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Genome-Wide Identification, Characterization, and Expression Profile of SWEETs Gene Family in Grapevine (*Vitis vinifera* L.)

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Abstract: SWEET (Sugars Will Eventually Be Exported Transporter) proteins, identified recently as a novel class of sugar transporters, play pivotal roles in the transport and distribution of photosynthetic products in plants. They are integral to physiological processes such as response to biotic and abiotic stress, growth and development, and fruit quality formation. In this study, leveraging the latest grapevine genomic data, we identified 18 members of the grapevine *SWEET* family and named them based on their homologs in Arabidopsis. We conducted a detailed analysis of these protein-encoding genes, focusing on their structure, conserved domains, and phylogenetic relationships. Phylogenetic analysis revealed that the grapevine *SWEET* family members could be categorized into four clades, with the majority of members displaying relatively conserved gene structures and motifs. Chromosomal localization and homology analysis indicated an uneven distribution of *VvSWEETs* across 11 chromosomes, with evidence of two segmental duplication events during evolution. Furthermore, we investigated the transcription levels of *SWEET* genes across different tissues, organs, and developmental stages of fruit, as well as their response patterns under abiotic stress (drought, cold, and salt stress) and biotic stress (*Botrytis cinerea* infection). Expression profiling demonstrated strong tissue-specificity and temporal-spatial specificity of *VvSWEETs*, correlated with their respective clades. It is noteworthy that the expression levels of most members within Clade 1 of the *VvSWEET* gene family, especially *VvSWEET1*, were markedly upregulated in response to a broad range of stress conditions. Our results provide a comprehensive bioinformatic characterization and analysis of the grapevine *SWEET* gene family, unveiling the potential functions of grapevine *SWEET* genes and offering a vital reference for further functional studies.

Keywords: grape; VvSWEET; gene family; expression profile; abiotic and biotic stress

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1. Introduction

Sugar serves as an essential carbon source for plant growth and development, participating in numerous physiological processes such as energy metabolism and signal transduction as substrates for the production of primary and secondary metabolites. This participation is crucial for maintaining plant growth and development, as well as responding to stress [1,2]. Consequently, the transport and distribution of sugars are vital in plants. However, sugars cannot independently cross the plant's biomembrane systems for transport and require the assistance of specific sugar transporter proteins [3].

In plants, three main families of sugar transport proteins exist: Monosaccharide Transporters (MSTs) [4], Sucrose Transporters (SUTs) [5], and Sugars Will Eventually be Exported Transporters (SWEETs) [6]. SWEETs represent a newly discovered sugar transport protein family (PF03083) in recent years. In comparison to the former two, SWEETs can transport sugars bidirectionally across membranes without depending on the environmental pH and along concentration gradients [5,7]. In eukaryotic organisms, SWEET proteins consist of a typical 7-transmembrane α-helical structure domain (7-TMs), including two conserved MtN3/saliva domains. Each domain is comprised of 3-TMs forming triple-helix bundles (THB). In prokaryotic organisms, SWEET homologs, known as semi-SWEET, only contain a single 3-TMs domain but still possess the ability to transport sucrose.

SWEET proteins are ubiquitously present in prokaryotes, plants, humans, and other animals, and exhibit a high degree of conservation. With the completion of plant genome sequencing, *SWEET* genes in multiple species have been successively identified. To date, the identification and analysis of the *SWEET* gene family have been reported in a variety of plants (Table 1). Phylogenetic analysis reveals that this family is generally divided into four clades, with members of Clade I and Clade II primarily transporting hexoses, Clade III members mainly transporting sucrose, and Clade IV members localized to the vacuolar membrane, tending to transport fructose [8].

Numerous studies have demonstrated that SWEET sugar transport proteins participate in essential physiological processes related to plant growth and development by regulating the transportation, distribution, and storage of sugar compounds within plants. These processes include pollen development, fruit ripening, and leaf senescence, among others [9,10]. Mutation in the *AtSWEET8* gene results in male sterility, as it fails to transport glucose for pollen nutrition, leading to pollen unviability [6]. Similarly, in rice, *OsSWEET11* supports pollen vitality; silencing of the *OsSWEET11* gene reduces starch content in pollen and may lead to male sterility in rice [11]. In pineapple, *AnmSWEET5* and *AnmSWEET11* are highly expressed in the early stages of fruit development [12]. Likewise, in the apple genome, nine *MdSWEET* genes are highly expressed throughout fruit development, with *MdSWEET9b* and *MdSWEET15a* likely involved in the regulation of sugar accumulation in apples [13]. Overexpression of *OsSWEET5* in rice results in stunted plant growth [14].

The *SWEET* family also extensively responds to various stress conditions. Overexpression of *AtSWEET16* and *AtSWEET17* in Arabidopsis enhances the transgenic plants' cold tolerance [15]. In tea plants, the expression of *CsSWEET2*, *CsSWEET3*, and *CsSWEET16* is significantly suppressed under cold stress, whereas the expression of *CsSWEET1* and *CsSWEET17* dramatically increases [16]. Transgenic Arabidopsis overexpressing *AtSWEET15(SAG29)* is more sensitive to salt stress, and *AtSWEET15* mutants exhibit enhanced salt tolerance [17]. Heat stress negatively affects the SWEET functions in phloem loading, unloading, and long-distance transport of sugars, ultimately leading to abnormal plant growth and development [18]. Drought stress induces the expression of *AtSWEET11* and *AtSWEET12*, promoting the transport of sucrose from leaves to roots [19]. In tomatoes, the *SlSWEET* genes have multiple cis-acting elements related to stress and hormone responses in their promoter regions. The expression levels of several *SlSWEET* genes change significantly in leaves, roots, mature green fruits, and ripe red fruits under high-sugar, high-salt, high-temperature, and low-temperature conditions [20]. In addition to the abiotic stresses mentioned, most pathogens require glucose from the host plant as a carbon source for their growth before successful invasion. SWEET sugar transport proteins control the sugar competition at the plant–microbe interface, and their regulation determines the outcome of the interaction. For example, in rice, the pathogen Xanthomonas oryzae pv. oryzae secretes specific transcription activator-like (TAL) effecto rs after infection, which bind precisely to the cis-regulatory elements of the rice *OsSWEET11* gene promoter, regulating transcription and increasing sugar efflux, which is ultimately exploited by the pathogen, leading to plant susceptibility [21].

Grapes (*Vitis vinifera* L.) are one of the world's most economically valuable perennial fruit crops. Breeding for improved varieties through conventional breeding and biotechnological approaches holds great promise, but progress is limited due to a general lack of

understanding of key genes involved in stress responses, restricting the selection process. Research on gene families is critical for analyzing gene origins and predicting gene functions. The publication of the grape genome [22] has facilitated the identification of gene families. In this study, we identified members of the *SWEET* family from the grape genome and conducted a bioinformatics analysis of the *SWEET* genes, including phylogenetic relationships, chromosomal localization, exon–intron structures, motif composition, and collinearity analysis. Furthermore, we extracted related transcriptome data to analyze the expression patterns of the *SWEET* family in different grape tissues and organs, at various fruit developmental stages, and under abiotic stress conditions. In summary, this work provides important information for future research on the biological functions of grape *SWEET* family members and lays a foundation for further utilization of these genes in breeding high-quality new grape varieties.

Table 1. SWEET families of several plant species.

Type	Species	Number of SWEET Members	Reference
Monocotyledons	*Oryza sativa*	21	[23]
	Sorghum bicolor	23	[24]
	Musa acuminata	25	[25]
	Ananas comosus	39	[12]
	Bletilla striata	17	[26]
	Saccharum spontaneum	22	[27]
	Zea mays	24	[28]
	Prunus mume	17	[29]
	Triticum aestivuml	59	[30]
	Hordeum vulgare	23	[31]
Dicotyledons	*Arabidopsis thaliana*	17	[6]
	Manihot esculenta	23	[32]
	Citrus sinensis	16	[33]
	Eucalyptus grandis	52	[34]
	Glycine max	52	[35]
	Solanum lycopersicum	29	[20]
	Solanum tuberosum	35	[36]
	Pyrus bretschneideri	18	[37]
	Gossypium hirsutum	55	[38]
	Malus domestica	25	[13]
	Camellia sinensis	13	[39]
	Litchi	16	[40]
	Medicago truncatula	25	[41]
	Brassica oleracea	30	[42]
	Juglans regia	25	[43]
	Citrullus lanatus	22	[44]
	Punica granatum	20	[45]
	Dimocarpus longan	20	[46]
	Beta vulgaris	16	[47]
	Rosa rugosa	25	[48]
	Prunus salicina	15	[49]
	Capsicum annuum	33	[50]
	Medicago polymorpha	23	[51]
	Ziziphus jujuba	19	[52]
	Betula platyphylla	13	[53]
	Potentilla anserina	23	[54]

2. Materials and Methods

2.1. Identification and Annotation of SWEET Genes in the Grapevine Genome

To pinpoint potential *SWEET* genes in grapevine, the newest grape genome iteration (12X.v2) along with the VCost.V3 gene annotations were retrieved from the URGI portal (https://urgi.versailles.inra.fr/Species/Vitis/Annotations, accessed on 2 January 2023) [55]. The Hidden Markov Model (HMM) profile specific to the MtN3_saliva domain (accession:PF03083) was acquired from the Pfam database (http://pfam.xfam.org/family/PF03083) (accessed on 2 January 2023) [56], and the search for prospective *SWEET* genes in the grape genome was conducted using the HMMER3.0 tool, setting the E-value $\leq$ 0.01 [57,58]. CRIBI v2.1 ID and Locus ID information were sourced from the Phytozome v13 database

(https://phytozome-next.jgi.doe.gov) (accessed on 2 January 2023) [59] and the Grape Genome Browser (12X) (https://www.genoscope.cns.fr/vitis) (accessed on 3 January 2023) [22]. Verification of the MtN3_saliva domain's authenticity was carried out using the SMART resource (http://smart.embl-heidelberg.de) (accessed on 3 January 2023) [60] and the Conserved Domain Database (https://www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) (accessed on 3 January 2023) [61]. Predictions on the molecular weight (Mw) and isoelectric point (pI) of the identified SWEET proteins were made via the ProtParam utility (http://web.expasy.org/protparam/) (accessed on 3 January 2023) [62].

