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Genome-Wide Identification, In Silico Analysis and Expression Profiling of SWEET Gene Family in Loquat (*Eriobotrya japonica* Lindl.)

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Abstract: SWEETs (sugars will eventually be exported transporters) have various physiological and biochemical roles in plant growth, including pollen development, seed nourishment, nectar secretion, and longer-distance sugar transportation. The *SWEET* genes were identified in various plant species, but they have not yet been thoroughly characterized. Here, we discovered 21 putative *SWEET* genes from the *Eriobotrya japonica* Lindl. genome. For further elucidation, comprehensive bioinformatics analysis was utilized to determine the physicochemical properties, gene organization, conserved motifs, *cis*-regulatory elements, gene duplication, and phylogenetic relationships of *EjSWEET* genes. Most of the SWEET proteins were predicted to be located on the plasma membrane or vacuole. Gene organization and motif analysis showed that the numbers of exons and motifs in each gene ranged strikingly, between 5 and 6 and between 5 and 8, respectively. Synteny analysis showed that the tandem or segmental duplication played a dynamic role in the evolution of *SWEET* genes in loquat. Likewise, we analyzed the expression patterns of *EjSWEET* genes in the root, stem, leaf, flower, and fruit of loquat. Some genes exhibited varying expression in loquat tissues, indicating their potential roles in plant development. The relative expression levels of *EjSWEET1*, *EjSWEET3*, and *EjSWEET16* were noticeably higher in ripened fruits, suggesting their possible role in the transportation and unloading of sugars in fruits. The present study provides initial genome-wide identification and characterization of the *SWEET* gene family in loquat and lays the foundation for their further functional analysis.

Keywords: sugar transporters; gene expression; Rosaceae; bioinformatics; phylogeny; tandem

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1. Introduction

Sugars are primarily produced in response to the photosynthetic process, which is a prerequisite for the maintenance of the plant growth cycle, plant signaling, molecule transportation, and energy storage carbon skeletons [1]. Synthesized sugars from leaves are then needed to be transported into different plant tissues (seeds, roots, and fruits) to fulfil sufficient plant growth [2–6]; such transportation and cellular exchange of sugars are undertaken mainly by three major sugar transporter families specifically, *SWEETs* (sugars will eventually be exported transporters), sucrose transporters (*SUTs*), and monosaccharide

transporters (*MSTs*) [7]. These transporters are now widely known for sugar facilitation into several plant tissues upon source to sink demand, therefore keenly regulating plant growth and fruit quality [1,7–9].

Among the sugar transporters, *SUTs* and *MSTs* are major facilitator super-families that are characterized by 12 transmembrane domains [8–10]. Additionally, recently acknowledged SWEET proteins for sugar transportation which belong to the MtN3/saliva family (PF03083) regulate selective efflux for disaccharides or monosaccharides between intracellular membranes [3,7,11,12]. SWEET family proteins were characterized for several plant and animal species as well as prokaryotes [13,14]; SWEET proteins from eukaryotes are predicted to be tandem repeats of two 3-α-helical transmembrane (TM) domains (having two conserved MtN3/saliva motifs) and separated by a less conserved single TM [12]. SWEET proteins identified in prokaryotes (also called as SemiSWEETs) hold only a single 3-TM, reflecting that evolution for SWEETs in eukaryotes was undertaken via duplication and fusion of the basic 3-TM unit that exists in prokaryote SemiSWEETs [12].

In recent years, several pieces of evidence linked to *SWEETs* have shown that they are involved in plant biochemical and physiological process regulation; in maintaining sugar supply and demand for longer-distance sugar transportation, pollen nourishment, and nectar secretion; and in response to pathological stress [3,7,15]. In *Arabidopsis thaliana*, two genes localized at the plasma membrane, named *AtSWEET11* and *AtSWEET12*, are responsible for the exportation of sucrose from phloem parenchyma cells into the apoplast [16]. *AtSWEET9* was reported to directly influence the production of nectar and additionally work as a transporter of sucrose [17]. Moreover, *AtSWEET5*, also known as *VEX1*, is noticed to be expressed during the development of pollen [18]; *AtSWEET8*, also known as *RPG1*, is highly expressed in the male tapetum as well as microspores during meiosis [19]; and *AtSWEET13*, also known as *RPG2*, is noticed to have more expression in plant anther tissues [20].

Similar studies in rice also observed higher expression of *OsSWEET5* in anthers [21]. A significant role of *SWEETs* was also observed in seeds; genes *SWEET11*, *SWEET12*, and *SWEET15* from *A. thaliana* were expressed spatiotemporally throughout seed development, while seed defects were only observed in a triple knockout mutant, causing seed wrinkling in mature seeds [22,23]. Other defects include retarded embryo development, reduced seed weight, and lesser starch and lipid contents [24]. In comparison to Arabidopsis, genes *OsSWEET4* in rice and *ZmSWEET4c* in maize are responsible for seed filling as well as hexose transportation across the basal endosperm transfer layer [25]. In addition, pathogen interactions also alter the expression pattern of *SWEETs*, which is modified according to their needs for carbohydrates to complete their growth cycle [26–30]. *SWEETs* were studied in many species at genomic and protein levels, mainly in rice and Arabidopsis. Besides providing a better understanding, studies have shown that *SWEET* genes may cover extensive functional divergence in plants [31].

Loquat (*Eriobotrya japonica* Lindl.) is an evergreen fruit tree that originated from China [32]. It belongs to the family Rosaceae, subfamily Maloideae [33]. It is a rich source of vitamin A, vitamin B6, potassium, magnesium, and dietary fiber [34,35]. It is most widely grown in Japan, Korea, India, Pakistan, and the south-central region of China [36]. The availability of the loquat (*Eriobotrya japonica* Lindl.) genome published by Jiang et al. [37] facilitated genomic, proteomic, and functional studies. To expand our knowledge of the *SWEET* gene family in loquat, we systematically identified 21 *SWEETs* and further investigated their phylogenetic relationship, subcellular localization, gene duplication, and expression patterns. This investigation helped us to understand the evolutionary patterns and roles of *SWEETs* in loquat growth and development, including sugar transport in different plant tissues.

2. Materials and Methods

2.1. Identification and Characterization of EjSWEET Genes

In order to identify the *SWEET* genes in loquat, we explored the corresponding *Eriobotrya japonica* Lindl. Genome Project from the GigaScience Database (http://gigadb.org/dataset/view/id/100711, accessed on 22 December 2020) [37]. For phylogenetic analysis, the apple genome sequence [38] was acquired from the Phytozome website (http://phytozome.jgi.doe.gov/pz/portal.html, accessed on 16 June 2021) and the genome sequences of *Arabidopsis thaliana* were downloaded from the Arabidopsis Information Resource (TAIR) (http://www.arabidopsis.org/, accessed on 22 December 2020). Arabidopsis *SWEET* genes were deployed as a query sequence to run BLAST against the genome databases of the species as mentioned above. Additionally, using the HMMER3 software package, the seed alignment file for the MtN3/saliva domain (PF03083) was retrieved from the Pfam database [39]. HMMER software suite was then used to run HMM searches against the local protein databases of the species as mentioned above [40].

Furthermore, we evaluated the physical locations of all putative *SWEET* genes and ruled out redundant sequence repeats with the same chromosome location. Additionally, all retrieved SWEET protein sequences were re-analyzed in the Pfam database using the SMART programs (http://smart.embl-heidelberg.de, accessed on 22 January 2022) to identify the presence of the MtN3/saliva domain. The protein sequences that lacked the MtN3/saliva domain were discarded. ExPASy Proteomics Server (http://web.expasy.org/compute_pi/, accessed on 25 January 2022) was used to determine the physicochemical characteristics of EjSWEET proteins. The subcellular localization of EjSWEETs was predicted using the WoLF PSORT web server (https://wolfpsort.hgc.jp/, accessed on 25 January 2022).

2.2. Gene Structure, Conserved Motif, and Promoter Region Analyses of EjSWEET Genes

The exon–intron structure of *EjSWEET* genes was identified by aligning coding sequences with the respective genomic sequences. TBtools software package (v0.6655) was used to generate diagrams [41]. The MEME suite server (http://meme-suite.org/, accessed on 25 January 2022) was used to identify conserved motifs in the sequences of *SWEET* genes. The following parameters were set up: maximum numbers of different motifs, 10; minimum width, 10; maximum width, 50. The promoter region analysis was carried out through the online PlantCARE database (http://bioinformatics.psb.ugent.be/webtools/plantcare/html/, accessed on 28 January 2025) and illustrated using TBtools software package v0.6655 [41].

2.3. Chromosomal Mapping and Syntenic Analysis of SWEETs in Loquat

Using Tbtools (v0.6655), a gff3-file of the *E. japonica* genome was employed to evaluate the distribution and mapping of *EjSWEET* genes on all chromosomes [41]. The MCScanX software was used to identify the duplicated *SWEET* genes in the loquat genome [42]. BLASTP was used to compare all of the protein sequences from loquat (http://www.ncbi.nlm.nih.gov/blast/blast.cgi, accessed on 14 February 2022) with an e-value less than 1×10^{-5}. With default settings, the BLASTP outputs with gene-location files were processed as input for MCScanX to identify syntenic gene pairs and duplication types.

2.4. K_a and K_s Calculation

The values of K_a (nonsynonymous) and K_s (synonymous) of syntenic gene pairs were annotated using MCScanX downstream analysis tools. Briefly, K_a and K_s were calculated using KaKs_Calculator (v2.0) with the Nei–Gojobori (NG) method [43,44].

2.5. Multiple Sequence Alignment and Phylogenetic Analysis

Molecular Evolutionary Genetics Analysis X (MEGA-X v10.2.6) was used to perform phylogenetic and molecular evolutionary genetics studies [45]. Multiple sequence alignment was conducted with MEGA-X (default settings) using Multiple Sequence Caparison

by Log-Expectation (MUSCLE). The conserved or similar amino acid sequences were highlighted with GeneDoc (v2.7). The neighbor-joining (NJ) approach was used to generate different SWEET trees with a bootstrap of 1000 repetitions, *p*-distance, and pairwise deletion using MEGA-X.

