Genome-wide identification and expression profiling of the SWEET family in *Actinidia polygama* (Sieb. & Zucc.) Maxim.

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Abstract

Sugar was transported from photosynthetic source cells to sink cells, sugar efflux transporter protein (sugars will eventually be exported to transporters, SWEETs) play an important role in the process. Although *SWEET* family members had been identified in many plants, transcriptome or genomics analysis of *Actinidia polygama SWEET* genes remains uncharacterized. In this study, 14 *SWEET* genes of *Actinidia polygama* were identified by protein Blast. The structural characteristics of *SWEET* genes showed that the number of amino acids encoded by the gene family was between 233 and 304, the relative molecular weight was between 25,918.83 and 33,192.12, the isoelectric point was within the range of 6.96 to 9.71, 14 *ApSWEET* from *Actinidia polygama* and the known grape and Arabidopsis *SWEETs* were divided into four clades (I, II, III, and IV) according to the phylogenetic relationships. The gene structure analysis showed that most of *ApSWEET* genes have six exons and five introns except *ApSWEET5* and *ApSWEET14*. All ApSWEET proteins also contained P-loop, MtN3-slv, and transmembrane domain. Expression patterns of 14 *ApSWEET* in different organs and at different fruit developmental stages were analyzed. *ApSWEET1* and *ApSWEET5* exhibited tissue-specific expression, whereas other genes were more ubiquitously expressed. *ApSWEET1*, *ApSWEET10*, and *ApSWEET11* exhibited higher expression in fruit. The results of this study provide insights into the characteristics of the *SWEET* genes in *Actinidia polygama* and may serve as a basis for further functional studies of such genes.

Introduction

Sugars as the main soluble solid component are important nutrients and a key factor influencing the flavor quality of fruits[1]. They also play a crucial role in regulating the expression of fruit-related genes, as well as plant growth and development[2], stress responses[3], and other developmental processes. In leaves, sugars are produced as important photo-assimilates and are loaded into the phloem system through the symplasm pathway. They are then unloaded in sink organs, such as fruits and flower, through the apoplast pathway[4]. Sugar transporters that play an indispensable role in phloem loading, nectar secretion, and reproductive tissue development. Many studies have explored the mechanism of sucrose transport from source cells to sink cells, which involves the synergistic effects of multiple transporters[4–8]. At present, sugar transporters in plants are classified into two types: Sugars Will Eventually be Exported Transporters (SWEETs) and major facilitator superfamily (MFS) transporters. Sugars Will Eventually be Exported Transporters (SWEETs) have been newly identified in plants in recent years[9]. Sugar transporters of MFS are further classified into MSTs and SUTs[10], which primarily facilitate sugar influx into the cytosol. However, some MSTs, namely the tonoplast sugar transporter (TST) and the vacuolar glucose transporter (VGT) involved in transporting sugars from the cytosol to vacuoles and act as H+/sugar antiporters[11]. Both MSTs and SUTs consist of 12 transmembrane α-helices and mediate membrane transport of different sugars[12,13].

SWEET transporters differ from the classic 12 transmembrane structural domains of the major facilitator superfamily. The typical characteristics of SWEET genes include seven TM domains, including two MtN3_saliva domains, which are connected to a low conserved single TM, forming a 3-1-3 symmetric structure[14]. Phylogenetic analysis shows that members of SWEET can be divided into four clades. Clades I, Clades II and Clades IV are mainly hexose transporters, while Clade III is mainly a sucrose transporter[15]. There is substantial evidence suggesting that SWEET protein in Clade I and II may transport glucose, most of the Clade III SWEET proteins are sucrose transporters, and Clade IV evolved from other SWEET clades to primarily act as vacuolar transporters, regulating fructose transport[14]. Members of the SWEET family are widely distributed, and SWEET genes are found not only in plants but also in prokaryotes and animals[16]. With the development of plant genome research, the identification and functional study of SWEET family genes have been carried out in many plant species, and the number of SWEET family members varies significantly among different plants. For example, the SWEET family consists of 17 members in Arabidopsis, 22 in *Saccharum spontaneum*, 20 in strawberry, 15 in pomegranate, 19 in jujube, 29 in tomato, and 33 in apple[17–23]. Previous studies have reported that members of the *SWEET* gene family are involved