2.2. Phylogenetic and Conserved Domain Alignment Analysis

The alignment of the amino acid sequences within the MtN3_saliva domain was executed employing DNAMAN (Version 7.0.2, Lynnon Biosoft, San Ramon, CA, USA), followed by the generation of sequence logos utilizing WebLogo 3 (http://weblogo.threeplusone.com) (accessed on 4 January 2023) [63,64]. To align full-length protein sequences, the Muscle tool integrated into the MEGA 7.0 software suite was utilized, facilitating the subsequent construction of a phylogenetic tree through the Neighbor-Joining (NJ) method [65], supported by 1000 bootstrap iterations. The analysis was conducted under the following parameters: application of the Poisson correction model, assumption of uniform evolutionary rates across sites, consideration of sites as homogeneous (identical), and the implementation of pair-wise deletion for handling gaps. Sequences of SWEET proteins from various species, including *Arabidopsis* (*AtSWEET*), *Oryza sativa* (*OsSWEET*), *Cucumis sativus* (*CsSWEET*), and *Hemerocallis* (*HfSWEET*), were procured from their respective species-specific genome repositories.

2.3. Analysis of Exon–Intron Structure and Conserved Motifs

The exon–intron configurations of validated *SWEET* genes were ascertained through comparison of their coding sequences against the complete genomic sequences found in the Grapevine Genome (12X) repository (https://www.genoscope.cns.fr/vitis/) (accessed on 5 January 2023) [22]. Illustrations of these exon–intron configurations were produced via the Gene Structure Display Server 2.0, accessible online (http://gsds.cbi.pku.edu.cn) (accessed on 5 January 2023) [66]. In addition, the conserved motifs within all SWEET proteins were delineated employing the MEME analysis platform available online (http://meme-suite.org/tools/meme) (accessed on 5 January 2023) [67], setting the limit for the number of motifs to 16 while maintaining default settings for all other parameters. Only motifs exhibiting an E-value inferior to 0.05 were considered for presentation. Furthermore, TBtools software (version 1.098) was engaged to visualize the distribution of these conserved motifs [68].

2.4. Chromosomal Localization and Synteny Analysis

Based on the physical location information from the latest version of the grape genome annotation, the chromosomal positions of each *VvSWEET* gene were determined [69]. MCScanX software (version 1.1.11) was used to identify and analyze the collinearity blocks between grape *SWEET* genes and between grape and Arabidopsis. The collinearity analysis and chromosomal localization maps were drawn using the Circos-0.69-6 program (http://circos.ca) (accessed on 10 January 2023) [70]. The non-synonymous (Ka) and synonymous (Ks) substitution rates for each gene pair were calculated using TBtools software [69]. The divergence time (T) was calculated using the Ks values with the formula: $T = Ks/(2\lambda)$, where λ is the rate of divergence for the grape species, estimated at 6.5×10^{-9} [71].

2.5. Analysis of Expression Profiles in Various Organs and Different Berry Developmental Stages

VvSWEET microarray expression data from different vegetative and reproductive organs at various developmental stages were acquired from the GEO datasets from the GSE36128 series [72] (see Supplementary File: Table S1). The microarray expression data from different developmental stages of berries were obtained from the GEO database series GSE98923 [73] (see Supplementary File: Table S2).

2.6. Analysis of Expression Profiles in Different Abiotic Stress Conditions

The RNA-seq data of *VvSWEET* exhibit responses to cold, drought, and salt stress, derived from datasets available in the published literature as detailed below:

- Leaves from one-year-old potted grapevine plants with cold-resistant (*V. amurensis S'huangyou'*) and cold-sensitive (*V. vinifera cultivar R'ed Globe'*) varieties after cold stress (0 °C) for 3, 12, 48, and 72 h [74].
- Leaves of two-year-old potted cutting seedlings from the drought-resistant (*V. yeshanensis 'Yanshan-1'*) and the drought-sensitive (V. *riparia' He'an'*) varieties, after drought stress for 0, 8, 16, and 24 days [75].
- Two-year-old potted grapevine rootstocks, including the salt-tolerant varieties 3309C (*V. riparia* × *V. rupestris*), 520A (*V. berlandieri* × *V. riparia*), and 1103P (*V. berlandieri* × *V. rupestris*), as well as the salt-sensitive varieties 5BB (*V. berlandieri* × *V. riparia*), 101–14 (*V. riparia* × *V. rupestris*), and Beta (*V. riparia* × *V. labrusca*), were irrigated with a 130 mmol L^{-1} NaCl solution for two consecutive days to induce salt stress [76].

The RPKM (Reads Per Kilobase of transcript per Million mapped reads) values were employed to evaluate the expression of *VvSWEET*, and all heatmaps were produced utilizing the software package R version 4.2.2 (https://www.r-project.org/) (accessed on 10 January 2023).

2.7. Expression Profiles to Biotic Stress

Eight grapevine cultivars were selected for resistance/susceptibility to *Botrytis cinerea* from the grapevine germplasm resource vineyard of Northwest A&F University, Yangling, Shaanxi, China (34°20′ N, 108°24′ E). Specifically, young leaves of four resistant varieties (*R'ed Globe'*, *G'ebixinxiu'*, *T'hompson Seedless'*, *J'ingXiangyu'*) and four susceptible varieties (*S'huangyou'*, *B'eihong'*, *B'eimei'*, *G'old Finger'*) grown under natural environmental conditions [77–79]. Among these, young leaves from *S'huangyou'* and *R'ed Globe'* were inoculated with *Botrytis cinerea* as previously described [77] and collected at 0, 24, and 48 h post-inoculation. All samples were rapidly frozen in liquid nitrogen and stored at −80 °C.

2.8. RNA Extracted and qRT–PCR Analysis

Total RNA was extracted and purified using EZNA Plant RNA Kit (R6827–01, Omega Bio-tek, Norcross, GA, USA). RNA purity and quantity were determined using a NanoDrop 2000 Spectrophotometer (Thermo Fisher Scientific, Wilmington, DE, USA). First-strand cDNA synthetization was performed using the Prime Script RT reagent Kit (TaKaRa Biotechnology, Dalian, China). The resulting cDNA was diluted sixfold for use in quantitative RT-PCR experiments. Three biological replicates were set up. Relative expression levels were calculated using the $2^{-\Delta\Delta CT}$ method with the grapevine *ACTIN1* (Vitvi04g01613.t01) as a reference gene [80]. GraphPad Prism 8.0 software was used for Student's *t*-test ($p < 0.01$) and correlation analysis ($p < 0.01$).

3. Results

3.1. Identification and Characterization of Grape SWEET Genes

Through the search and filtration of grapevine genomic data (https://www.genoscope.cns.fr/vitis) (accessed on 10 January 2023), we have identified a total of putative 18 *SWEET* genes. Given the consistency of the grapevine naming system [81] and their homology with *Arabidopsis*, these 18 genes were designated as *VvSWEET1*-*VvSWEET17d* (Table 2). The analysis of physicochemical properties revealed that the sizes of grapevine *SWEET* genes range from 606 bp (*VvSWEET17a*) to 897 bp (*VvSWEET17d*), encoding proteins between 201 to 289 amino acids in length, with an average length of 254.3 amino acids. The predicted molecular weights ranged from 22.0 to 32.8 kDa, with an average molecular weight of 28.4 kDa, and theoretical isoelectric points varied from 5.1 to 9.7. All identified grapevine SWEET proteins exhibited an average hydrophobicity index greater than 0, classifying them as hydrophobic proteins.

Table 2. Detailed information of *VvSWEET* gene family members in grapevine.