2.6. Plant Sampling, RNA Isolation, and Quantitative RT-PCR Analysis

The plant tissues were sampled from 10-year-old loquat trees of the Jiefangzhong cultivar growing in a private orchard located in Fuqing county, Fujian province, China. Total RNA was isolated from root, stem, mature leaf, full-bloom flower, and ripened fruit of loquat using a Total RNA kit (TianGen Biotech, Beijing, China). A NanoDrop N-1000 spectrophotometer (NanoDrop Technologies, Wilmington, DE, USA) and agarose gel electrophoresis were used to assess the quantity and quality of RNA. The first-strand cDNA was synthesized from 1 μg RNA using Prime Script RT Reagent Kit with a gDNA Eraser (TaKaRa, Dalian, China). Quantitative RT-PCR analysis was carried out using high-performance real-time PCR (LightCycler 96, Roche Applied Science, Penzberg, Germany). The $2^{-\Delta\Delta CT}$ method was used to calculate the relative expression levels of *EjSWEET* genes with 3 biological and 3 technical replicates. An actin gene (EVM0004523.1) described in previous studies [43,46,47] was selected as a constitutive control, and all the primers used for qRT-PCR are listed in Table 1.

Table 1. Primer sequences of *EjSWEET* genes.

Gene Name	Gene ID	Forward Primer (5′–3′)	Reverse Primer (5′–3′)
EjSWEET1	EVM0038444.1	CCCCAATGCCAACATTTAAG	GGAAAACAGCTCCGATTGAA
EjSWEET2	EVM0001232.1	CCGAAAGAGCGGTTAAGATG	TGGCGAACCATACATGAAAA
EjSWEET3	EVM0035130.1	TCTTTCTCTCGCGACGTTTT	TGGCTCTCTTGAGGCATCTT
EjSWEET4	EVM0038533.1	CGCCTTTCTCGATTATGAGC	TCCCAAACCCATTTGGAATA
EjSWEET5	EVM0008081.1	TGTCACCAATGCCAACATTT	GAGCAATCCCGATATCCTCA
EjSWEET6	EVM0039968.1	TTGCGTCCATTGTCTTTGTC	GGCATGAACTCAACGCTTTT
EjSWEET7	EVM0006793.1	TTGTGTCCGCAAACAACATT	AGAACAGAGCAAGCACAGCA
EjSWEET8	EVM0033338.1	TCCCCAATCCCAACATTTTA	ACCTTCAAGCGTTGTGAACC
EjSWEET9	EVM0009105.1	TTGGTGCGGTGTTTCAATTA	CTTTGTCCGGATCACCAAGT
EjSWEET10	EVM0029629.1	ATGGAATTTTGTGGCTCGTC	TTTGGCCTTTCATCATCCTC
EjSWEET11	EVM0019072.1	ATGGGCTGGGCTTACTTTTT	CATGTCCGGTTGTTCTGATG
EjSWEET12	EVM0043755.1	TTTGCACACTGCTCAACTCC	CCACATCCAAGGTTCCAATC
EjSWEET13	EVM0005220.1	TCATCGAAAGGGTTCCAATC	CCGAAAGCGGCTACATTTAC
EjSWEET14	EVM0003016.1	CGTGGTATGGATCGCCTATT	AATCTGCAAGCTCCCAAAGA
EjSWEET15	EVM0006392.1	CATGTCCTTCCCTTTGTCGT	CATTTTCTGCAACCCCATTT
EjSWEET16	EVM0043307.1	CTCCTTCGGGCTTTTTCTCT	CAAGAGTGCTGTCAGGGTGA
EjSWEET17	EVM0019370.1	GCTCGTTTTCATGGCTCTTC	CATATGTGAACCAGGCAACG
EjSWEET18	EVM0020014.1	TGACTCGCTTTCTAGCCACA	TGATAACGGAAATGGCATGA
EjSWEET19	EVM0044002.1	CCGGTCTTTGGTAGTTGGAA	GACCAGCCAAACAACTCCAT
EjSWEET20	EVM0026582.1	AAGCAACAAATGGGAAAACG	GCTGAGATAATGGCGGTGAT
EjSWEET21	EVM0012820.1	TCTTCACCATTAACGGCACA	AACGACGGCTGAGATAATGG
EjAct	EVM0004523.1	GGAGCGTGGATATTCCTTCA	GCTGCTTCCATTCCAATCAT

2.7. Statistical Analysis

The obtained qRT-PCR data were statistically analyzed through one-way ANOVA using the software "Statistix v8.1". The relative expression levels of *EjSWEET* genes in different tissues of loquat were compared using Fisher's LSD technique.

3. Results

3.1. Identification and Characterization of EjSWEET Genes in Loquat

In total, 21 *EjSWEET* genes were identified in the *E. japonica* genome, and the members of the *EjSWEET* gene family were named *EjSWEET1* to *EjSWEET21* based on their positions on 17 chromosomes. Table 2 shows detailed information about gene location on chromosomes and the peptide and CDS sequence lengths of *EjSWEETs*.

Table 2. The basic information about *SWEET* genes in loquat.

Gene Name	Gene ID	Chromosome	Start Site	End Site	Strand	CDS (bp)
EjSWEET1	EVM0038444.1	1	5761008	5763757	+	699
EjSWEET2	EVM0001232.1	1	5771748	5773852	+	717
EjSWEET3	EVM0035130.1	1	5776406	5778843	+	708
EjSWEET4	EVM0038533.1	1	46469811	46472452	+	792
EjSWEET5	EVM0008081.1	2	6408991	6411071	+	699
EjSWEET6	EVM0039968.1	2	6430524	6432707	+	717
EjSWEET7	EVM0006793.1	2	41029948	41032274	+	792
EjSWEET8	EVM0033338.1	3	39566451	39568463	−	711
EjSWEET9	EVM0009105.1	4	3837836	3844089	−	708
EjSWEET10	EVM0029629.1	6	8278274	8279519	−	708
EjSWEET11	EVM0019072.1	6	20451938	20455969	−	765
EjSWEET12	EVM0043755.1	8	27415495	27429282	−	720
EjSWEET13	EVM0005220.1	12	889913	891387	+	804
EjSWEET14	EVM0003016.1	12	34849459	34851971	+	708
EjSWEET15	EVM0006392.1	12	37885082	37886461	−	825
EjSWEET16	EVM0043307.1	13	35717082	35718865	+	726
EjSWEET17	EVM0019370.1	14	25165282	25168251	−	786
EjSWEET18	EVM0020014.1	14	28293338	28295358	+	888
EjSWEET19	EVM0044002.1	14	31729522	31732348	−	711
EjSWEET20	EVM0026582.1	16	1749505	1750777	−	762
EjSWEET21	EVM0012820.1	16	1768563	1769839	−	750

CDS: coding sequence (DNA); bp: base pair.

The specific physicochemical properties of *EjSWEET* genes are shown in Table 3. The protein length of 21 *EjSWEET* genes was estimated between 232 to 295 amino acids, and the maximum and minimum molecular weights were 33.08 and 25.35 kDa, respectively. The lowest isoelectric point value (4.93) was recorded in *EjSWEET6*, and the isoelectric point of *EjSWEET4* was the highest (9.77). The GRAVY values of all proteins were predicted to be more than 0, indicating their hydrophobic nature. The GRAVY values of *EjSWEETs* were found to be between 0.502 and 0.992. Additionally, SWEET proteins showed an instability index from 24.63 to 48.15, and the aliphatic index was also found to be from 98.97 to 135.64.

Table 3. Summary information of physicochemical properties of the SWEET proteins in loquat.

Gene Name	Protein Length (A.A.)	MW (kDa)	pI	Instability Index	Aliphatic Index	GRAVY
EjSWEET1	232	25.35819	8.71	34.89	128.1	0.936
EjSWEET2	238	26.6245	5.42	43.89	115.42	0.758
EjSWEET3	235	25.87286	8.91	32.61	126.89	0.926
EjSWEET4	263	28.84401	9.77	36.02	98.97	0.502
EjSWEET5	232	25.37417	8.55	38.09	127.28	0.929
EjSWEET6	238	26.69948	4.93	45.91	114.62	0.777
EjSWEET7	263	28.63981	9.57	30.76	102.36	0.521
EjSWEET8	236	26.58405	9.03	39.64	132.33	0.992
EjSWEET9	235	25.99204	9.03	45.23	121.53	0.871
EjSWEET10	235	26.53263	8.99	34.12	121.53	0.716
EjSWEET11	254	28.66118	9.41	30.9	117.76	0.63
EjSWEET12	239	26.42614	5.67	43.5	118.28	0.714
EjSWEET13	267	29.88251	9.34	24.63	111.69	0.59
EjSWEET14	235	25.92469	6.55	45.96	123.19	0.857
EjSWEET15	274	30.57331	8.24	41.78	111.79	0.596
EjSWEET16	241	26.69083	9.22	31.14	121.29	0.687
EjSWEET17	261	29.34506	9.71	38.69	124.33	0.628
EjSWEET18	295	33.0874	5.97	48.15	120.24	0.664
EjSWEET19	236	26.84237	9.57	41.76	135.64	0.911
EjSWEET20	253	28.09724	9.15	40.88	115.06	0.532
EjSWEET21	249	27.58569	9.36	39.2	117.31	0.584

MW: molecular weight of amino acid sequence; pI: theoretical isoelectric point; GRAVY: grand average of hydropathicity.

3.2. Protein Conserved Domain, Subcellular Localization, and Gene Structural Analysis of EjSWEET Genes

Conserved domain analysis showed that all *EjSWEET* genes contained two MtN3/saliva domains, except *EjSWEET9* and *EjSWEET14*. Two genes, *EjSWEET9* and *EjSWEET14*, also contained another domain of the DUF2070 superfamily (Figure S1). Most of the *EjSWEET* genes were predicted to be positioned in the vacuole and plasma membrane, and a few genes were positioned in mitochondria, chloroplasts, and endoplasmic reticulum (Figure 1A). The structural analysis of *EjSWEET* genes indicated that all *EjSWEET* genes contained six exons, except *EjSWEET11* and *EjSWEET17* (Figure 1B). They contained only five exons and four introns.