in many important physiological processes of plant growth and development, including nectar production, seed, and pollen development, and the regulation of phloem loading, phloem transport, phloem unloading, abiotic stress, and pathogen interaction by regulating carbohydrate compounds[18,24–26]. SWEET proteins are also associated with flower, fruit, and seed development. For example, *AtSWEET8* mainly affected fertility during early inflorescence development, and *AtSWEET13* mainly affected fertility during late inflorescence development[27]. The *SWEET* gene family was also involved in plant interactions with pathogens, such as *VvSWEET4*, which shows strong up-regulation of expression with infection of *Botrytis cinerea*[28,29].

Furthermore, SWEET proteins are key factor in regulating the distribution of soluble sugars, which is closely related to plant stress resistance[26]. Overexpression of two homologues *AtSWET16* and *AtSWEET17* in *Arabidopsis*[26,30]. Overexpression of *AtSWEET15* show more sensitivity to salt stress, and loss of function mutations in *AtSWEET15* show higher salt tolerance[31]. Drought stress affects the redistribution of carbohydrates in plants[32]. Under water deficiency conditions, the expression of *AtSWEET11* and *AtSWEET12* in leaves and roots increased, accompanying an increase in the transportation capacity of sucrose from leaves to roots, indicating that plants regulate the redistribution of carbohydrates by regulating the expression of *SWEETs* under water deficiency conditions[33].

Actinidia polygama (Sieb. & Zucc.) Maxim. is a perennial vine plant, its fruit contains many unique and interesting flavors and many nutrients, including organic acids, amino acids, flavonoids, dietary fiber, and vitamins C and E. In addition, the leaves, fruits, stems, and roots also were exploited as nutraceuticals or medicine. Therefore, *A. polygama* as a valuable wild resource has very high nutritional and medicinal value. However, the current domestication of *A. polygama* is not complete, particularly in terms of improving fruit quality. During the ripening process of *A. polygama* fruit, sugar accumulation is closely related to fruit quality and edible ability. In this study, we identified the *SWEET* gene family members of *A. polygama*, and analyzed the physical and chemical properties, phylogenetic relationship, gene structure, and promoter elements of *SWEET* family proteins. We also studied the expression of *SWEET* gene family members in different organs of *A. polygama*, which laid a foundation for further study on the structure and function of the *SWEET* gene family.

Results

Identification and physical and chemical analysis of SWEET

By searching the *SWEET* gene on the website (ID: PRJDB13926), a total of 23 *SWEET* gene family members of *A. polygama* were screened out, and nine *SWEET* subfamily members were eliminated because of lack of the conserved structures. According to the physicochemical property table (Table 1), the amino acid quantity of the *SWEET* gene family ranged from 233 to 304. The molecular weight ranges from 25,895.58 to 33,192.12. The isoelectric point is between 6.96 and 9.71, and the stability coefficient of *ApSWEET4*, *ApSWEET5* and *ApSWEET14* proteins was greater than the remaining 11 proteins. The aliphatic index was 94.76~118.24, which was a stable protein. Furthermore, most of the *SWEET* genes were found to be present on the cell membrane, rarely on the chloroplast and Golgi apparatus.

Phylogenetic and structural analysis of SWEET gene family members in *A. polygama*