Gene Name	VCost. v3 ID	Gene Locus ID	Accession No	CDS (bp)	ORF (aa)	Chromosome	MW	pI	Instability Index	Aliphatic Index	GRAVY
VvSWEET1	Vitvi18g01215.t01	GSVIVT01010015001	XP_002265836.1	747	248	Chr18: 13476398—-13478625 (−)	27,352.74	9.64	28.21	111.29	0.682
VvSWEET2	Vitvi10g00679.t01	GSVIVT01021317001	XP_002269484.1	699	232	Chr10: 7519651—7522710 (+)	25,777.62	8.84	53.21	115.99	0.839
VvSWEET2b	Vitvi19g00024.t01	GSVIVT01014088001	XP_010644065.1	708	235	Chr19: 278130—280739 (−)	26,117.95	9.30	47.26	117.32	0.741
VvSWEET3	Vitvi16g01984.t01	GSVIVT01028713001	XP_002267886.1	750	249	Chr16: 21023729—21025870 (+)	28,036.27	9.13	39.81	116.14	0.535
VvSWEET4	Vitvi14g01783.t01	GSVIVT01032489001	XP_002274582.1	765	254	Chr14: 27825326—27827944 (−)	27,899.02	9.36	38.85	108.98	0.517
VvSWEET5a	Vitvi17g00791.t01	GSVIVT01007779001	XP_002283068.1	705	234	Chr17: 9249190—9250708 (+)	26,160.48	9.43	29.69	124.06	0.693
VvSWEET5b	Vitvi17g00793.t01	GSVIVT01007777001	XP_002279850.1	699	232	Chr17: 9294434—9295940 (+)	25,967.16	9.49	30.31	119.66	0.698
VvSWEET7	Vitvi02g00181.t01	GSVIVT01019601001	XP_002263697.1	783	260	Chr02: 1670296—1672808 (−)	28,845.49	9.62	43.99	116.54	0.695
VvSWEET9a	Vitvi04g01075.t01	GSVIVT01026399001	XP_002267792.1	837	278	Chr04: 15790881—15792999 (−)	31,515.37	9.05	39.12	112.16	0.576
VvSWEET9b	Vitvi04g01077.t01	-	RVW45685.1	795	264	Chr04: 15798938—15800244 (+)	29,875.24	8.34	31.28	100.80	0.454
VvSWEET9c	Vitvi07g00250.t01	GSVIVT01010993001	XP_002270131.1	831	276	Chr07: 2749916—2751667 (−)	31,004.89	9.30	34.08	106.05	0.535
VvSWEET10a	Vitvi17g00069.t01	GSVIVT01008597001	XP_002280599.1	852	283	Chr17: 678049—680301 (+)	31,699.78	9.34	38.63	112.93	0.575
VvSWEET10b	Vitvi17g00070.t01	GSVIVT01008595001	XP_002284244.1	813	270	Chr17: 682658—684513 (+)	30,608.80	8.90	34.63	127.70	0.836
VvSWEET15	Vitvi01g01719.t01	GSVIVT01000938001	NP_001384792.1	864	289	Chr01: 23092093—23093857 (−)	32,149.03	9.08	35.66	110.00	0.492
VvSWEET17a	Vitvi05g00013.t01	GSVIVT01035138001	XP_010649584.1	606	201	Chr05: 123990—126791 (−)	21,952.92	5.06	36.17	122.69	0.772
VvSWEET17b	Vitvi14g00147.t01	GSVIVT01031172001	RVW97796.1	714	237	Chr14: 1525182—1527230 (−)	26,266.34	6.29	48.06	128.23	0.845
VvSWEET17c	Vitvi14g00148.t01	GSVIVT01031172001	RVW97798.1	717	238	Chr14: 1533798—1535492 (−)	26,473.40	6.90	46.38	124.79	0.782
VvSWEET17d	Vitvi14g00149.t01	GSVIVT01031170001	XP_002279031.1	897	298	Chr17: 1541544—1545073 (−)	32,764.71	9.68	37.96	107.58	0.424

CDS: coding sequence, ORF: open reading frame, MW: molecular weight, pI: isoelectric points, and GRAVY: grand average of hydropathicity.

3.2. Phylogenetic Analysis of the Grape SWEET Proteins

To analyze the evolutionary relationships and potential functional divergences within the *VvSWEET* gene family, we compiled a dataset consisting of 94 SWEET protein sequences from various species, including 17 from Arabidopsis, 21 from rice, 17 from cucumber, 19 from daylily, and 18 from grape. A phylogenetic tree was constructed using the Neighbor-Joining (NJ) method (Figure 1). These SWEET proteins were divided into four typical clades. Clade I includes 4 VvSWEETs (1, 2a, 2b, 3), Clade II comprises 4 VvSWEETs (4, 5a, 5b, 7), Clade III consists of 6 VvSWEETs (9a–c, 10a, 10b, 15), and Clade IV includes 4 VvSWEETs (7a–d). Among these, five pairs of proteins, VvSWEET2a/2b, 5a/5b, 10b/15, 9a/9b, and 17b/17c, exhibit high amino acid sequence similarity and are closely branched on the phylogenetic tree, suggesting possible functional redundancy among these *VvSWEET* genes. In contrast, five other VvSWEET proteins (VvSWEET1, VvSWEET13, VvSWEET17, VvSWEET10a, VvSWEET17a) show lower homology compared to other family members, resulting in their distant and independent branches on the phylogenetic tree.

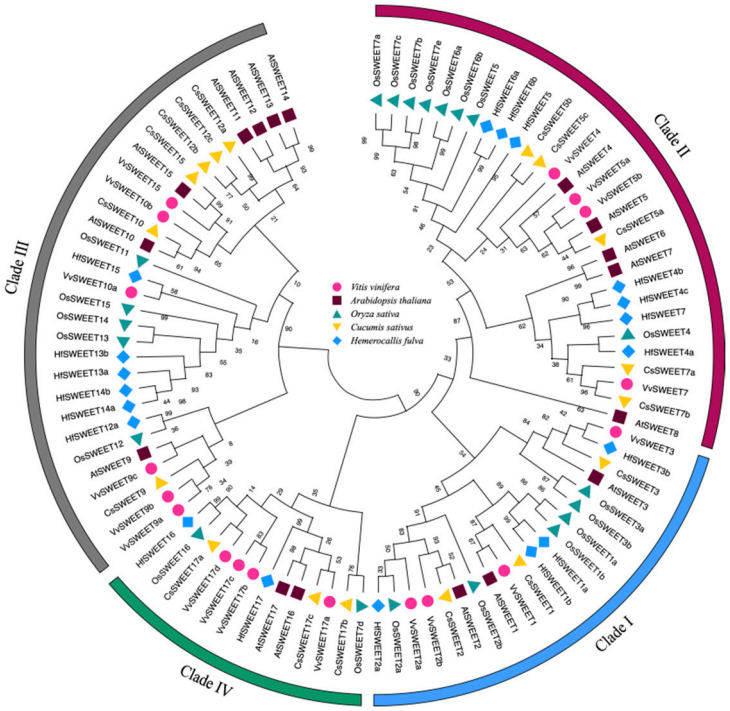


Figure 1. Phylogenetic analysis of SWEET proteins from grapevine, Arabidopsis, Rice, Cucumber, and Daylily. The tree was divided into four clades, which are marked by different colors and named as Clade I, II, III and IV. The bootstrap values are indicated at each node.

3.3. Analysis of Conserved Protein Motifs and Exon–Intron Structure of Grape SWEET Genes

To further understand the conservation and diversity of the grape *SWEET* gene family, we analyzed the conserved protein motifs encoded by the genes, identifying a total of 11 conserved motifs (Figure 2B). The phylogenetic tree constructed from the conserved domain sequences of the 18 grape SWEET proteins (Figure 2A) shares a generally consistent topology with the evolutionary tree constructed from SWEET proteins of five plant species (Figure 1). Members with similar motif compositions clustered into groups, indicating functional similarities among SWEET proteins within the same group. Motifs 1, 2, 3, 4, and 6 were found in almost all VvSWEET proteins, demonstrating their high conservation in the VvSWEET protein sequences. Motif 11 is unique to Clade II, which may be a reason for the functional diversification of *VvSWEET* genes. An analysis of the exon–intron structures of the 18 *VvSWEET* genes was also conducted (Figure 2C). The number of introns in most *VvSWEET* genes is relatively stable, with 14 genes having 5 introns, accounting for 77.8% of the total number of family members. Notably, *VvSWEET9b* exhibits the lowest intron

count with only three introns. The distribution of introns shows a phase order of 1, 2, 0. *VvSWEETs* with closer evolutionary relationships often have similar exon–intron structures, such as the 4 members of Clade I (*VvSWEET1~VvSWEET3*) which have the same number of exons.