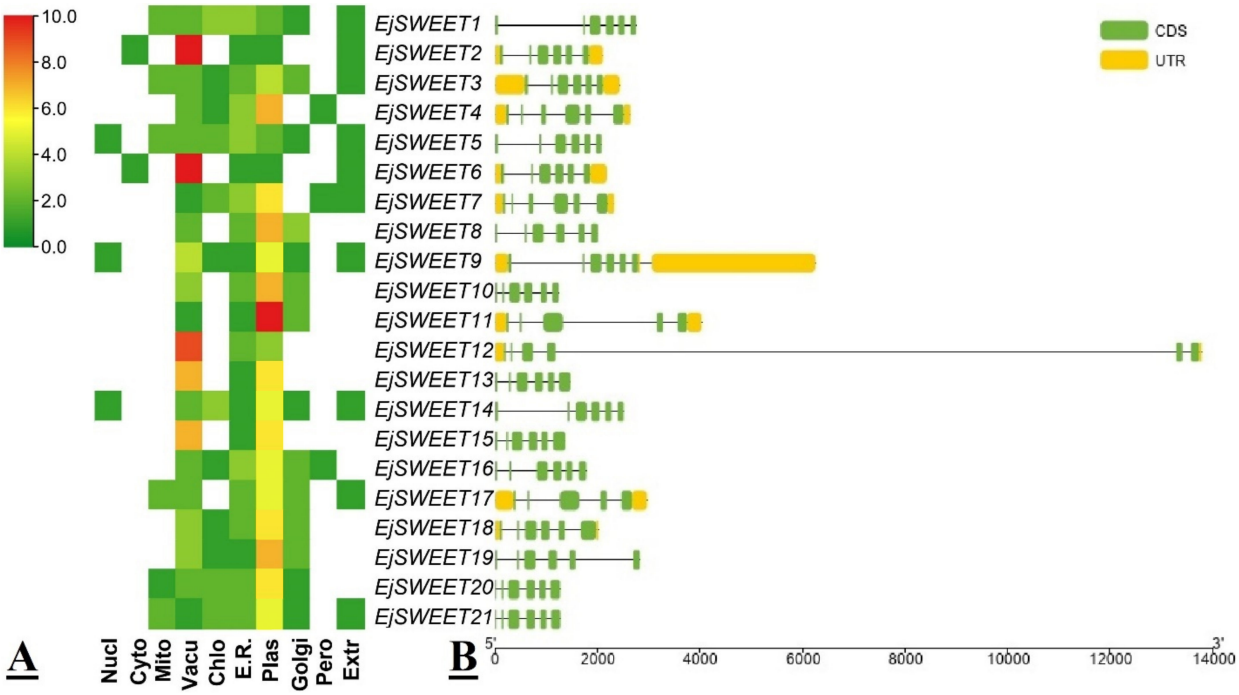

Figure 1. (**A**) Protein subcellular localization prediction of loquat SWEETs. Maximum and minimum signals are shown with red and green color boxes, respectively, while white color boxes represent no available data. Abbreviations: Nucl, nucleus; Cyto, cytoplasm; Mito, mitochondria; Vacu, vacuole; Plas, plasma membrane; Chlo, chloroplast; Golgi, Golgi apparatus; E.R., endoplasmic reticulum; Pero, peroxisomes; Extr, extracellular region. (**B**) Gene organization of *EjSWEETs*. The green boxes represent exons and the black lines represent introns, while untranslated regions (UTR) are denoted by yellow areas.

3.3. Phylogenetic and Conserved Motif Analysis of EjSWEET Genes

The phylogenetic relationship of 21 *EjSWEET* genes was constructed by MEGA-X. The *EjSWEET* genes were divided into three subgroups, according to the similarity level between them. By utilizing online servers of MEME, the distribution of conserved motifs for *EjSWEETs* was thoroughly assessed; a range of five to eight presumed conserved motifs was acknowledged among EjSWEET proteins. Figure 2 indicates that the *EjSWEETs* present in all three groups of phylogenetic analysis (A–C) exhibited similarity in their motifs' organization and composition. The *EjSWEETs* present in phylogenetic groups A, B, and C showed 7–8, 6–7, and 5–6 motifs. Thus, it can be assumed that during the evolutionary process, *EjSWEETs* evidently exhibited extreme conservation.

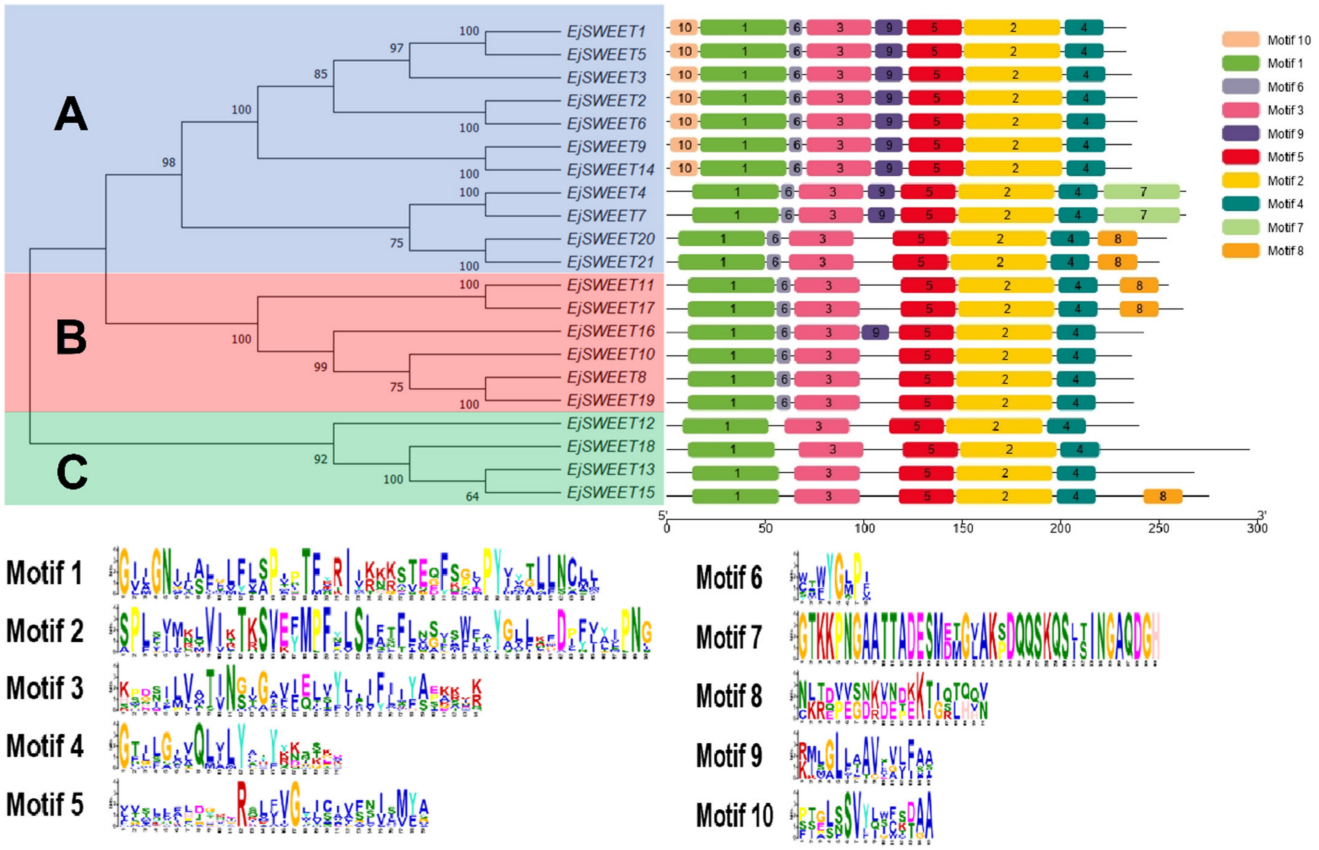

Figure 2. Phylogenetic relationship and conserved motif analysis of *EjSWEET* genes.

3.4. Chromosomal Mapping and Syntenic Analysis of EjSWEET Genes

The chromosomal mapping of 21 *EjSWEET* genes is displayed in Figure 3A. Among these genes, four genes (*EjSWEET1*, *EjSWEET2*, *EjSWEET3*, and *EjSWEET4*) are positioned on Chromosome 1, three genes (*EjSWEET5*, *EjSWEET6*, and *EjSWEET7*) are on Chromosome 2, one gene (*EjSWEET8*) is on Chromosome 3, one gene (*EjSWEET9*) is on Chromosome 4, two genes (*EjSWEET10* and *EjSWEET11*) are on Chromosome 6, one gene (*EjSWEET12*) is on Chromosome 8, three genes (*EjSWEET13*, *EjSWEET14*, and *EjSWEET15*) are on Chromosome 12, one gene (*EjSWEET16*) is on Chromosome 13, three genes (*EjSWEET17*, *EjSWEET18*, and *EjSWEET19*) are on Chromosome 14, and two genes (*EjSWEET20* and *EjSWEET21*) are on Chromosome 16.

The syntenic analysis of the *EjSWEET* genes showed that there are two pairs of tandemly repeated sequences (*EjSWEET13* and *EjSWEET15*, *EjSWEET20* and *EjSWEET21*), among the 21 *EjSWEET* genes (Figure 3B). Meanwhile, the major duplication was observed as "whole-genome (WGD) or segmental-duplication". We found six pairs of WGD repeated genes (*EjSWEET1* and *EjSWEET5*, *EjSWEET2* and *EjSWEET6*, *EjSWEET4* and *EjSWEET7*, *EjSWEET8* and *EjSWEET19*, *EjSWEET9* and *EjSWEET14*, *EjSWEET11* and *EjSWEET17*).

To further analyze whether these tandem or segmental repeated genes are under selection pressure during evolution, we calculated the K_a and K_s values of these genes [48]. The analysis results show that the K_a/K_s values of these *EjSWEET* sequences are less than 1 (Table 4), indicating that these genes have undergone purifying selection during the evolution process.