To study the phylogenetic relationships among *SWEET* genes in *A. polygama* and other plant species, a neighbor-joining phylogenetic tree was constructed by aligning 14 *ApSWEET* sequences, 17 *AtSWEET* sequences, and 14 *VvSWEET* sequences (Supplemental Tables S1–S3). Apparently, 45 proteins were clustered into four different groups (Fig. 1). In detail, six *ApSWEETs* (*ApSWEET9*, *10*, *11*, *12*, *13*, *14*) showed high homology with three *AtSWEETs* (*AtSWEET1–3*) and three *VvSWEETs* (*VvSWEET2a*, *2b*, *3*) in group I. In group II, three *ApSWEETs* (*ApSWEET5*, *7*, *8*) were clustered with five *AtSWEETs* (*AtSWEET4–8*) and three *VvSWEETs* (*VvSWEET5a*, *5b*, *7*). *ApSWEET1/4* were homologous to seven *AtSWEETs* (*9–15*) and five *VvSWEETs* (*VvSWEET9*, *10*, *11*, *12*, *15*) in group III. Three *ApSWEET* (*ApSWEET2*, *3*, *6*), two *AtSWEETs* (*AtSWEET16*, *17*) and three *VvSWEETs* (*VvSWEET17a*, *17b*, *17d*) were included in group IV. The exon-intron structural evolution showed that most of the *ApSWEET* contained six exons, except for *ApSWEET5* and *ApSWEET14* which contained five exons (Fig. 2). *ApSWEET2* and *ApSWEET4* had the shortest and longest sequence, respectively. *ApSWEET11*, *ApSWEET12*, and *ApSWEET 13* had similar exon-intron structures. In addition, *ApSWEET1* and *ApSWEET4*, *ApSWEET3*, and *ApSWEET6* also showed similar structures, these genes belong to the same group. These results suggested that *ApSWEETs* in the same group shared similar exon-intron organizations.

Table 1. Physicochemical properties of SWEET gene family proteins.

| Gene ID	Number of amino acids	Molecular weight	pI	Asp + Glu	Arg + Lys	Instability index	Predicted location
ApSWEET1	275	31,093	8.17	17	19	37.35 (stable)	Cell membrane
ApSWEET2	244	27,049.86	6.86	16	16	35.79 (stable)	Cell membrane
ApSWEET3	304	33,192.12	9.49	19	30	36.79 (stable)	Chloroplast
ApSWEET4	254	29,132.9	7.61	19	20	46.56 (unstable)	Cell membrane
ApSWEET5	259	28,602.2	9.71	13	24	45.53 (unstable)	Cell membrane
ApSWEET6	234	25,895.58	8.48	14	16	35.70 (stable)	Cell membrane
ApSWEET7	238	26,891.1	9.18	13	19	36.22 (stable)	Cell membrane
ApSWEET8	237	26,653.71	8.87	13	17	34.25 (stable)	Cell membrane
ApSWEET9	253	27,530.9	9.5	12	23	29.02 (stable)	Cell membrane Golgi apparatus
ApSWEET10	252	27,529.8	9.49	12	20	25.31 (stable)	Cell membrane
ApSWEET11	236	25,918.83	9.26	8	14	36.71 (stable)	Cell membrane
ApSWEET12	234	25,977.87	9.03	9	14	36.26 (stable)	Cell membrane
ApSWEET13	238	26,391.35	9.01	9	14	38.49 (stable)	Cell membrane
ApSWEET14	233	26,641.9	9.36	15	24	43.15 (unstable)	Cell membrane
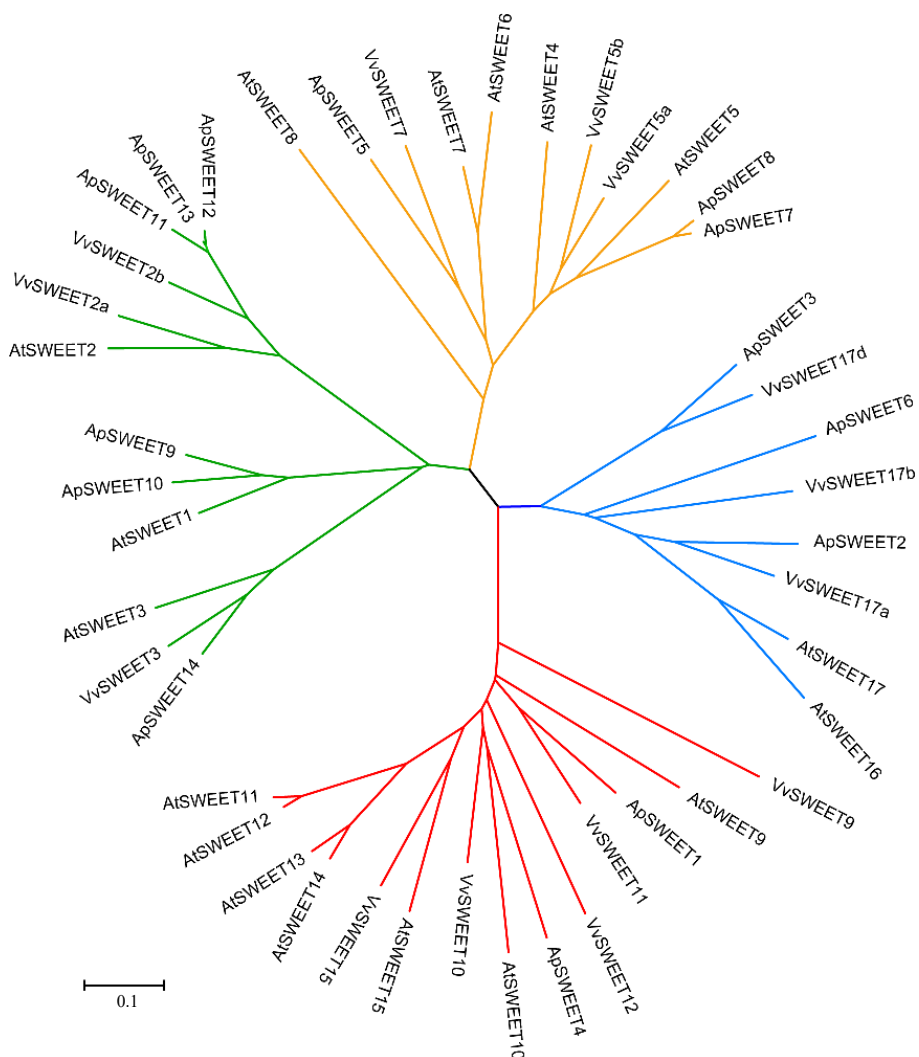
Fig. 1 Phylogenetic analysis of the ApSWEETs from *A. polygama*, *Arabidopsis thaliana*, and *Vitis vinifera*. The Neighbor-joining tree was drawn using MEGA7.0 with 1,000 bootstraps. The roman numbers (I–IV) labeled with various colors indicate different clades: green – Clade I, orange – Clade II, red – Clade III, blue – Clade IV.