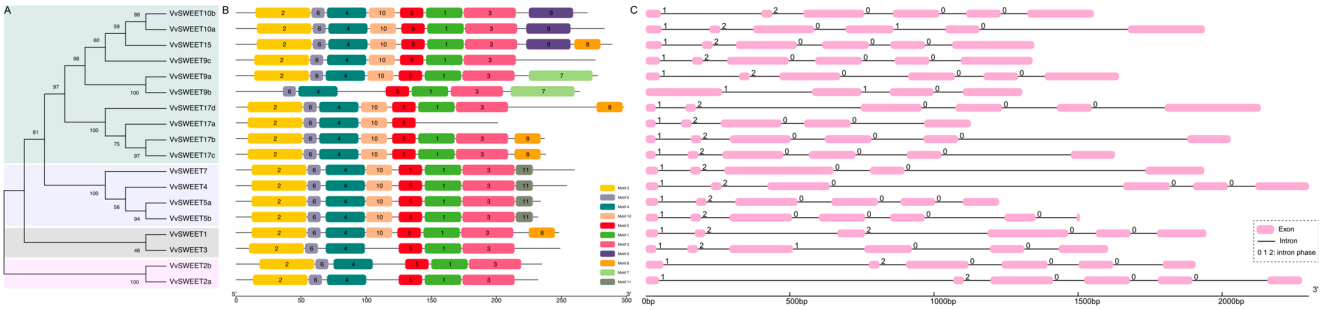
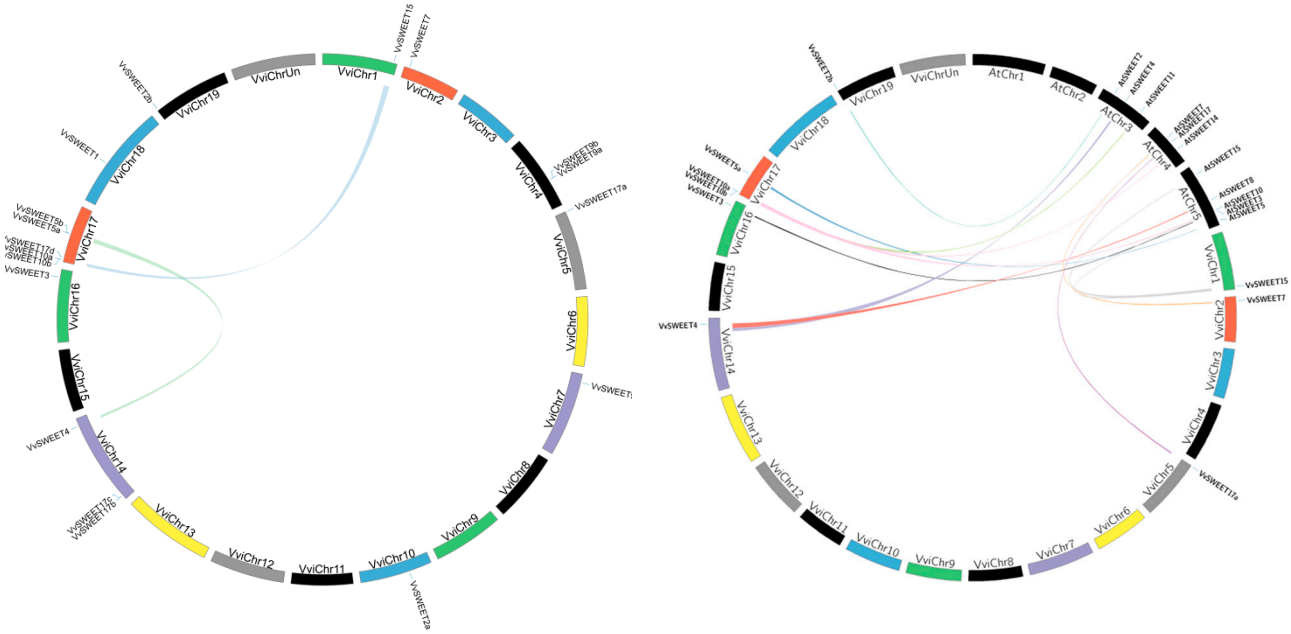


Figure 2. Characterization of grapevine *SWEET* genes. (**A**) Phylogenetic analysis of SWEET proteins in grapevine; (**B**) distribution of conserved motifs identified in the 18 VvSWEET proteins; and (**C**) exon–intron structure of grapevine *SWEET* genes. Exons are represented by pink boxes and black lines connecting two exons represent an intron.

3.4. Chromosomal Distribution and Synteny Analysis among Grape SWEET Genes

Based on genomic localization data, the 18 *VvSWEET* genes are dispersed throughout 11 chromosomes of the grapevine (Figure 3). Chromosome 17 harbors the largest number of *VvSWEET* genes, totaling five, whereas Chromosome 14 and Chromosome 4 contain three and two *VvSWEET* genes, respectively. Chromosomes 1, 2, 5, 7, 10, 16, 17, and 19 each accommodate a single *VvSWEET* gene.



(**a**) (**b**)

Figure 3. Chromosomal distribution and synteny analysis among grape *SWEET* genes. (**a**) Distribution and synteny analysis of *VvSWEET* genes on grapevine chromosomes. The approximate chromosomal locations of the *SWEET* genes are indicated on the periphery. The colored lines linking genes from different chromosomes denote segmental duplication events. (**b**) Synteny analysis of *SWEET* genes between grapevine and Arabidopsis. The chromosomes of grapevine and Arabidopsis are arranged as a circle. Syntenic occurrences of *SWEET* genes are represented by colored lines.

Segmental and tandem duplications contribute to the evolution of gene families [82]. As observed in Figure 3a, there were two segmental duplication events from *VvSWEET4* to *VvSWEET5a* and from *VvSWEET10* to *VvSWEET15a*, with no tandem duplications detected. This suggests that segmental duplication alone participated in the evolution of the grape *SWEET* gene family, indicating that large segmental chromosomal duplications are the primary mode of expansion for members of the grape SWEET family. Phylogenetic and chromosomal localization analyses revealed that all collinear gene pairs are located on different chromosomes, leading to the speculation that genes with collinearity mainly originate from inter-chromosomal segmental duplications or whole-genome duplication events. To further understand the evolutionary relationship between *VvSWEET* and *AtSWEET* genes, a collinearity analysis between the grape and Arabidopsis genomes was conducted, identifying 12 pairs of genes with collinear relationships, involving 9 *VvSWEETs* and 11 *AtSWEETs* (Figure 3b). This indicates that the large-scale expansion of these *SWEET* genes occurred before the divergence of grape and Arabidopsis. Phylogenetic analysis shows that each pair of collinear genes is positioned within the same branch in evolutionary terms, further supporting the reliability of the grouping. Among the *VvSWEET* and *AtSWEET* genes, there are 6 pairs with a one-to-one collinear relationship, where one *AtSWEET* gene corresponds to one *VvSWEET* gene, such as *AtSWEET2*-*VvSWEET2b* and *AtSWEET3*-*VvSWEET3*. Moreover, there are one-to-many and many-to-one collinear relationships, with the former referring to one *AtSWEET* gene corresponding to two or more *VvSWEET* genes, such as *AtSWEET10*-*VvSWEET10b*/*VvSWEET15*, and the latter referring to two or more *AtSWEET* genes corresponding to one *VvSWEET* gene, such as *AtSWEET4*/*AtSWEET8*-*VvSWEET4*.

To investigate the potential selective pressures on the duplication events of *VvSWEET* genes, we calculated the rates of nonsynonymous (Ka) and synonymous (Ks) substitutions. In grape and Arabidopsis, or exclusively in grape, all segmentally duplicated gene pairs exhibited a Ka/Ks ratio less than 1, indicating that they primarily evolved under purifying selection. Specifically in grape, the divergence time for segmental duplication events was estimated to be approximately 150 to 200 million years ago (Mya) (Table S3); between grape and Arabidopsis, the divergence times ranged from approximately 132 to 263 Mya, with an average of 179.7 Mya (Table S4). This suggests that the *SWEET* gene family was subjected to strong purifying selection following the divergence of the grape genome.

3.5. Expression Analysis of Grape SWEET Genes in Different Tissues

An expression atlas for all *VvSWEET* genes was constructed utilizing microarray data from 54 unique combinations of organs/tissues at varying developmental stages (Figure 4). We observed that *VvSWEET7* exhibited broad expression throughout grape development, particularly showing elevated expression levels in tendrils, mature leaves, and senescing leaves. Conversely, *VvSWEET17a* predominantly displayed expression in young buds, leaves, and fruit skins. Notably, *VvSWEET10b* and *VvSWEET15*, closely related in the evolutionary tree of the grape *SWEET* gene family, demonstrated similar expression patterns, with pronounced expression in the flesh and skin of fruits during veraison and maturity. Moreover, compared to other tissue types, distinct expression patterns were observed for specific *VvSWEET* genes: *VvSWEET1* showed heightened expression in woody stems and buds; *VvSWEET5a* exhibited increased expression in floral organs such as the stamen; *VvSWEET9c* displayed enhanced expression in seeds; and *VvSWEET3* had elevated expression in the fruit peduncle.

