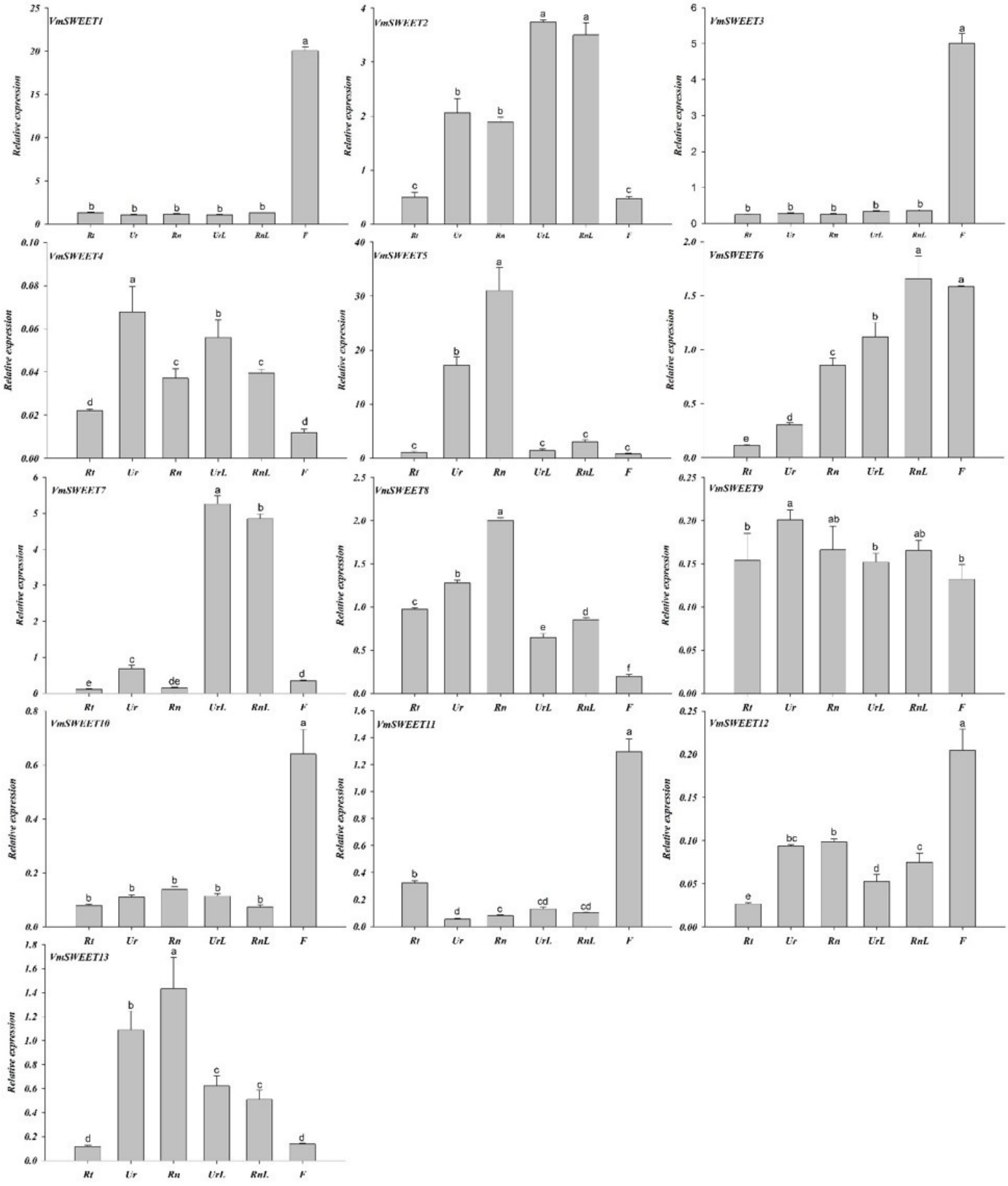
VmSWEET1
VmSWEET2
VmSWEET3
VmSWEET4
VmSWEET5
VmSWEET6
VmSWEET7
VmSWEET8
VmSWEET9
VmSWEET10
VmSWEET11
VmSWEET12
VmSWEET13
Relative expression
Rt
Ur
Rn
UrL
RnL
F