Analysis of conserved motif of *ApSWEET* gene family

The Motif1~Motif10 conserved motifs were found in the *SWEET* gene of *A. polygama*. (Fig. 3), whereas motifs 1, 2, 4, and 5 were observed in all *ApSWEET* members. In addition, Motif3 was observed in 13 *ApSWEET* members except *ApSWEET4*. Six genes lacked Motif6, and five genes lacked Motif7. Only *ApSWEET13*, *ApSWEET12* and *ApSWEET2* genes contained Motif8, *ApSWEET10*, *ApSWEET5* and *ApSWEET4* contained Motif9, *ApSWEET1* and *ApSWEET14* contained Motif10. Most of the conserved motifs had a relatively consistent relationship with the evolutionary tree with the same order of number, suggesting that these genes had strong conserved structures and similar gene functions. We confirmed that the *ApSWEET* proteins also contained P-loop, MtN3-slv, and transmembrane domain (Fig. 4). The typical structure of plant SWEET proteins consists of seven predicted transmembrane (7-TM) helices forming two MtN3_slv domains (triple-helix bundles, THB) connected by a linker transmembrane helix (TM4). All *ApSWEET* genes comprise two sugar transporter domains for intercellular exchange.

Secondary and tertiary structure analysis of *ApSWEET* gene family members

SOPMA was used to analyze the secondary structure of the *SWEET* gene family in *A. polygama*, including Alpha helix, chain extension structure, Beta turn, and Random coil (Table 2). These results showed that Alpha helix and Random coil were significantly higher than the chain extension structure and Beta turn. In addition, the Alpha helix proportion of *ApSWEET6* was the highest, and *ApSWEET9* was the lowest. Meanwhile, the proportion of chain extension structure in *ApSWEET9* was the highest, and *ApSWEET3* was the lowest. The beta turn of *ApSWEET8* was the highest, and *ApSWEET4* was the lowest. A random coil of *ApSWEET4* was the highest, but *ApSWEET6* was the lowest.