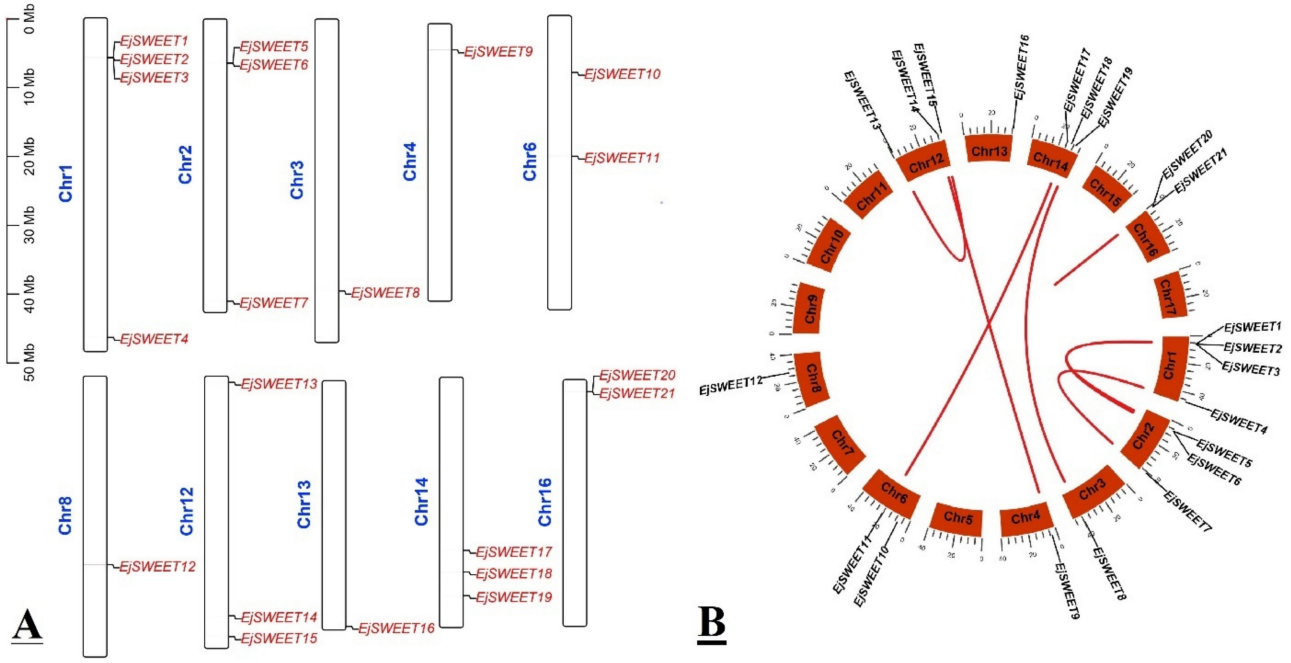

Figure 3. Chromosomal mapping (**A**) and gene duplication (**B**) of *EjSWEETs*.

Table 4. The K_a/K_s ratio of duplicated *EjSWEET* genes.

Gene 1	Gene 2	K_a	K_s	K_a/K_s	Duplication
EjSWEET1	*EjSWEET5*	0.02442	0.1683363	0.1450679	segmental
EjSWEET2	*EjSWEET6*	0.048297	0.1254252	0.3850654	segmental
EjSWEET4	*EjSWEET7*	0.046486	0.2183978	0.2128524	segmental
EjSWEET8	*EjSWEET19*	0.096019	0.1580628	0.6074743	segmental
EjSWEET9	*EjSWEET14*	0.052542	0.0856763	0.6132632	segmental
EjSWEET11	*EjSWEET17*	0.086059	0.135099	0.6370108	segmental
EjSWEET13	*EjSWEET15*	0.452987	2.3006035	0.1968992	tandem
EjSWEET20	*EjSWEET21*	0.014411	0.032882	0.4382501	tandem

3.5. Analysis of Cis-Acting Regulatory Elements in EjSWEET Promoter Region

To further understand the transcription process of *EjSWEET* genes, 1000 bp of the upstream region of *EjSWEETs* were analyzed. The promoter area of *EjSWEETs* contains a large number of *cis*-acting elements, mainly divided into four types: light-response-related component type, hormone-response-related component type, stress-response-related component type, and component type of plant growth and development (Figure 4). Several growth-hormone-related *cis*-elements (i.e., GARE, SARE, AuRE, MeJA, AARE) which regulate different hormones, i.e., gibberellins, methyl jasmonate, salicylic acid, abscisic acid, and auxins, were identified in the promoter regions of *EjSWEETs*. In addition, low temperature stress response *cis*-element LTR was also identified in several genes. The LRE was found as a light-responsive *cis*-element. Apart from the aforementioned *cis*-elements, circadian was also identified in two *EjSWEET* genes, having a role in circadian control.

3.6. Multiple Sequence Alignment of EjSWEET Proteins

Sequence alignment revealed that the five motifs were relatively conserved (Figure 5). Furthermore, there were two possible serine phosphorylation sites located on the inner side of the Motif 2 area. However, Motifs 1 and 2 started after alanine. In Motif 1, glycine was highly conserved with isoleucine and asparagine. In addition, leucine, proline, threonine, phenylalanine, and tyrosine were also found highly conserved in Motif 1. Phosphorylation can occur for all of these amino acids but not for alanine. Valine, isoleucine, threonine, and serine were found highly conserved in the case of Motif 2. Motif 3 showed the conservation

of phenylalanine and asparagine. Arginine and glutamine were the only amino acid bases conserved in all EjSWEET proteins in Motif 4 and Motif 5, respectively.

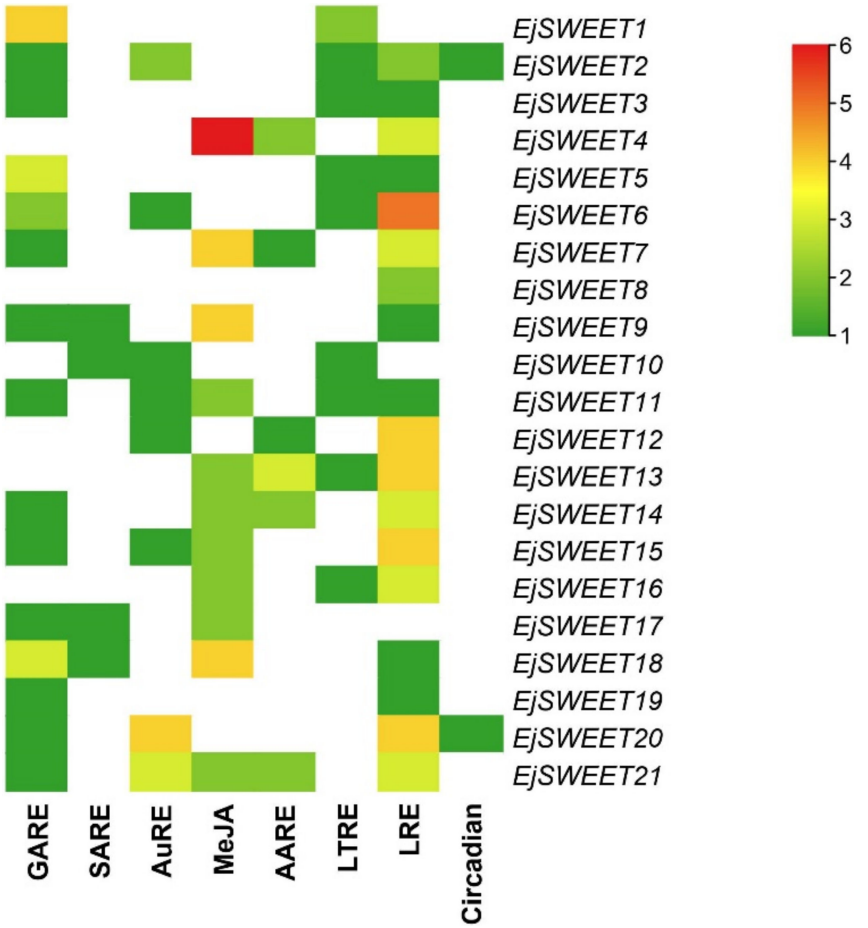

Figure 4. The promoter region analysis (*cis*-regulatory elements) of *EjSWEET* genes. Maximum and minimum number of *cis*-elements are denoted by red and green boxes, respectively.

3.7. Phylogenetic Analysis of the EjSWEET Genes in Eriobotrya Japonica, Malus Domestica, and Arabidopsis Thaliana

The phylogenetic tree of 63 *SWEET* genes (21 for *Eriobotrya japonica*, 25 for *Malus domestica*, and 17 for *Arabidopsis thaliana*) was constructed by MEGA-X. These proteins were divided into three groups (A–C) based on their sequence similarity (Figure 6), and the 21 *EjSWEET* genes were distributed into three groups. Among the *EjSWEET* gene family, groups A, B, and C contained 11, 6, and 4 genes, respectively.

The grouping results of the integrated phylogenetic tree were basically consistent with the phylogenetic tree based on the *EjSWEET* protein sequences. According to the results of conserved motifs, the same subgroup has a similar number and type of conserved motifs, which further verifies the grouping results of the phylogenetic tree (Figure 2). In addition, the number and types of conserved motifs and protein conserved domains of groups B and C are significantly less than those of group A.