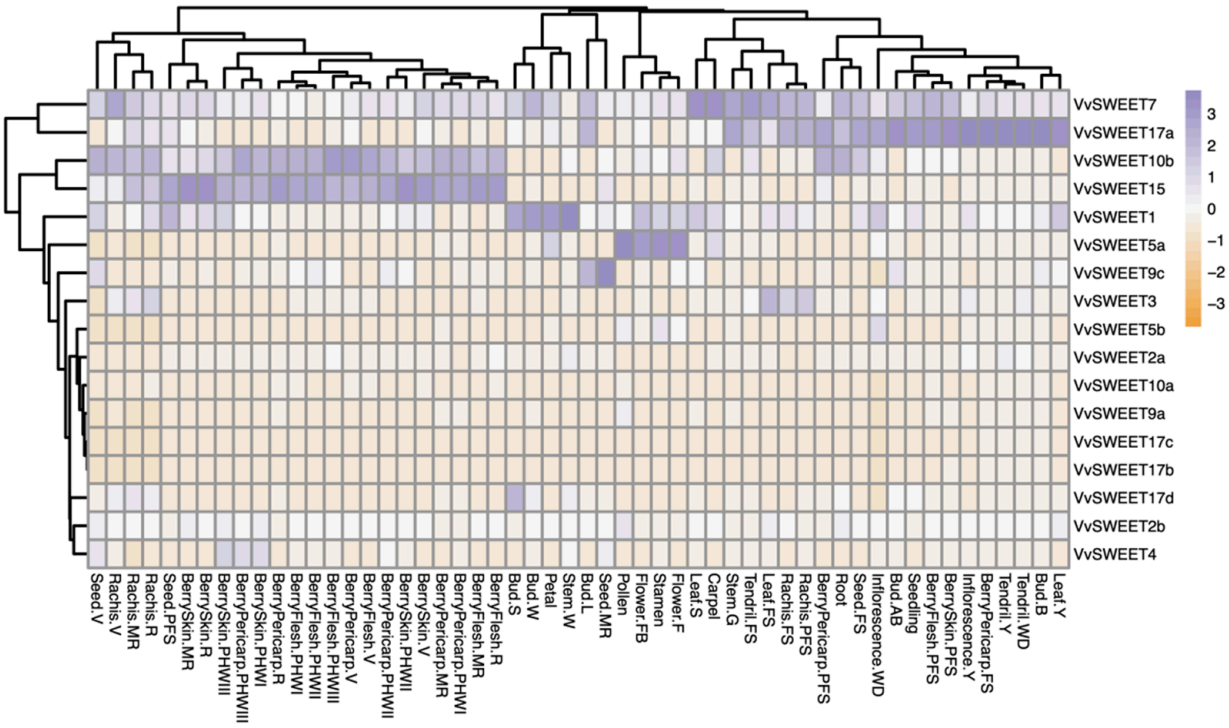


Figure 4. *VvSWEET* expression profiles in various tissues at different developmental stages. *VvSWEET* transcript levels in various tissues were investigated based on the mean expression value of each gene in a public transcriptome database [73]. The violet and orange colors represent the higher and lower relative expression levels, respectively. Bud (-L: latent bud, -W: winter bud, -S: bud swell, -B: bud burst, -AB: after-burst); inforescence (-Y: young inforescence, -WD: well developed inforescence); flower (-FB: fowering begins, -F: fowering); tendril (-Y: young tendril, -WD: well developed tendril, -FS: mature tendril); leaf (-Y: young leaf, -FS: mature leaf, -S: senescencing leaf); berry pericarp (-FS: fruit set, -PFS: post-fruit set, -V: véraison, -MR: mid-ripening, -R: ripening, -PHWI: post-harvest withering I, -PHWII: post-harvest withering II, -PHWIII: post-harvest withering III); berry skin/flesh (-PFS: post-fruit set, -V: véraison, -MR: mid-ripening, -R: ripening, -PHWI: post-harvest withering I, -PHWII: post-harvest withering II, -PHWIII: post-harvest withering III); seed (-FS: fruit set, -PFS: post-fruit set, -V: véraison, -MR: mid-ripening); rachis (-FS: fruit set, -PFS: post-fruit set, -V: véraison, -MR: mid-ripening, -R: ripening); and stem (-G: green stem, -W: woody stem).

3.6. Expression Analysis of Grape SWEET Genes during Fruit Developmental Stages

To elucidate the potential roles of *VvSWEET* genes in the development and ripening of grape berries, we utilized RNA sequencing data from the Gene Expression Omnibus (GEO) database. Our analysis focused on the expression patterns of *VvSWEET* genes across various developmental stages of grape berries (Figure 5). We identified fourteen genes that were active at different phases of berry maturation, categorizing them into two groups. The first group comprised eight *VvSWEET* genes, such as *VvSWEET10b* and *VvSWEET17d*, which exhibited lower expression levels during the early green fruit stage and véraison in the T'*angwei*', followed by a marked increase at the ripening phase. Interestingly, this expression trajectory was not consistent across all varieties; for instance, *VvSWEET10b* did not demonstrate a notable change in expression in the T'*onghua-3*'. Meanwhile, genes like *VvSWEET4* and *VvSWEET7* showed varying degrees of upregulation in both T'*angwei*' and T'*onghua*'. The second group, including six genes such as *VvSWEET17a* and *VvSWEET17b*, displayed higher expression during the green fruit stage, with a subsequent decrease as the fruit developed.

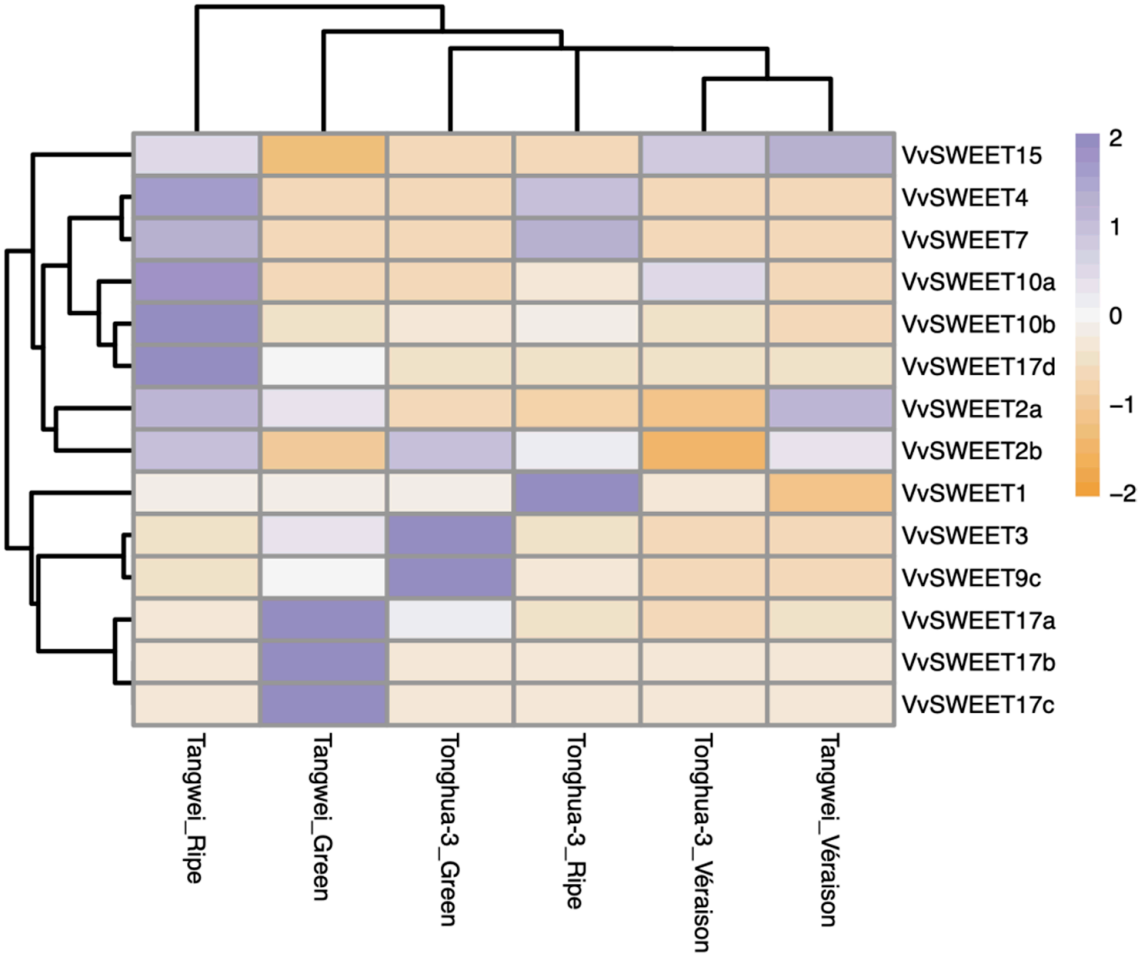


Figure 5. Expression of the *VvSWEET* gene family in grapefruits during the green fruit stage, veraison, and ripening based on transcriptome data.

3.7. Expression Patterns of Grape SWEET Genes under Abiotic Stress Conditions

Utilizing published grapevine transcriptome datasets, we analyzed the expression patterns of *VvSWEET* under various abiotic stress conditions, including cold, drought, and salt stress. The datasets include numerous resources from wild grape species in China, such as *Vitis amurensis*, *Vitis yeshanensis*, and *Vitis bryoniifolia*, among others. Due to the diversity of their native environments, these wild grapes typically exhibit superior adaptability and resistance. These characteristics make them ideal subjects for studying the response of grapevines to abiotic stress. Utilizing transcriptomic data from these representative varieties helps us to uncover the expression differences of the *SWEET* gene family members under abiotic stress conditions.