SWISS-MODEL was used for homology modeling analysis (Fig. 5), and it was found that the three-dimensional structure of ApSWEET proteins could be roughly divided into two categories. *ApSWEET1*, *ApSWEET2*, *ApSWEET4*, *ApSWEET5*, *ApSWEET6*, *ApSWEET7* and *ApSWEET8* was clustered into one group, and *ApSWEET3*, *ApSWEET9*, *ApSWEET10*, *ApSWEET11*, *ApSWEET12*, *ApSWEET13* and *ApSWEET14* was clustered into one group.
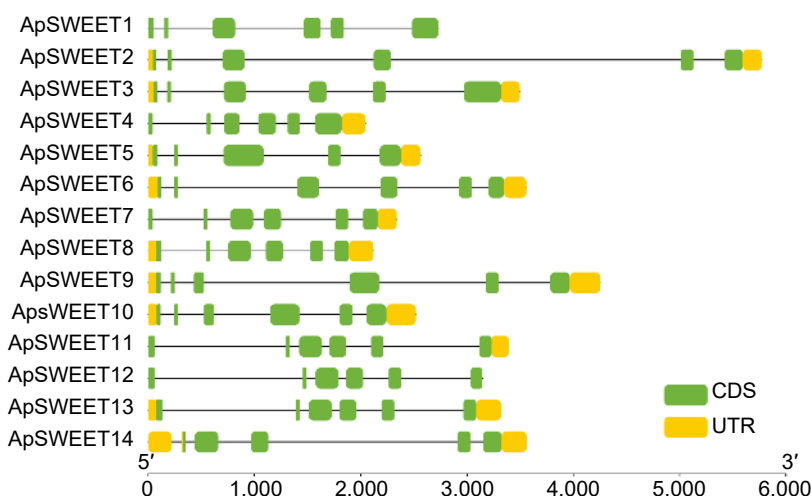
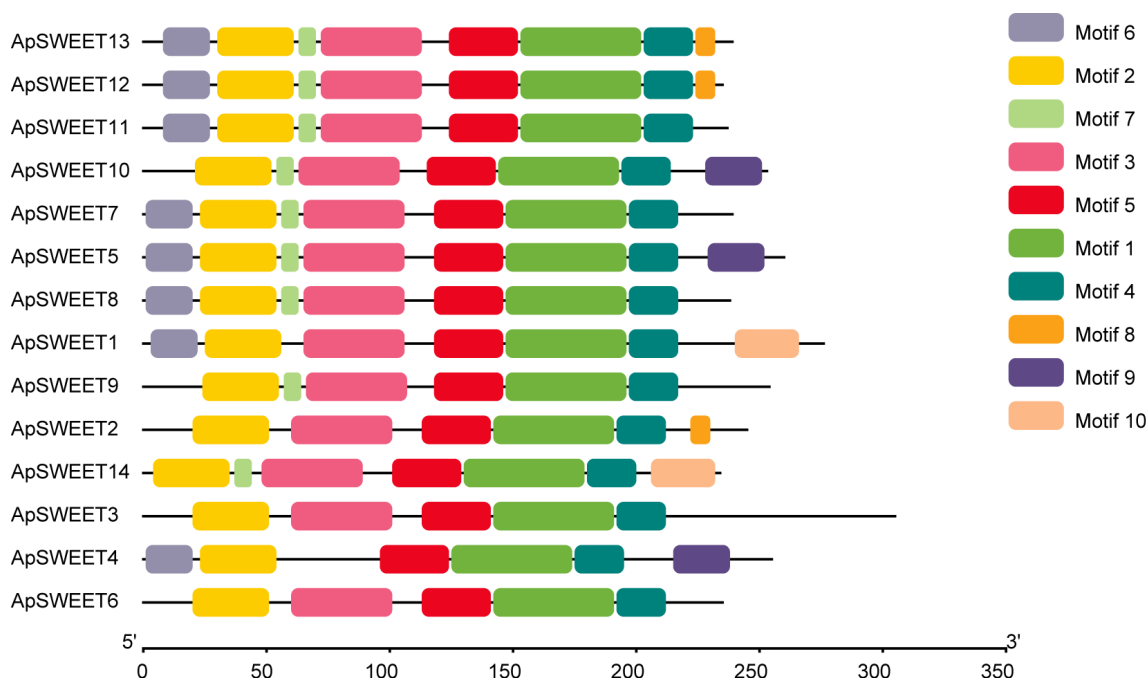**Fig. 2** *ApSWEET* gene structure of *A. polygama*.