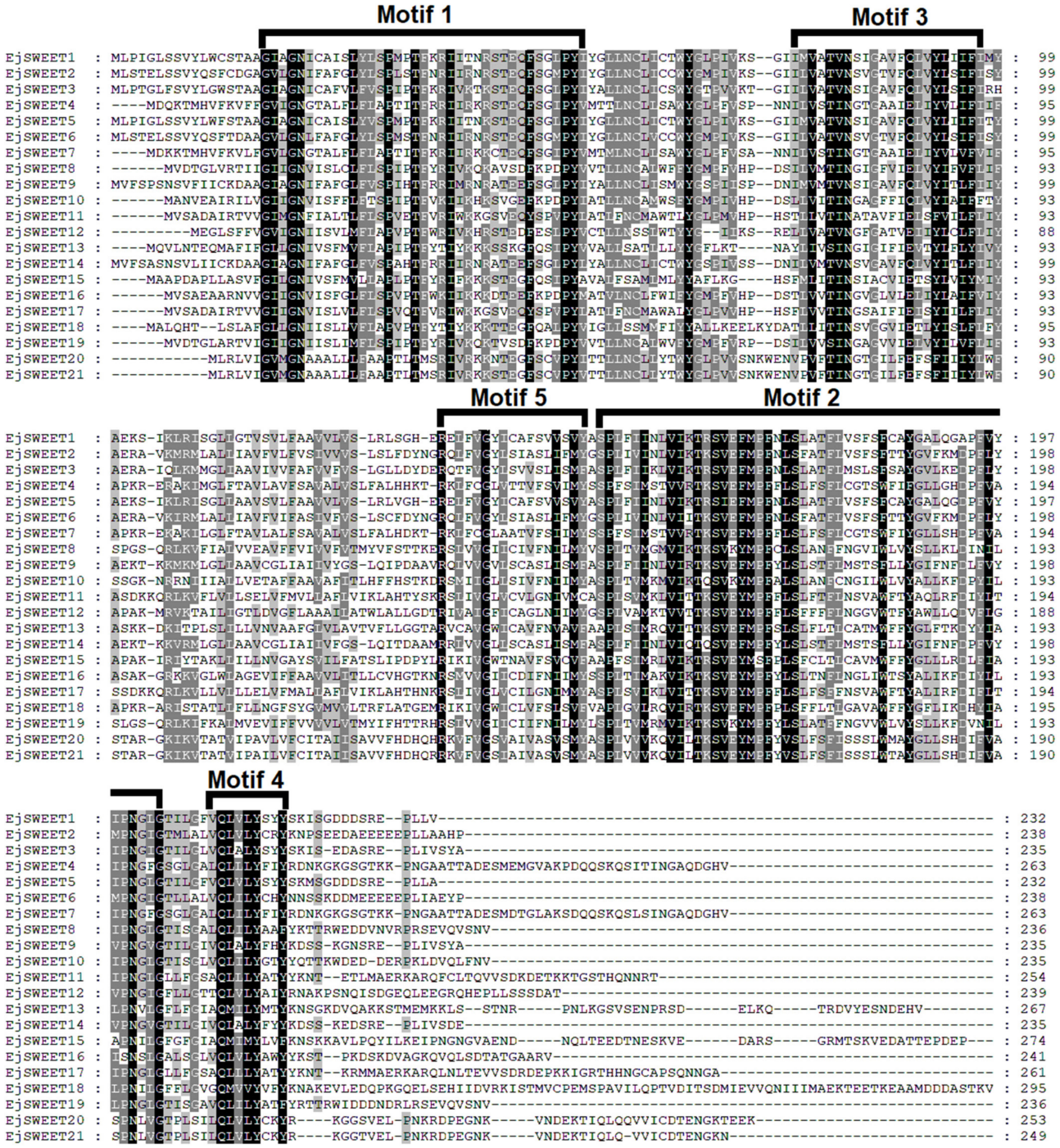

Figure 5. Multiple sequence alignment of the *EjSWEETs*. The positions of the seven motifs are indicated above the sequences. Highly conserved amino acids (>80%) are shaded with black, moderate conservation (60–80%) is with grey, while the non-shaded amino acids represent less conservation (<60%).

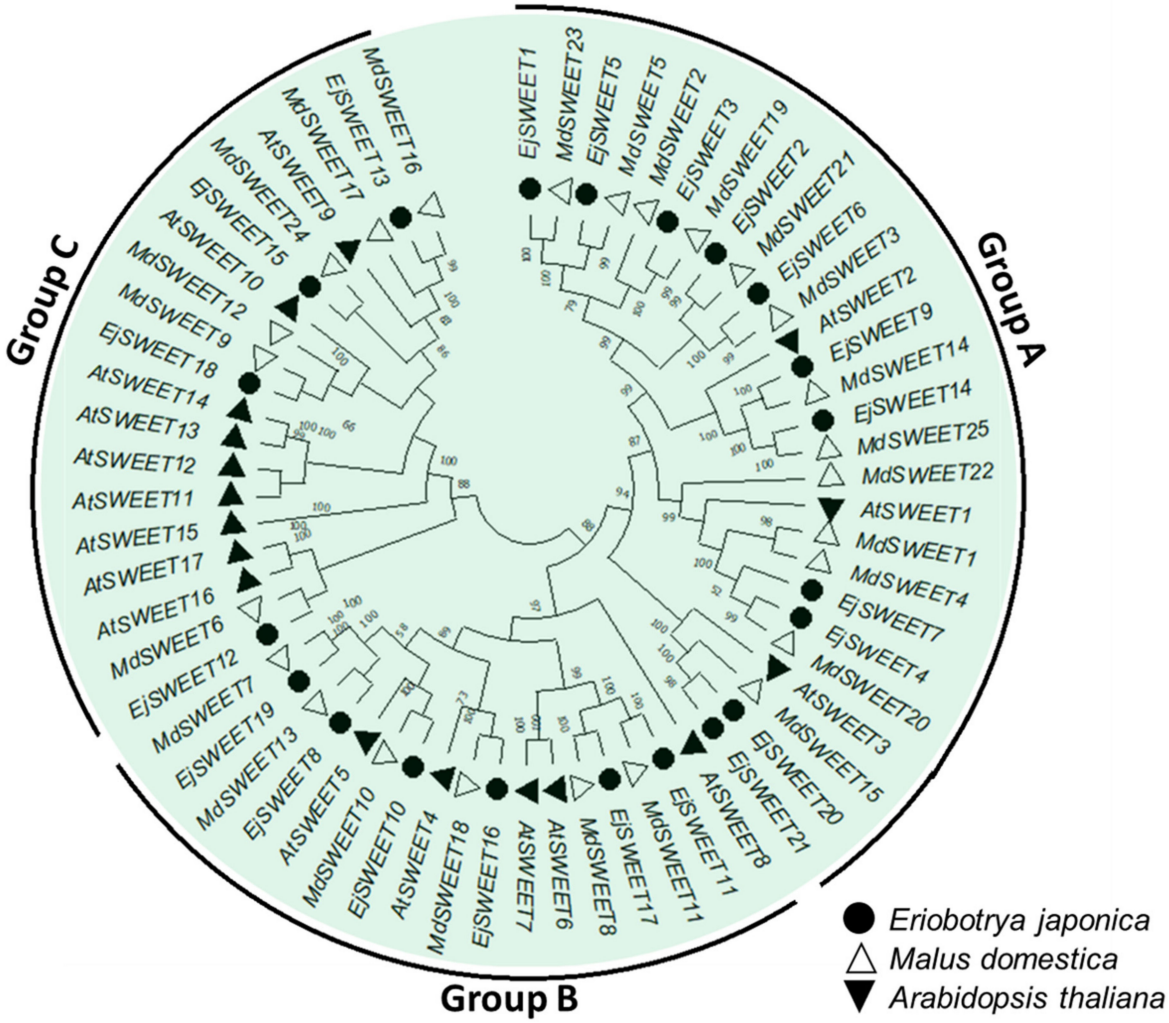

Figure 6. Phylogenetic associations of *SWEET* genes among loquat, Arabidopsis, and apple. *SWEET* genes fall into three groups labeled A, B, and C. Ej—loquat; Md—apple; At—Arabidopsis. The gene IDs of apple and Arabidopsis *SWEET* genes are provided in Table S1.

3.8. Expression Profiling of EjSWEET Genes in Different Tissues of Loquat

Different tissues from loquat were selected for expression profile analysis among 21 *SWEET* genes, including mature leaves, stems, roots, flowers, and fruits (Figure 7). All genes exhibited significantly different expression levels among different plant tissues. Among 21 loquat *SWEETs*, 12 genes (*EjSWEET1*, *EjSWEET2*, *EjSWEET5*, *EjSWEET7*, *EjSWEET8*, *EjSWEET9*, *EjSWEET11*, *EjSWEET14*, *EjSWEET17*, *EjSWEET19*, *EjSWEET20*, and *EjSWEET21*) were maximally expressed in full-bloom flowers of loquat, while ripened fruits exhibited the maximum expression of 3 genes, i.e., *EjSWEET1*, *EjSWEET3*, and *EjSWEET16*. Similarly, *EjSWEET4*, *EjSWEET10*, *EjSWEET15*, and *EjSWEET18* were maximally expressed in loquat stem, while loquat leaves showed maximum transcript levels of *EjSWEET3*, *EjSWEET6*, and *EjSWEET15*. The expression level of *EjSWEET13* was recorded maximum in loquat roots, among all other examined tissues.