Under salt stress, Clade I members (*VvSWEET1*, *VvSWEET2b*, *VvSWEET3*) and Clade IV's *VvSWEET17a* showed variable upregulation, with VvSWEET1 and VvSWEET2b experiencing notably significant increases (Figure 6A). Conversely, Clade II and III members experienced slight downregulation across various grape varieties. Similarly, under drought stress, the expression patterns of *SWEET* genes mirrored those observed under salt stress. Specifically, *VvSWEET1*, *VvSWEET2b*, *VvSWEET3*, and *VvSWEET17a* were upregulated to varying extents, while the expression of other members decreased following stress induction (Figure 6B). After exposure to low temperatures, Clade I genes again showed differential upregulation, with *VvSWEET1* registering the most significant increase. In contrast, cold stress led to the suppression of most *VvSWEET* genes' expression levels (Figure 6C).

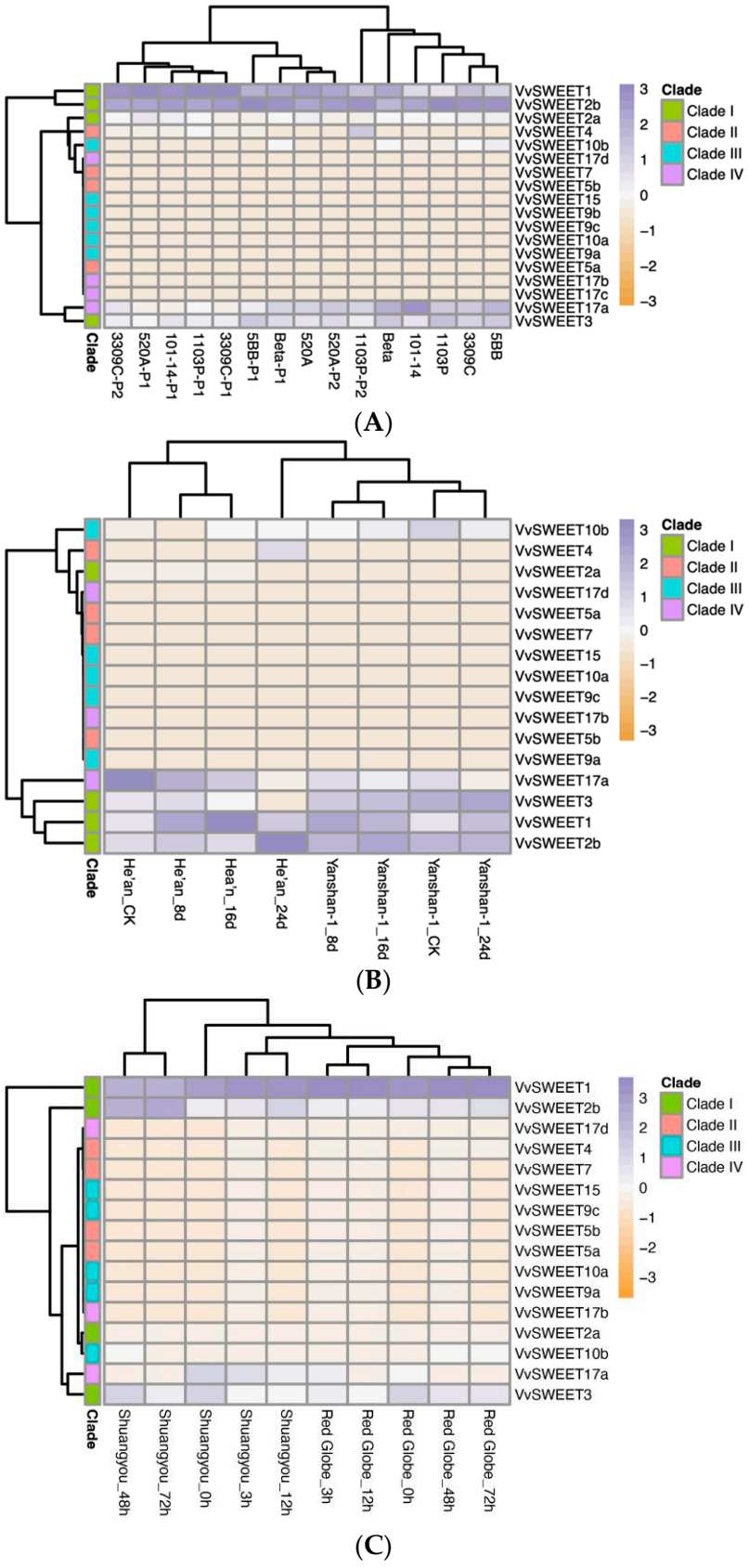
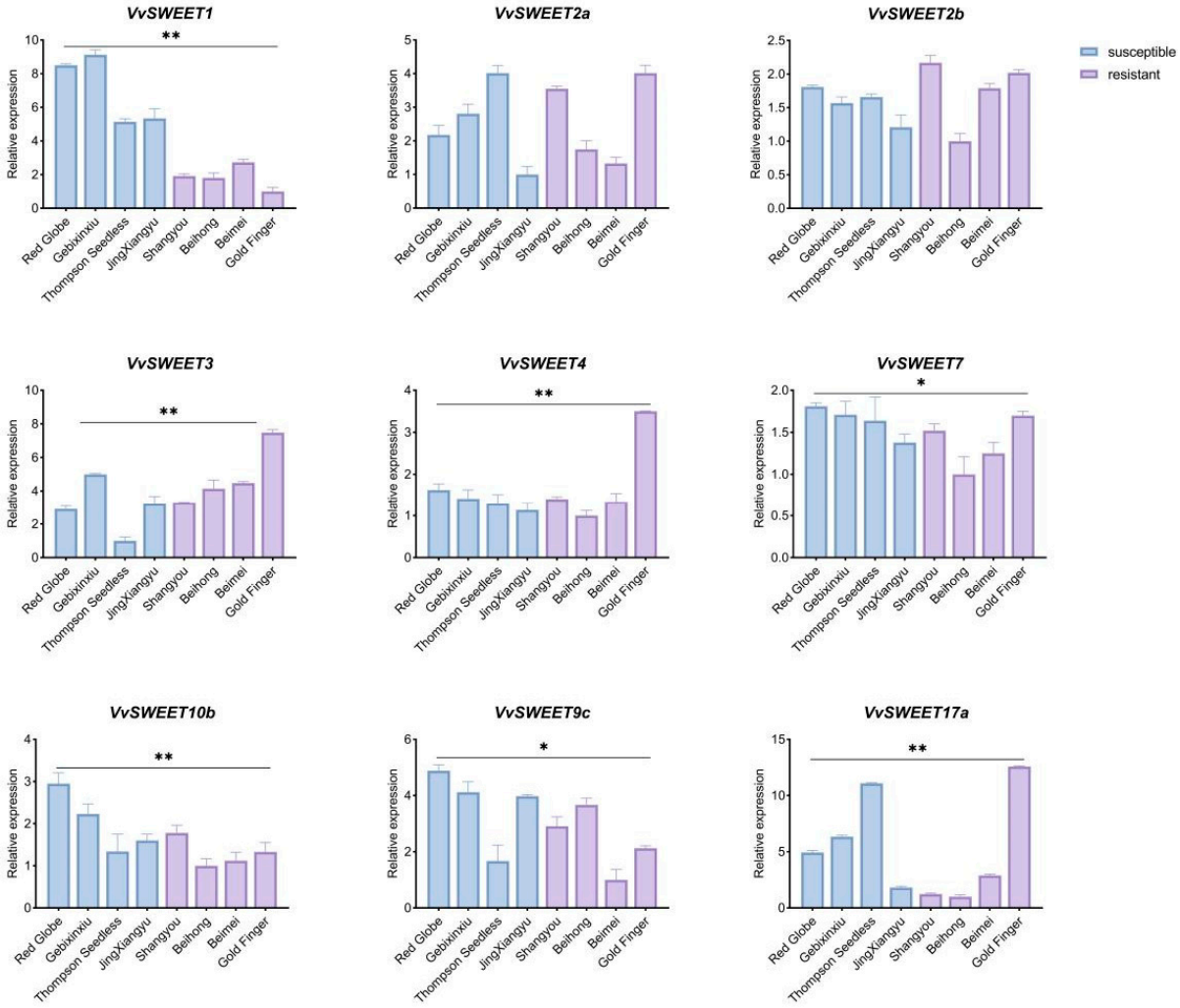


Figure 6. *VvSWEET* expression analysis in response to various abiotic stresses including cold (**A**), drought (**B**) and salt (**C**) treatments. The color scale represents log2(FPKM + 1) normalized transformed counts where orange indicates low expression and violet indicates high expression.