Fig. 3 The conserved motif analyses of ApSWEETs proteins.

Chromosomal localization and collinear analysis of the *ApSWEET* gene

Chromosome analysis of genes (Fig. 6) showed that except for chromosome ApChr19, other genes were evenly distributed on 11 chromosomes. Three *ApSWEETs* genes were distributed in clusters on chromosomes ApChr19, *APSWEET12,* and *ApSWEET13* may be due to gene replication. According to the collinearity analysis diagram (Fig. 7), *ApSWEET2* and *ApSWEET6*, *ApSWEET4* and *ApSWEET7*, *ApSWEET11* and *ApSWEET3/12/13* exist collinearity, which may be obtained by chromosome fragment replication.

Promoter analysis of the *ApSWEET* gene family

In order to study the potential regulatory factors of the *ApSWEET* gene, the 2,000 bp promoter region of this family was analyzed (Fig. 8), and 88 elements in promoter regions of all *ApSWEETs* genes were predicted. The results showed that response elements such as low temperature, light, and hormone appeared in most gene promoter regions, indicating that genes may be affected by low temperature, light, and hormone levels. They were classified into three groups based on their functional associations: stresses (ARE, DRE, STRE, LTR, MBS, and MYC), hormones (ABRE, TATC-box, CGTCA motif/ TGACG motif, HD-Zip1, P-Box, GARE-motif, GA-motif, ERE, and TCA-element) and light (GT1-motif, TCCC-motif, TCT-motif, G-Box, Gap-box, LAMP-element). Among these elements, six elements were responsive to stress, ten elements were responsive to hormones, and six elements were responsive to light. Four development-related elements are responsive to meristem expression (CAT-box), cis-regulatory element involved in endosperm expression (GCN4), involved in endosperm-specific negative expression (AACA), and seed-specific regulation (RY). These findings indicated that *ApSWEETs* may respond to hormones or be involved in plant growth and stress resistance.
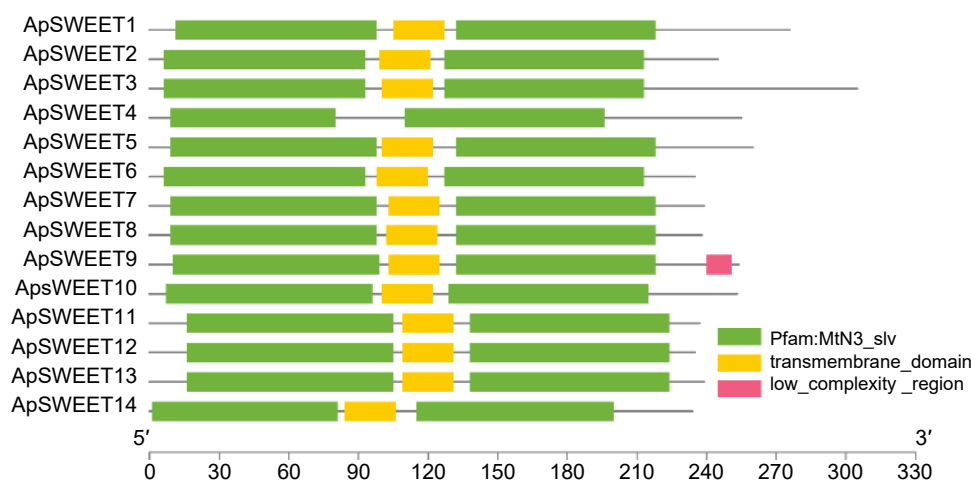
Fig. 4   Conserved structural domains of ApSWEETs.

Table 2.   Secondary structure analysis of *ApSWEET family* members.