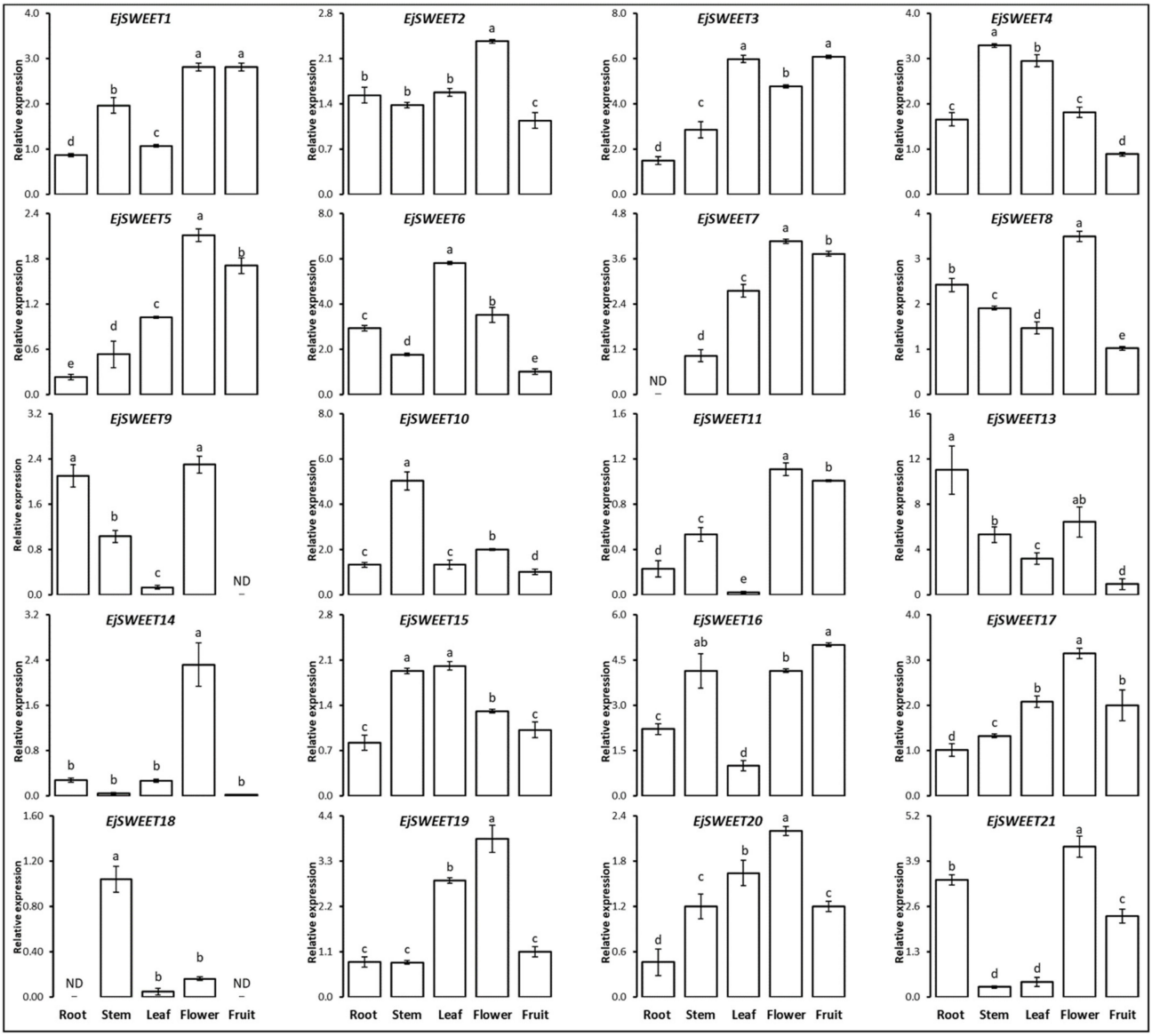

Figure 7. Relative expression levels of *EjSWEETs* in five tissues of loquat, i.e., root, stem, leaf, flower, and fruit. Significant difference among different tissues is denoted by different letters following the least significant difference (LSD) test ($p \leq 0.05$). Vertical bars show average ± standard error (6 replicates). ND—not detected. The genetic expression of *EjSWEET12* was not detected in all loquat tissues.

4. Discussion

SWEET proteins are widely distributed in plant species and have a basic contribution to numerous processes during the life cycle of the plant [7,13,14,17,24]. To date, studies about the genomic and functional characterization of *SWEET* genes have only been conducted in a few species, including Arabidopsis, rice, tomato, soybean, and cucumber [11,14,31,49–51]. Meanwhile, except for apple, no systematic investigation had been conducted for the *SWEET* gene family in Rosaceae species until now [52]. Following a combination of available analytical techniques, we identified 21 *EjSWEET* genes in terms of their gene structure, chromosome distribution, phylogeny, *cis*-acting regulatory elements, domain architecture, and expression profiles among different tissues of loquat.

As every organism went through evolution, it is proposed that tandem and WGD/segmental duplication both play an integral role in gene duplication and the evolutionary process [46]. Likewise, recently it was suggested that *SWEETs* also underwent gene

duplication during the evolution of rice [49] and soybean [14]. We also found that two pairs of *EjSWEETs* (*EjSWEET13* and *EjSWEET15*, *EjSWEET20* and *EjSWEET21*) were regarded as tandem duplication (Figure 3). The *cis*-elements available in promotor regions of *EjSWEETs* were also investigated, giving at least one *cis*-element responsible for hormone regulation, and may be integral for corresponding hormone regulation within loquat (Figure 4). Other plant species, e.g., cucumber and rice, were also reported to have tandem duplications among *SWEET* genes [49,50]. Conserved regions in SWEET proteins could be important for their basic functioning in plants [50]. We also found a relatively conserved region among 21 identified gene sequences in loquat. Moreover, on the inner side of Motif 2 proteins, we observed two possible serine phosphorylation sites (Figure 5), suggesting that EjSWEET proteins may exhibit key functions in regulating reversible dephosphorylation/phosphorylation, controlled by protein phosphatase/kinase [53]. Such outcomes might be a breakthrough for *SWEET* gene family functional analysis in loquat.

The phylogenetic tree of *SWEET* genes of loquat, apple, and Arabidopsis was constructed, and these genes were divided into three groups based on their sequence similarity (Figure 6). A similar grouping has been reported earlier when *SWEET* genes were identified in apple [52,54]. The selection of the apple genome for phylogenetic analysis was due to its similarity with the genome of loquat [37,55]. *SWEET* genes are well known for their diversified functional regulation throughout the plant life cycle. Though functional characterization of loquat *SWEET* genes has not been performed yet, there is a high possibility they are likely to have similar features to rice and Arabidopsis *SWEET* genes. Previously, Arabidopsis and rice *SWEET* genes were found to be associated with the development of reproductive tissues [11,13,19,56,57]. In such relation, expression profiles were analyzed among different tissues of the loquat *SWEET* family, and 11 genes showed higher relative expression in flowers (Figure 7), implying that most of the *EjSWEET* genes may be closely associated with growth and development of reproductive tissues. Paralogs from the *SWEET* gene family within different species could have functional redundancy or precisely regulate the plant life cycle throughout key developmental stages. Hence, in order to keenly study the functional profiles of *SWEET* genes, further comprehensive analyses are required, while our experimental outcomes provide a foundation for future studies.

In model plant species, Arabidopsis and rice, sucrose is known as the main form of carbohydrates circulated via phloem sap upon sink requirements. For the transportation of photoassimilates, such as sugars, from leaves to sink organs, phloem loading is the basic step in terms of longer-distance sugar transport [58]. In *A. thaliana*, two genes localized at the plasma membrane, named *AtSWEET11* and *AtSWEET12*, are responsible for the exportation of sucrose from phloem parenchyma cells to the apoplast [16]. Besides sucrose transportation, raffinose family oligosaccharides (RFOs) are also translocated as primary carbohydrates in many plant species [59,60]. In plants exhibiting RFO transportation, an extensive symplasmic pathway known as a polymer trap is followed for phloem loading [58]; it mechanizes sucrose diffusion into intermediary cells from the mesophyll symplasm, as sucrose molecules are smaller than RFOs and are apparently unable to diffuse back to the mesophyll through plasmodesmata intermediary cells [58,61]. In current study, *EjSWEET3*, *EjSWEET6*, and *EjSWEET15* exhibited a relatively higher expression in loquat leaves (Figure 7), indicating their possible involvement in RFO transport. Chen et al. [24] also identified the role of the *AtSWEET2* gene, as it was to be involved in glucose accumulation in the root and leaf tonoplast of Arabidopsis. The genes *EjSWEET3*, *EjSWEET6*, and *EjSWEET15* may not directly regulate the phloem loading, but they are noticeably involved in monosaccharide regulation in loquat leaves, having a significant impact on carbon allocation as well as flux transportation between heterotrophic plant tissues. However, specific functional studies still need to be conducted.

Sucrose and hexoses (glucose and fructose) are major sugars found in fruits and plant stems of loquat [36], which suggests that sucrose is translocated into the fruit [5]. Following the apoplastic pathway, sucrose is unloaded from the phloem into the fruits of loquat [62]. Cell wall invertase (*cwINV*) is subsequently important for sucrose hydrolysis

to form hexoses, which are then taken into fruit parenchyma cells for storage via hexose transporters (*HT3*) [63]. Still, the process contains several unanswered questions; for example, there is an unknown key transporter that exports sucrose from sieve companion cell complexes into apoplastic spaces, and fructose transportation taking place from the cleavage of sucrose in the apoplast remains unclear because *HT3* could be a key facilitator only for glucose intake, not fructose [63,64]. We observed that some *SWEET* genes such as *EjSWEET1*, *EjSWEET3,* and *EjSWEET16* have higher expression levels in loquat fruits (Figure 7), suggesting their possible role in the transportation and unloading of sugars into fruits for sink storage. Nevertheless, additional experimentations are required to keenly determine the basic functions of *SWEET* genes for sugar regulation during the fruit development of loquat, as they could be major facilitators for not only sucrose but also fructose and glucose [11,65]. Additionally, *SWEET* genes were identified and expressed in some fruiting species such as apple [52], tomato [66], and sweet orange [67], but their particular contribution remains unclear.

5. Conclusions

In the present study, 21 putative *SWEET* genes were identified in the loquat genome, and a comprehensive genome-wide in silico analysis was performed in terms of their possible characteristics, including subcellular localization, gene organization, chromosomal distribution, synteny, phylogeny, multiple sequence alignment, conserved motifs, and *cis*-regulatory elements in promoter regions. In addition, their relative expression levels were examined in different tissues of loquat. The results revealed the basic understanding of *EjSWEET* genes and may also facilitate future research that may elucidate the detailed roles of *SWEET* genes in loquat and other Rosaceae crops.

Supplementary Materials: The following supporting information can be downloaded at: https://www.mdpi.com/article/10.3390/agriculture12091312/s1, Figure S1: The conserved domain analysis of *EjSWEETs*; Table S1: The gene IDs of apple and Arabidopsis SWEET genes.