Figure 10

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

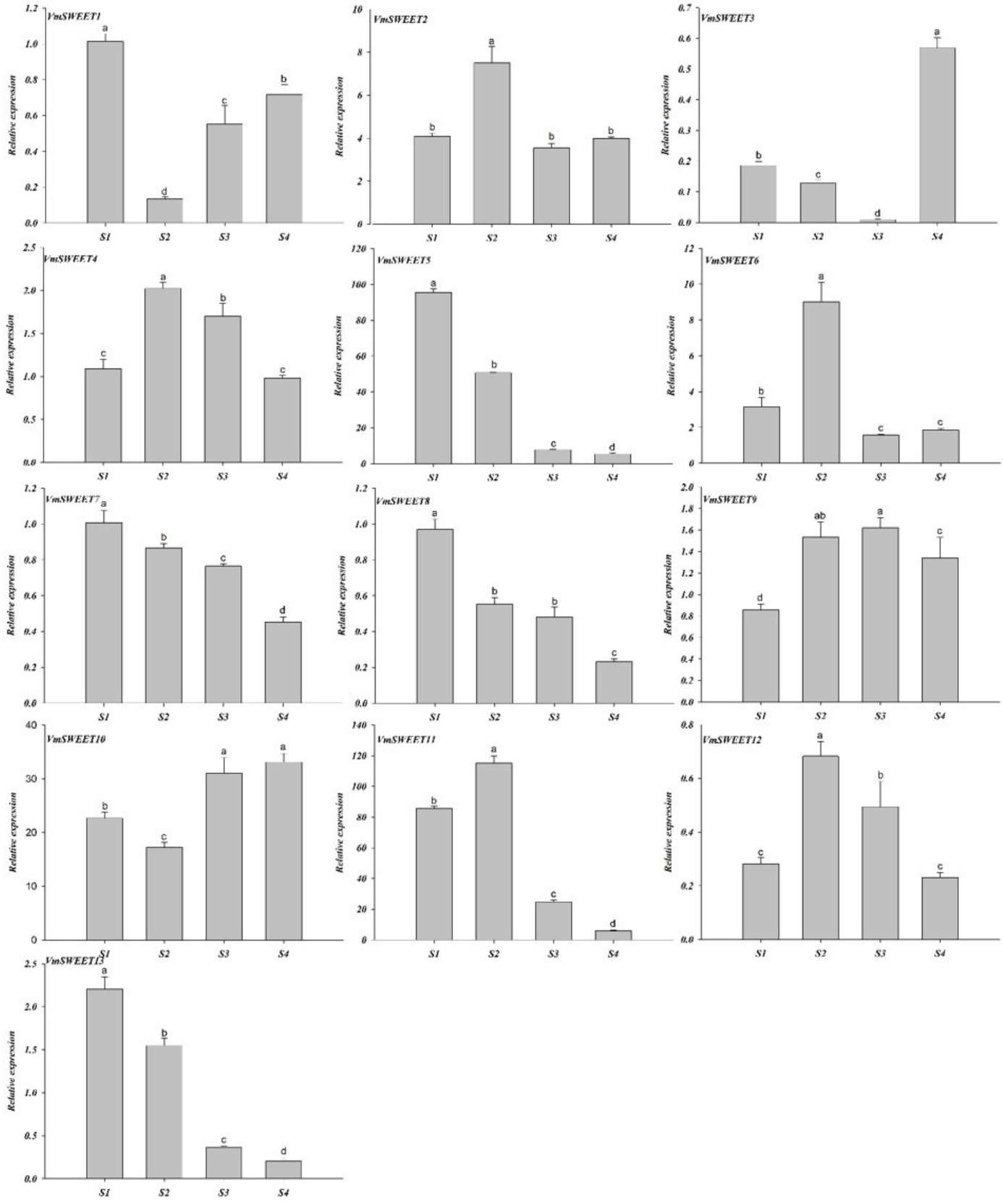
VmSWEET1
VmSWEET2
VmSWEET3
VmSWEET4
VmSWEET5
VmSWEET6
VmSWEET7
VmSWEET8
VmSWEET9
VmSWEET10
VmSWEET11
VmSWEET12
VmSWEET13
Relative expression
S1
S2
S3
S4