3.8. qRT-PCR Analysis of VvSWEET Genes in Relation to Grape Botrytis cinerea Infection

Beyond abiotic stress, our research further investigated the expression dynamics of *VvSWEET* genes within grapes subjected to biotic stress. Employing qRT-PCR for rigorous quantification, we analyzed the expression profiles of *VvSWEET* genes in four *Botrytis cinerea*-resistant grape varieties versus four susceptible varieties (Figure 7A). The analysis unveiled notable disparities in the expression levels of select *VvSWEET* genes between the resistant and susceptible groups. Specifically, *VvSWEET1* and *VvSWEET10b* exhibited elevated expressions in the susceptible varieties, whereas *VvSWEET3* demonstrated enhanced expression in the resistant varieties. To delve deeper into the role of *VvSWEET* genes in grape leaf response to *Botrytis cinerea* infection, we inoculated leaves from the susceptible *R'ed Globe'* variety alongside those from the highly resistant *S'huangyou'* variety, observed the entity of the disease (Figure S1) and subsequently analyzed *VvSWEET* gene expression patterns at various intervals post-inoculation. The qRT-PCR findings highlighted a significant induction of *VvSWEET1*, *VvSWEET2b*, *VvSWEET3*, and *VvSWEET4* expression levels post-infection. Particularly, from 0 to 48 h post-inoculation, *R'ed Globe'* leaves manifested consistently higher expression levels of *VvSWEET1* and *VvSWEET2a* compared to *S'huangyou'* leaves (Figure 7B); conversely, the expression trends of *VvSWEET3* and *VvSWEET17c* exhibited an inverse relationship.



(A)

Figure 7. *Cont.*

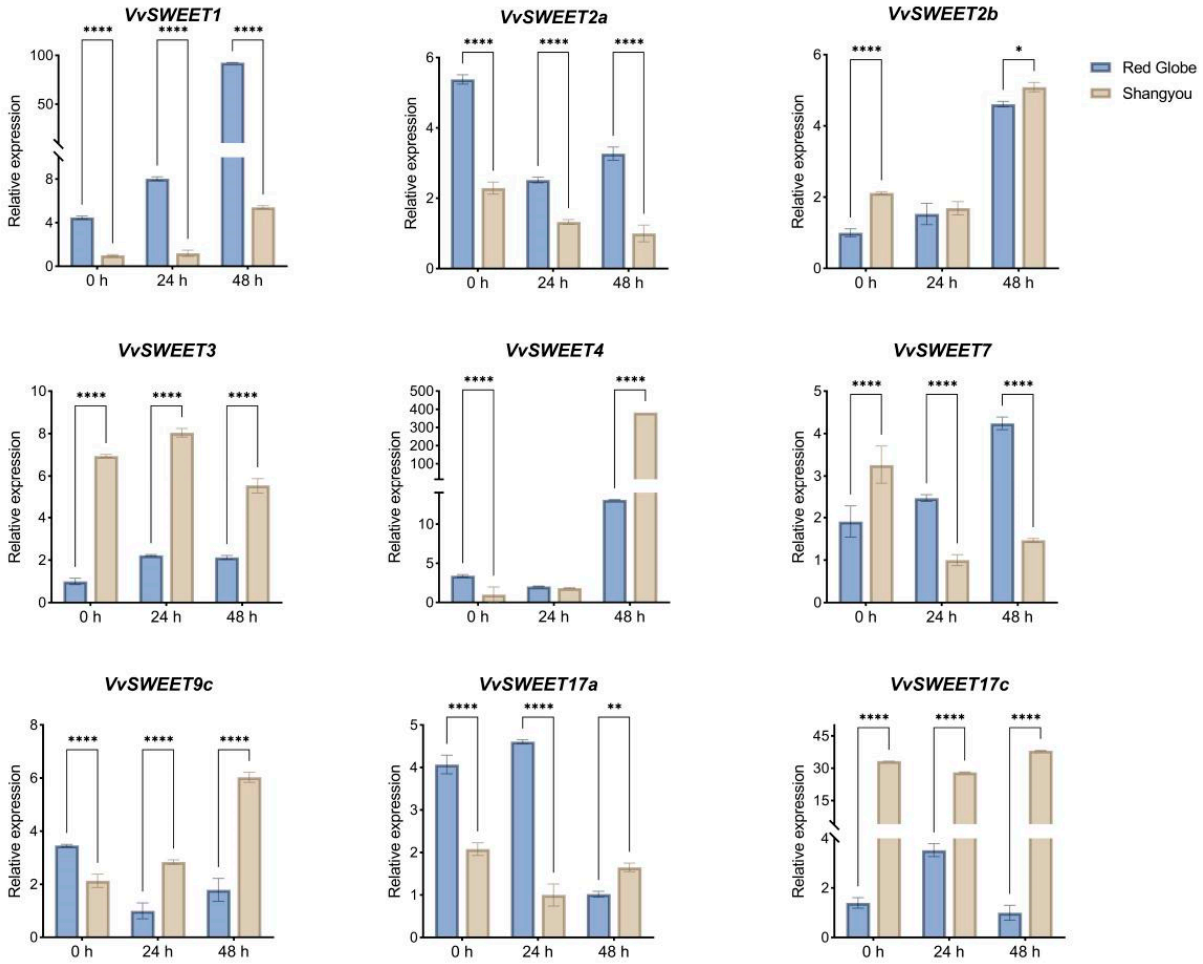


Figure 7. qRT-PCR analysis of expression of selected *VvSWEET* genes. (**A**) Expression of *VvSWEET* genes in *Botrytis cinerea*-resistant and -susceptible varieties. (**B**) Expression of *VvSWEET* genes following *Botrytis cinerea* inoculation. The grapevine *ACTIN1* gene was used as an internal control to normalize expression levels. Mean values and standard deviations (SDs) are indicated by error bars. Asterisks indicate significance of the indicated differences in gene expression according to the *t*-test (* $p < 0.05$, ** $p < 0.01$,**** $p < 0.0001$).

4. Discussion

In this study, bioinformatics methods were utilized to identify a total of 18 *VvSWEET* genes from the grape genome. These genes were named according to the naming convention as *VvSWEETs* (Table 2), and their genomic locations were annotated on specific chromosomes (Figure 3). Table 2 provides detailed information about these genes, including gene ID, accession number, and the physicochemical properties of the encoded proteins. This research identified one more gene than previously reported studies, thanks to the utilization of a newly assembled grape genome. Furthermore, we discovered that among plants, wheat (*Triticum aestivum*) has the highest number of SWEET members, totaling 105, while loquat (*Eriobotrya japonica*) has the fewest, with only 7 members. In allohexaploid species such as bread wheat, the presence of each genome may contribute to additional copies of *SWEET* genes. Consequently, compared to other diploid or lower ploidy plant species, there is a greater likelihood of increased expression and functional diversification of *SWEET* genes. The significant variation in gene numbers across different plants may be attributed to genomic sequences that have not yet been sequenced, or it may reflect species-specific duplications or deletions that occurred during the evolutionary process.

We conducted a phylogenetic analysis of SWEET protein sequences from grapes and four other plant species. The phylogenetic tree indicates that grape SWEET proteins are more closely related to those from Arabidopsis and cucumber, suggesting a closer evolutionary relationship with dicots than with monocots like rice and daylily, which might be attributed to the more recent divergence of dicotyledonous plants. The 18 grape SWEET proteins can be classified into four distinct clades. Within the same clade, the distribution of motifs among VvSWEET proteins was generally similar, though there were exceptions (Figure 3). For instance, Motif 11 was unique to members of Clade II, while in Clade III, Motif 7 was only present in two genes, *VvSWEET9a* and *VvSWEET9b*, and Motif 9 was exclusive to three genes, *VvSWEET10a*, *VvSWEET10b*, and *VvSWEET15*; Motif 2 was absent only in *VvSWEET9b*. These findings suggest that these VvSWEET proteins may possess unique functions.

The structure of introns and exons is closely related to gene evolution [83]. We found that the number of exons in the 18 *VvSWEET* genes was stable, with most *SWEET* genes containing 6 exons, and a minority, constituting 22.2% of the family, having only 4–5 exons. This is similar to the proportions observed in the SWEET families of pear [37], tomato [20], soybean [35], and pineapple [12], indicating a conserved number and position of introns across different species. *VvSWEET* genes with similar exon–intron structures clustered together in the phylogenetic tree. Specifically, two pairs of genes (*VvSWEET4*/*VvSWEET5a* and *VvSWEET10*/*VvSWEET15a*) had the same number of exons and almost identical exon lengths (Figure 2C), suggesting they may have arisen from segmental or tandem duplications, a hypothesis supported by collinearity analysis results (Figure 3). These results further demonstrate the evolutionary conservation of SWEET proteins. Additionally, the gain or loss of introns can lead to structural complexity, a key evolutionary mechanism in most gene families. The first two exons of *VvSWEET* genes are relatively short and may be easily lost during evolution. We observed exon–intron loss, with *VvSWEET9a* containing six exons while its paralog, *VvSWEET9b*, contained only four exons (Figure 2C). This indicates that *VvSWEET9b* may have lost two exons during evolution, undergoing a deletion of genetic information. Similar findings were reported in two members of the pear SWEET family, *PbSWEET8* and *PbSWEET16* [37]. This evolutionary mechanism leads to a more complex gene structure and diverse gene functions, preventing functional redundancy within the gene family. Notably, the distribution of introns in the grape *SWEET* gene family exhibits a phase pattern of 1, 2, 0, perhaps explaining why introns with phase 2 in the middle sequences of genes are conserved and do not undergo structural loss. From the results of the conserved domain distribution, most *SWEET* genes contain eight conserved domains, with some experiencing the loss of individual domains, resulting in only seven or six conserved domains. The loss of sequences at both ends of the genes led to the corresponding loss of conserved domains, with both results being interrelated. The distribution of domains on the genes, showing their appearance order and location to be largely consistent, indirectly reflects the high level of conservation in the grape *SWEET* gene family.