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References

1. Chen, L.-Q.; Cheung, L.S.; Feng, L.; Tanner, W.; Frommer, W.B. Transport of Sugars. *Annu. Rev. Biochem.* **2015**, *84*, 865–894. [CrossRef] [PubMed]
2. Sadat-Hosseini, M.; Bakhtiarizadeh, M.R.; Boroomand, N.; Tohidfar, M.; Vahdati, K. Combining independent de novo assemblies to optimize leaf transcriptome of Persian walnut. *PLoS ONE* **2020**, *15*, e0232005. [CrossRef] [PubMed]
3. Chen, L. SWEET sugar transporters for phloem transport and pathogen nutrition. *New Phytol.* **2014**, *201*, 1150–1155. [CrossRef] [PubMed]
4. Lalonde, S.; Wipf, D.; Frommer, W.B. Transport mechanisms for organic forms of carbon and nitrogen between source and sink. *Annu. Rev. Plant Biol.* **2004**, *55*, 341–372. [CrossRef] [PubMed]

5. Yu, X.; Ali, M.M.; Li, B.; Fang, T.; Chen, F. Transcriptome data-based identification of candidate genes involved in metabolism and accumulation of soluble sugars during fruit development in 'Huangguan' plum. *J. Food Biochem.* **2021**, *45*, e13878. [CrossRef] [PubMed]
6. Pan, T.; Ali, M.M.; Gong, J.; She, W.; Pan, D.; Guo, Z.; Yu, Y.; Chen, F. Fruit Physiology and Sugar-Acid Profile of 24 Pomelo (*Citrus grandis* (L.) Osbeck) Cultivars Grown in Subtropical Region of China. *Agronomy* **2021**, *11*, 2393. [CrossRef]
7. Eom, J.-S.; Chen, L.-Q.; Sosso, D.; Julius, B.T.; Lin, I.; Qu, X.-Q.; Braun, D.M.; Frommer, W.B. SWEETs, transporters for intracellular and intercellular sugar translocation. *Curr. Opin. Plant Biol.* **2015**, *25*, 53–62. [CrossRef]
8. Kühn, C.; Grof, C.P. Sucrose transporters of higher plants. *Curr. Opin. Plant Biol.* **2010**, *13*, 287–297. [CrossRef]
9. Ayre, B.G. Membrane-Transport Systems for Sucrose in Relation to Whole-Plant Carbon Partitioning. *Mol. Plant* **2011**, *4*, 377–394. [CrossRef]
10. Slewinski, T.L. Diverse Functional Roles of Monosaccharide Transporters and their Homologs in Vascular Plants: A Physiological Perspective. *Mol. Plant* **2011**, *4*, 641–662. [CrossRef]
11. Chen, L.-Q.; Hou, B.-H.; Lalonde, S.; Takanaga, H.; Hartung, M.L.; Qu, X.-Q.; Guo, W.-J.; Kim, J.-G.; Underwood, W.; Chaudhuri, B.; et al. Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* **2010**, *468*, 527–532. [CrossRef]
12. Xuan, Y.H.; Hu, Y.B.; Chen, L.-Q.; Sosso, D.; Ducat, D.C.; Hou, B.-H.; Frommer, W.B. Functional role of oligomerization for bacterial and plant SWEET sugar transporter family. *Proc. Natl. Acad. Sci. USA* **2013**, *110*, 3685–3694. [CrossRef]
13. Yuan, M.; Wang, S. Rice MtN3/Saliva/SWEET Family Genes and Their Homologs in Cellular Organisms. *Mol. Plant* **2013**, *6*, 665–674. [CrossRef]
14. Patil, G.; Valliyodan, B.; Deshmukh, R.; Prince, S.; Nicander, B.; Zhao, M.; Sonah, H.; Song, L.; Lin, L.; Chaudhary, J.; et al. Soybean (*Glycine max*) SWEET gene family: Insights through comparative genomics, transcriptome profiling and whole genome re-sequence analysis. *BMC Genom.* **2015**, *16*, 520. [CrossRef]
15. Chandran, D. Co-option of developmentally regulated plant SWEET transporters for pathogen nutrition and abiotic stress tolerance. *IUBMB Life* **2015**, *67*, 461–471. [CrossRef]
16. Chen, L.-Q.; Qu, X.-Q.; Hou, B.-H.; Sosso, D.; Osorio, S.; Fernie, A.R.; Frommer, W.B. Sucrose Efflux Mediated by SWEET Proteins as a Key Step for Phloem Transport. *Science* **2012**, *335*, 207–211. [CrossRef]
17. Lin, I.W.; Sosso, D.; Chen, L.-Q.; Gase, K.; Kim, S.-G.; Kessler, D.; Klinkenberg, P.M.; Gorder, M.K.; Hou, B.-H.; Qu, X.-Q.; et al. Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. *Nature* **2014**, *508*, 546–549. [CrossRef]
18. Engel, M.L.; Holmes-Davis, R.; McCormick, S. Green Sperm. Identification of Male Gamete Promoters in Arabidopsis. *Plant Physiol.* **2005**, *138*, 2124–2133. [CrossRef]
19. Guan, Y.-F.; Huang, X.-Y.; Zhu, J.; Gao, J.-F.; Zhang, H.-X.; Yang, Z.-N. RUPTURED POLLEN GRAIN1, a Member of the MtN3/saliva Gene Family, Is Crucial for Exine Pattern Formation and Cell Integrity of Microspores in Arabidopsis. *Plant Physiol.* **2008**, *147*, 852–863. [CrossRef]
20. Sun, M.-X.; Huang, X.-Y.; Yang, J.; Guan, Y.-F.; Yang, Z.-N. Arabidopsis RPG1 is important for primexine deposition and functions redundantly with RPG2 for plant fertility at the late reproductive stage. *Plant Reprod.* **2013**, *26*, 83–91. [CrossRef]
21. Zhou, Y.; Liu, L.; Huang, W.; Yuan, M.; Zhou, F.; Li, X.; Lin, Y. Overexpression of *OsSWEET5* in Rice Causes Growth Retardation and Precocious Senescence. *PLoS ONE* **2014**, *9*, e94210. [CrossRef]
22. Arab, M.M.; Brown, P.J.; Abdollahi-Arpanahi, R.; Sohrabi, S.S.; Askari, H.; Aliniaeifard, S.; Mokhtassi-Bidgoli, A.; Mesgaran, M.B.; Leslie, C.A.; Marrano, A.; et al. Genome-wide association analysis and pathway enrichment provide insights into the genetic basis of photosynthetic responses to drought stress in Persian walnut. *Hortic. Res.* **2022**, *9*, uhac124. [CrossRef]
23. Arab, M.M.; Marrano, A.; Abdollahi-Arpanahi, R.; Leslie, C.A.; Askari, H.; Neale, D.B.; Vahdati, K. Genome-wide patterns of population structure and association mapping of nut-related traits in Persian walnut populations from Iran using the Axiom J. regia 700K SNP array. *Sci. Rep.* **2019**, *9*, 6376. [CrossRef]
24. Chen, L.-Q.; Lin, I.W.; Qu, X.-Q.; Sosso, D.; McFarlane, H.E.; Londoño, A.; Samuels, A.L.; Frommer, W.B. A Cascade of Sequentially Expressed Sucrose Transporters in the Seed Coat and Endosperm Provides Nutrition for the Arabidopsis Embryo. *Plant Cell* **2015**, *27*, 607–619. [CrossRef] [PubMed]
25. Sosso, D.; Luo, D.; Li, Q.-B.; Sasse, J.; Yang, J.; Gendrot, G.; Suzuki, M.; Koch, K.E.; McCarty, D.R.; Chourey, P.S.; et al. Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport. *Nat. Genet.* **2015**, *47*, 1489–1493. [CrossRef]
26. Yuan, M.; Chu, Z.; Li, X.; Xu, C.; Wang, S. Pathogen-Induced Expressional Loss of Function is the Key Factor in Race-Specific Bacterial Resistance Conferred by a Recessive R Gene xa13 in Rice. *Plant Cell Physiol.* **2009**, *50*, 947–955. [CrossRef]
27. Streubel, J.; Pesce, C.; Hutin, M.; Koebnik, R.; Boch, J.; Szurek, B. Five phylogenetically close rice *SWEET* genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae*. *New Phytol.* **2013**, *200*, 808–819. [CrossRef]
28. Chong, J.; Piron, M.-C.; Meyer, S.; Merdinoglu, D.; Bertsch, C.; Mestre, P. The SWEET family of sugar transporters in grapevine: *VvSWEET4* is involved in the interaction with *Botrytis cinerea*. *J. Exp. Bot.* **2014**, *65*, 6589–6601. [CrossRef] [PubMed]
29. Cohn, M.; Bart, R.S.; Shybut, M.; Dahlbeck, D.; Gomez, M.; Morbitzer, R.; Hou, B.-H.; Frommer, W.B.; Lahaye, T.; Staskawicz, B.J. *Xanthomonas axonopodis* Virulence Is Promoted by a Transcription Activator-Like Effector–Mediated Induction of a SWEET Sugar Transporter in Cassava. *Mol. Plant Microbe Interact.* **2014**, *27*, 1186–1198. [CrossRef] [PubMed]