Figure 11

Gene expression heatmap of the *VmSWEET* genes in the leaf under abiotic stress

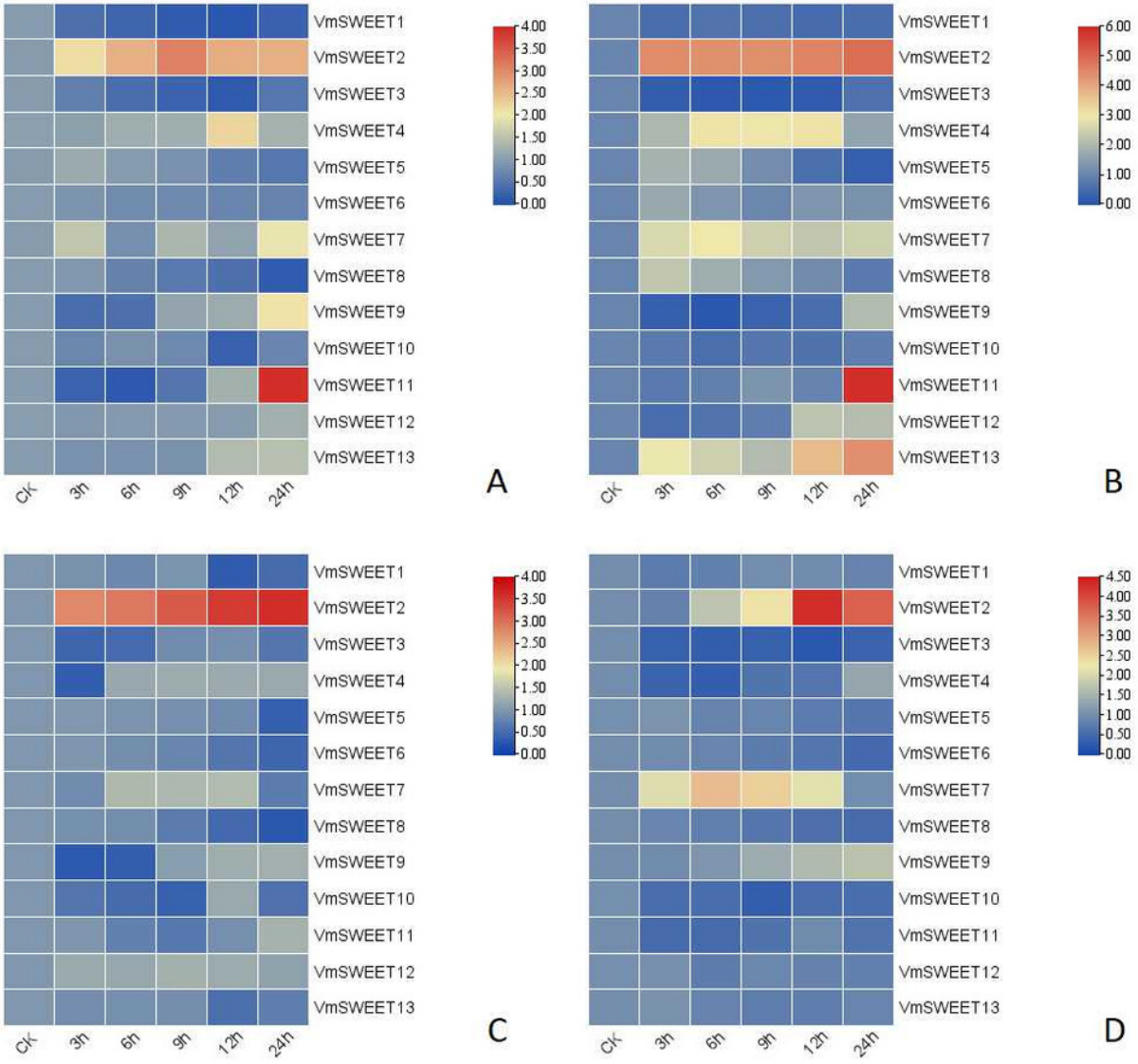

Figure 12

Schematic model of gene expression and role of *VmSWEET*s in different cranberry tissues and fruit development stages