Segmental duplication and tandem duplication are significant driving forces behind the expansion of gene families. In this study, we identified two pairs of segmentally duplicated genes on grape chromosomes 1, 14, and 17, but no tandem duplications were detected (Figure 3). Additionally, the segmentally duplicated gene pairs (e.g., *VvSWEET4*/*VvSWEET5a*) were located in the same group and exhibited similar exon–intron structures (Figure 2), indicating that segmental duplication has contributed to the expansion of the *SWEET* gene family in grape. We also identified 12 pairs of genes with collinearity, deriving from segmental duplications between grape and Arabidopsis, suggesting they may have a common ancestor. Therefore, the biological functions of grape *SWEET* genes can be preliminarily predicted based on the functions of their Arabidopsis *SWEET* homologs.

Previous studies have confirmed that the *SWEET* gene family plays roles in different tissues and organs during plant growth and development. Analysis of the expression

patterns of *SWEET* genes in different tissues of grape reveals that *VvSWEET10b* and *VvSWEET15* are highly expressed in the flesh and grape *SWEET* gene family skin of the fruit. The tissue-specific expression patterns of these two genes are very similar, which may be related to their close phylogenetic relationship within the and their nearly identical gene structures. *VvSWEET5a* is expressed significantly higher in flowers than in other tissues, leading to the preliminary prediction that this gene may be related to grape reproductive development. This suggests that the functional differentiation of *SWEET* genes is closely related to their expression patterns in different tissues, highlighting the importance of studying these genes for understanding the molecular mechanisms underlying plant development and stress responses.

Fruit maturation is a complex biological process. The sugars accumulated in grapefruits mainly consist of fructose and glucose, with a small amount of sucrose. During the growth and development of the fruit, sugars are transported from the source organs (leaves) to the fruit's vascular bundle phloem in the form of sucrose via long-distance transport through the phloem, and then from the phloem parenchyma cells to the phloem apoplast. Members of the *SWEET* gene family, localized on the plasma membrane, are required to transfer sucrose from the phloem parenchyma through the apoplast into the sieve tubes and companion cells. SWEET proteins have been reported to play a key role in the development and maturation of fruits, including apples [13], pears [37], tomatoes [20], citrus [33], and pineapple [12], especially in terms of sugar accumulation. In this study, the expression of nearly all *SWEET* genes changed during the fruit maturation process. *VvSWEET4*, *VvSWEET7*, *VvSWEET10a*, *VvSWEET10b*, and others were upregulated from the green fruit stage to the ripening process, showing a positive correlation with sugar accumulation in the fruit. Conversely, *VvSWEET3*, *VvSWEET17a*, *VvSWEET17b*, *VvSWEET17c*, etc., were downregulated, showing a negative correlation with sugar accumulation. We speculate that *VvSWEET4* and *VvSWEET7* genes mainly function in the transport and accumulation of sugars during fruit maturation, while *VvSWEET3* mainly functions in the transport and accumulation of sugars during the immature stage of fruit development. In apples, *MdSWEET1.1/2*, *MdSWEET2.4*, and *MdSWEET3.5* have higher expression levels in young fruits, while *MdSWEET3.6/7* are more expressed in larger fruits. Notably, unlike pineapple *SWEETs*, which show high levels of expression in the early stages of fruit development [11], in grapes, there are many genes that show high levels of expression during the maturation stage, suggesting that the *SWEET* gene family may play a more important role in the maturation period of grapefruit development. Overall, the study of the function of *SWEET* genes in grapefruits provide a new direction for future research, which is crucial for improving the quality of grapefruits.

Soluble sugars are crucial sources of energy and matter within cells. When plants face adverse environmental stress, the redistribution of soluble sugars in tissues can be modulated to maintain cellular osmotic potential balance, thereby enhancing their resistance to stress and ensuring normal growth. Sugar transport proteins are key factors in regulating the redistribution of soluble sugars, and the role of SWEET proteins in responding to various stress responses has been extensively documented. In Arabidopsis, several *SWEET* genes, such as *AtSWEET17*, *AtSWEET16*, *AtSWEET12*, *AtSWEET11*, and *AtSWEET4*, are closely linked to stress responses. They participate in the development of plant shoots or roots and regulate responses to abiotic stress through the modulation of sugar transport [84]. AtSWEET15 (SAG29), a plasma membrane-localized transporter, has its expression induced by osmotic stress through the ABA pathway and is associated with cell viability under high salinity and other osmotic stress conditions [17]. *AtSWEET4* enhances plant frost resistance; *AtSWEET4*-RNAi interference lines show reduced sugar accumulation and increased sensitivity to frost damage, whereas *AtSWEET4*-OE lines accumulate more sugar, exhibiting greater frost resistance [85]. In tea plants, the vacuolar-membrane-localized CsSWEET16 protein promotes the compartmentalization of sugars within vacuoles and improves drought resistance in Arabidopsis [40]; the expression of *CsSWEET1a* and *CsSWEET17* can be induced by cold stress, and their overexpression in Arabidopsis enhances

plant cold tolerance, further confirming their protective role against frost damage [86]. A genome-wide analysis of cabbage *SWEET* identified a candidate *SWEET* gene that could enhance plant cold tolerance through the promotion of sugar transport [42]. Additionally, in banana, the upregulation of *MaSWEETs* expression plays a significant role in responding to low temperature, salt stress, and osmotic stress [25]. Upon exposure to low temperature and other stress conditions, soluble sugars accumulate within cells. Vacuoles, which account for about 90% of plant cell volume, play a key role in the temporary and long-term storage of soluble sugars. The sugar storage capacity of vacuoles is crucial for regulating osmotic homeostasis; a significant increase in monosaccharide content within vacuoles can stabilize cell membranes, protect membrane proteins, and act as osmolytes and antifreeze agents, thereby enhancing plant stress resistance. We found that most genes in the grape *SWEET* family are downregulated under stress conditions such as salt, drought, and low temperature, while most members of Clade 1, such as *VvSWEET1*, *VvSWEET2b*, and *VvSWEET3*, are upregulated. Similarly, under the biotic stress of *Botrytis cinerea* infection, members of Clade 1 exhibit a higher level of induction compared to other *VvSWEET* members, with certain genes displaying significant differences between disease-resistant and susceptible varieties. Studies suggest that Clade 1 members, which are localized on vacuolar membranes and mainly transport glucose, may play a role in enhancing plant stress resistance. It is speculated that grapes might induce the expression of Clade 1 members of the *VvSWEET* family, particularly *VvSWEET1*, to transport more glucose into vacuoles, leading to feedback regulation of sugar metabolism and thus affecting the content changes of other carbohydrates, regulating plant stress resistance.

5. Conclusions

In this study, we identified 18 *VvSWEET* genes based on the latest version of the grape genome annotation. A comprehensive analysis of the *SWEET* gene family was conducted, encompassing phylogenetic relationships, gene structure, conserved motifs, chromosomal localization, gene collinearity, and expression patterns. The expression profiles of *VvSWEET* genes under various abiotic and biotic stress conditions, throughout fruit maturation, and in different tissues and organs suggest their significant roles in grape growth, development, and response to diverse stress conditions. Overall, this whole-genome analysis of the *VvSWEET* family provides a foundation for further research on the functions of *SWEET* genes in grapes, contributing to our understanding of the molecular mechanisms underlying grape development and stress adaptation.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/horticulturae10050428/s1, Figure S1: Disease development after inoculation of leaves from the highly susceptible *Vitis vinifera* Red Globe and the highly resistant *Vitis amurensis* shuangyou; Table S1: Description of sample of *Vitis vinifera* cultivar used for microarray analysis; Table S2: Description of sample used for microarray analysis; Table S3: Segmental duplications within grapevine *VvSWEET* genes and Ka/Ks ratios analysis of segmental duplicate gene pairs; Table S4: Segmental duplications of *SWEET* genes between grapevine and Arabidopsis and Ka/Ks ratios analysis of segmental duplicate gene pair.

Author Contributions: Data curation, S.X. and Y.L.; formal analysis, S.X; funding acquisition, M.G.; investigation, Z.Y.; proiect administration, M.G.; resources, X.C. and J.Z.; supervision, X.W. and M.G.; validation, C.S. and N.Z.; writing—original draft, L.Z.; writing—review & editing, S.X. and Z.L. All authors have read and agreed to the published version of the manuscript.

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Data Availability Statement: The original contributions presented in the study are included in the article and Supplementary Materials, further inquiries can be directed to the corresponding author.

Conflicts of Interest: The authors declare no conflicts of interest.

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