30. Hu, Y.; Zhang, J.; Jia, H.; Sosso, D.; Li, T.; Frommer, W.B.; Yang, B.; White, F.F.; Wang, N.; Jones, J.B. Lateral organ boundaries 1 is a disease susceptibility gene for citrus bacterial canker disease. *Proc. Natl. Acad. Sci. USA* **2014**, *111*, E521–E529. [CrossRef] [PubMed]
31. Xie, H.; Wang, D.; Qin, Y.; Ma, A.; Fu, J.; Qin, Y.; Hu, G.; Zhao, J. Genome-wide identification and expression analysis of *SWEET* gene family in Litchi chinensis reveal the involvement of LcSWEET2a/3b in early seed development. *BMC Plant Biol.* **2019**, *19*, 499. [CrossRef] [PubMed]
32. Ali, M.M.; Anwar, R.; Shafique, M.W.; Yousef, A.F.; Chen, F. Exogenous Application of Mg, Zn and B Influences Phyto-Nutritional Composition of Leaves and Fruits of Loquat (*Eriobotrya japonica* Lindl.). *Agronomy* **2021**, *11*, 224. [CrossRef]
33. Ali, M.M.; Li, B.; Zhi, C.; Yousef, A.F.; Chen, F. Foliar-Supplied Molybdenum Improves Phyto-Nutritional Composition of Leaves and Fruits of Loquat (*Eriobotrya japonica* Lindl.). *Agronomy* **2021**, *11*, 892. [CrossRef]
34. Ali, M.M.; Rizwan, H.M.; Yousef, A.F.; Zhi, C.; Chen, F. Analysis of toxic elements in leaves and fruits of loquat by inductively coupled plasma-mass spectrometry (ICP-MS). *Acta Sci. Pol. Hortorum Cultus* **2021**, *20*, 33–42. [CrossRef]
35. Badenes, M.L.; Canyamas, T.; Romero, C.; Soriano, J.M.; Martínez, J.; Llácer, G. Genetic diversity in european collection of loquat (*Eriobotrya japonica* Lindl.). *Acta Hortic.* **2003**, *620*, 169–174. [CrossRef]
36. Zhi, C.; Ali, M.M.; Zhang, J.; Shi, M.; Ma, S.; Chen, F. Effect of Paper and Aluminum Bagging on Fruit Quality of Loquat (*Eriobotrya japonica* Lindl.). *Plants* **2021**, *10*, 2704. [CrossRef]
37. Jiang, S.; An, H.; Xu, F.; Zhang, X. Chromosome-level genome assembly and annotation of the loquat (*Eriobotrya japonica*) genome. *GigaScience* **2020**, *9*, giaa015. [CrossRef]
38. Daccord, N.; Celton, J.-M.; Linsmith, G.; Becker, C.; Choisne, N.; Schijlen, E.; van de Geest, H.; Bianco, L.; Micheletti, D.; Velasco, R.; et al. High-quality de novo assembly of the apple genome and methylome dynamics of early fruit development. *Nat. Genet.* **2017**, *49*, 1099–1106. [CrossRef]
39. Finn, R.D.; Mistry, J.; Tate, J.; Coggill, P.; Heger, A.; Pollington, J.E.; Gavin, O.L.; Gunasekaran, P.; Ceric, G.; Forslund, K.; et al. The Pfam protein families database. *Nucleic Acids Res.* **2010**, *38*, D211–D222. [CrossRef]
40. Eddy, S.R. Accelerated Profile HMM Searches. *PLoS Comput. Biol.* **2011**, *7*, e1002195. [CrossRef]
41. Chen, C.; Chen, H.; Zhang, Y.; Thomas, H.R.; Frank, M.H.; He, Y.; Xia, R. TBtools: An Integrative Toolkit Developed for Interactive Analyses of Big Biological Data. *Mol. Plant* **2020**, *13*, 1194–1202. [CrossRef]
42. Wang, Y.; Tang, H.; DeBarry, J.D.; Tan, X.; Li, J.; Wang, X.; Lee, T.-H.; Jin, H.; Marler, B.; Guo, H.; et al. MCScanX: A toolkit for detection and evolutionary analysis of gene synteny and collinearity. *Nucleic Acids Res.* **2012**, *40*, e49. [CrossRef]
43. Gan, X.; Jing, Y.; Shahid, M.Q.; He, Y.; Baloch, F.S.; Lin, S.; Yang, X. Identification, phylogenetic analysis, and expression patterns of the SAUR gene family in loquat (*Eriobotrya japonica*). *Turk. J. Agric. For.* **2020**, *44*, 15–23. [CrossRef]
44. Wang, D.; Zhang, Y.; Zhang, Z.; Zhu, J.; Yu, J. KaKs_Calculator 2.0: A Toolkit Incorporating Gamma-Series Methods and Sliding Window Strategies. *Genom. Proteom. Bioinform.* **2010**, *8*, 77–80. [CrossRef]
45. Kumar, S.; Stecher, G.; Li, M.; Knyaz, C.; Tamura, K. MEGA X: Molecular Evolutionary Genetics Analysis across Computing Platforms. *Mol. Biol. Evol.* **2018**, *35*, 1547–1549. [CrossRef]
46. Ali, M.M.; Alam, S.M.; Anwar, R.; Ali, S.; Shi, M.; Liang, D.; Lin, Z.; Chen, F. Genome-Wide Identification, Characterization and Expression Profiling of Aluminum-Activated Malate Transporters in *Eriobotrya japonica* Lindl. *Horticulturae* **2021**, *7*, 441. [CrossRef]
47. Zhi, C.; Ali, M.M.; Alam, S.M.; Gull, S.; Ali, S.; Yousef, A.F.; Ahmed, M.A.A.; Ma, S.; Chen, F. Genome-Wide In Silico Analysis and Expression Profiling of Phospho*enol*pyruvate Carboxylase Genes in Loquat, Apple, Peach, Strawberry and Pear. *Agronomy* **2021**, *12*, 25. [CrossRef]
48. Akhunov, E.D.; Sehgal, S.; Liang, H.; Wang, S.; Akhunova, A.R.; Kaur, G.; Li, W.; Forrest, K.L.; See, D.; Šimková, H.; et al. Comparative Analysis of Syntenic Genes in Grass Genomes Reveals Accelerated Rates of Gene Structure and Coding Sequence Evolution in Polyploid Wheat. *Plant Physiol.* **2012**, *161*, 252–265. [CrossRef]
49. Yuan, M.; Zhao, J.; Huang, R.; Li, X.; Xiao, J.; Wang, S. Rice MtN3/saliva/SWEET gene family: Evolution, expression profiling, and sugar transport. *J. Integr. Plant Biol.* **2014**, *56*, 559–570. [CrossRef]
50. Hu, L.; Zhang, F.; Song, S.; Tang, X.; Xu, H.; Liu, G.; Wang, Y.; He, H. Genome-wide identification, characterization, and expression analysis of the *SWEET* gene family in cucumber. *J. Integr. Agric.* **2017**, *16*, 1486–1501. [CrossRef]
51. Feng, C.-Y.; Han, J.-X.; Han, X.-X.; Jiang, J. Genome-wide identification, phylogeny, and expression analysis of the *SWEET* gene family in tomato. *Gene* **2015**, *573*, 261–272. [CrossRef]
52. Wei, X.; Liu, F.; Chen, C.; Ma, F.; Li, M. The Malus domestica sugar transporter gene family: Identifications based on genome and expression profiling related to the accumulation of fruit sugars. *Front. Plant Sci.* **2014**, *5*, 569. [CrossRef]
53. Ardito, F.; Giuliani, M.; Perrone, D.; Troiano, G.; Lo Muzio, L. The crucial role of protein phosphorylation in cell signaling and its use as targeted therapy (Review). *Int. J. Mol. Med.* **2017**, *40*, 271–280. [CrossRef]
54. Zhen, Q.; Fang, T.; Peng, Q.; Liao, L.; Zhao, L.; Owiti, A.; Han, Y. Developing gene-tagged molecular markers for evaluation of genetic association of apple *SWEET* genes with fruit sugar accumulation. *Hortic. Res.* **2018**, *5*, 14. [CrossRef]
55. Lo, E.Y.Y.; Donoghue, M.J. Expanded phylogenetic and dating analyses of the apples and their relatives (Pyreae, Rosaceae). *Mol. Phylogenet. Evol.* **2012**, *63*, 230–243. [CrossRef]
56. Chu, Z.; Yuan, M.; Yao, J.; Ge, X.; Yuan, B.; Xu, C.; Li, X.; Fu, B.; Li, Z.; Bennetzen, J.L.; et al. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev.* **2006**, *20*, 1250–1255. [CrossRef]

57. Antony, G.; Zhou, J.; Huang, S.; Li, T.; Liu, B.; White, F.; Yang, B. Rice xa13 Recessive Resistance to Bacterial Blight Is Defeated by Induction of the Disease Susceptibility Gene Os- 11N3. *Plant Cell* **2010**, *22*, 3864–3876. [CrossRef]
58. Rennie, E.A.; Turgeon, R. A comprehensive picture of phloem loading strategies. *Proc. Natl. Acad. Sci. USA* **2009**, *106*, 14162–14167. [CrossRef]
59. Hu, L.-P.; Meng, F.-Z.; Wang, S.-H.; Sui, X.-L.; Li, W.; Wei, Y.-X.; Sun, J.-L.; Zhang, Z.-X. Changes in carbohydrate levels and their metabolic enzymes in leaves, phloem sap and mesocarp during cucumber (*Cucumis sativus* L.) fruit development. *Sci. Hortic.* **2009**, *121*, 131–137. [CrossRef]
60. Bachmann, M.; Matile, P.; Keller, F. Metabolism of the Raffinose Family Oligosaccharides in Leaves of *Ajuga reptans* L. (Cold Acclimation, Translocation, and Sink to Source Transition: Discovery of Chain Elongation Enzyme). *Plant Physiol.* **1994**, *105*, 1335–1345. [CrossRef] [PubMed]
61. Yadav, U.P.; Ayre, B.G.; Bush, D.R. Transgenic approaches to altering carbon and nitrogen partitioning in whole plants: Assessing the potential to improve crop yields and nutritional quality. *Front. Plant Sci.* **2015**, *6*, 275. [CrossRef] [PubMed]
62. Hu, L.; Sun, H.; Li, R.; Zhang, L.; Wang, S.; Sui, X.; Zhang, Z. Phloem unloading follows an extensive apoplasmic pathway in cucumber (*Cucumis sativus* L.) fruit from anthesis to marketable maturing stage. *Plant. Cell Environ.* **2011**, *34*, 1835–1848. [CrossRef] [PubMed]
63. Hayes, M.A.; Davies, C.; Dry, I.B. Isolation, functional characterization, and expression analysis of grapevine (*Vitis vinifera* L.) hexose transporters: Differential roles in sink and source tissues. *J. Exp. Bot.* **2007**, *58*, 1985–1997. [CrossRef]
64. Cheng, J.-T.; Li, X.; Yao, F.-Z.; Shan, N.; Li, Y.-H.; Zhang, Z.-X.; Sui, X.-L. Functional characterization and expression analysis of cucumber (*Cucumis sativus* L.) hexose transporters, involving carbohydrate partitioning and phloem unloading in sink tissues. *Plant Sci.* **2015**, *237*, 46–56. [CrossRef]
65. Guo, W.-J.; Nagy, R.; Chen, H.-Y.; Pfrunder, S.; Yu, Y.-C.; Santelia, D.; Frommer, W.B.; Martinoia, E. SWEET17, a Facilitative Transporter, Mediates Fructose Transport across the Tonoplast of Arabidopsis Roots and Leaves. *Plant Physiol.* **2014**, *164*, 777–789. [CrossRef]
66. Feng, L.; Frommer, W.B. Structure and function of SemiSWEET and SWEET sugar transporters. *Trends Biochem. Sci.* **2015**, *40*, 480–486. [CrossRef]
67. Zheng, Q.-M.; Tang, Z.; Xu, Q.; Deng, X.-X. Isolation, phylogenetic relationship and expression profiling of sugar transporter genes in sweet orange (*Citrus sinensis*). *Plant Cell Tissue Organ Cult.* **2014**, *119*, 609–624. [CrossRef]