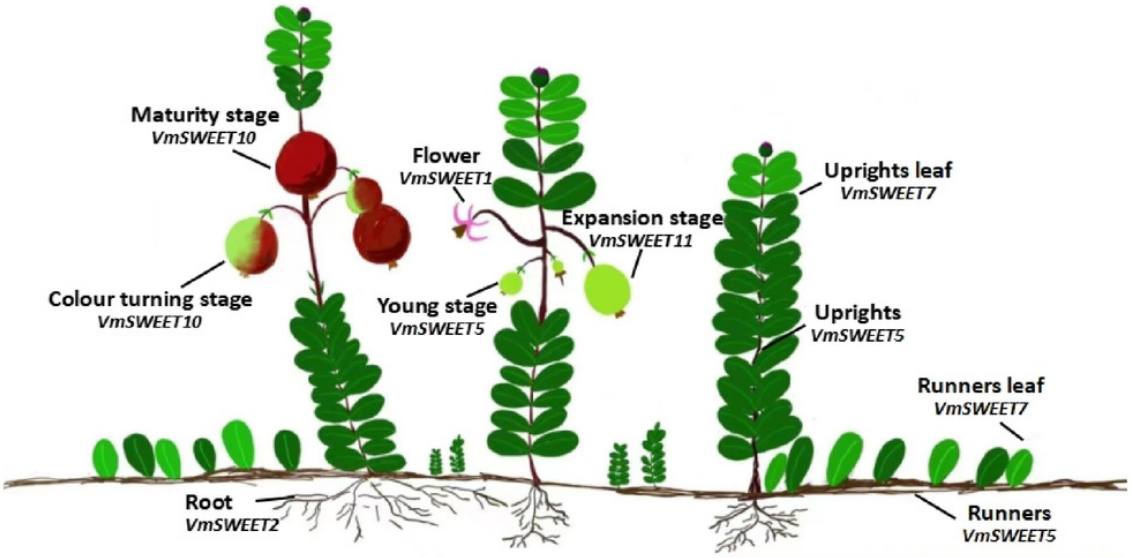
Maturity stage
VmSWEET10
Flower
VmSWEET1
Uprights leaf
VmSWEET7
Expansion stage
VmSWEET11
Colour turning stage
VmSWEET10
Young stage
VmSWEET5
Uprights
VmSWEET5
Runners leaf
VmSWEET7
Root
VmSWEET2
Runners
VmSWEET5

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 membrae

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	vmacro16733	879	292	32.48	8.56	38.24	128.53	0.861	7	PM
VmSWEET2	vmacro19147	867	288	32.42	8.94	36.98	117.36	0.666	7	PM
VmSWEET3	vmacro19148	807	268	30.41	9.44	39.65	111.31	0.512	7	PM
VmSWEET4	vmacro00890	741	246	26.91	9.2	30.53	106.95	0.628	7	TM
VmSWEET5	vmacro08173	681	226	24.93	6.82	41.97	116.95	0.613	6	PM
VmSWEET6	vmacro05470	768	255	28.11	9.61	30.52	107.49	0.507	6	ER
VmSWEET7	vmacro06571	1065	354	40.24	9.4	40.74	99.63	0.205	7	PM
VmSWEET8	vmacro09417	702	233	26.25	8.87	47.52	120.86	0.861	7	PM
VmSWEET9	vmacro16734	702	233	26.15	9.41	36.40	122.06	0.6	6	PM
VmSWEET10	vmacro18238	774	257	28.72	8.83	37.28	115.64	0.597	7	PM
VmSWEET11	vmacro01036	948	315	35.09	6.24	51.71	109.84	0.369	5	PM
VmSWEET12	vmacro19373	756	251	28.96	8.66	47.03	124.14	0.797	7	PM
VmSWEET13	vmacro03987	591	196	21.38	9.1	33.31	134.69	1.002	5	TM

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

4