	Alpha helix	Extended strand	Beta turn	Random coil
ApSWEET1	45.09%	16.73%	2.18%	36.00%
ApSWEET2	41.80%	22.54%	2.87%	32.79%
ApSWEET3	49.01%	15.46%	5.26%	30.26%
ApSWEET4	37.80%	18.11%	0.79%	43.31%
ApSWEET5	35.91%	21.24%	4.63%	38.22%
ApSWEET6	50.00%	21.79%	3.85%	24.36%
ApSWEET7	40.34%	21.85%	4.20%	33.61%
ApSWEET8	40.51%	21.94%	5.91%	31.65%
ApSWEET9	33.99%	25.30%	4.35%	36.36%
ApSWEET10	46.83%	17.06%	3.97%	32.14%
ApSWEET11	42.37%	19.49%	5.08%	33.05%
ApSWEET12	41.88%	20.94%	4.27%	32.91%
ApSWEET13	46.22%	19.33%	2.52%	31.93%
ApSWEET14	36.48%	21.89%	3.86%	37.77%

Expression analysis of *ApSWEET* gene

Real-time fluorescence PCR was used to detect the expression of *ApSWEET* members in leaves, stems, flowers, roots, mature fruits (Fig. 9) and fruits of different developmental stages (Fig. 10). These results showed that the expression level of *ApSWEET2*, *ApSWEET3*, *ApSWEET10*, *ApSWEET11* and *ApSWEET13* was higher in leaves, the expression level of *ApSWEET2*, *ApSWEET3*, *ApSWEET10*, *ApSWEET11*, *ApSWEET9,* and *ApSWEET13* was higher in stem, the expression level of *ApSWEET5* and *ApSWEET11* was higher in flower, the expression level of *ApSWEET3*, *ApSWEET10,* and *ApSWEET11* was higher in the root, the expression level of *ApSWEET1*, *ApSWEET10* and *ApSWEET11* was higher in fruit. Most of the *SWEET* genes were found to be ubiquitously expressed in all tissues except for *ApSWEET1* and *ApSWEET5*, the two genes were specifically expressed in the fruit and flower.

During fruit development, only *ApSWEET5* had higher expression at an early stage of fruit development, *ApSWEET1*, *ApSWEET2*, *ApSWEET10,* and *ApSWEET11* had higher expression at the mid and late stages of fruit development (Fig. 10). In addition, the glucose content in *A. polygama* is higher than the fructose and sucrose content from the initial measurement on June 11th 2022 (Fig. 10). In the final measurement on September 27th 2022, the glucose content is 2.7 times and 5.4 times higher than the fructose content and sucrose content.

Therefore, glucose content was the highest, followed by fructose, and sucrose content was the lowest during *A. polygama* development.

After the mature fruits are harvested, only *ApSWEET1* shows strong expression at different storage periods. *ApSWEET10* showed strong expression only on the first day after harvest, but the expression levels of *ApSWEET11* gradually decrease with prolonged fruit storage time (Fig. 11).

Discussion

Although *SWEET* genes have been extensively studied in various species, such as *Arabidopsis thaliana*[17], *Hemerocallis fulva*[34], *Chinese jujube*[14], *Malus*[35] and *Litchi chinensis*[36], the presence of *SWEET* gene family in *A. polygama* has not been reported. In this study, we identified 14 members of the *SWEET* gene family in *A. polygama*. The number is less than three in Arabidopsis and seven in rice[17,18]. Gene duplication has been shown to contribute to the expansion of *SWEET* genes in soybean and potato, enabling them to adapt to environmental changes. This could explain why these species have more SWEET proteins than others. Moreover, different predictions of their physical and chemical properties suggest that *SWEET* genes may have diverse functions in plants[17]. Eleven of the identified genes were located on different chromosomes, consistent with findings from Arabidopsis. It is speculated that different members of the gene family may perform specific biological functions in different plant tissues. However, further research is needed to elucidate their exact roles in plant growth and development.

The structure and number of conserved motifs among members of the *SWEET* gene family were similar, and there was also a relative relationship between the evolution tree and gene structure. For example, *ApSWEET11*, *ApSWEET12* and *ApSWEET13* exhibited similar gene structures and belonged to the same clade. However, some gene structures displayed noticeable differences, suggesting the occurrence of expansion, reduction, or mutation during the evolution of the SWEET gene family. These variations may be associated with the diverse functions of the genes. The SWEET family members generally consist of five to six exons in their structures. It is speculated that the diversity of gene function may arise from the loss or addition of exons during the evolutionary process of these family genes. Additionally, all the members of SWEET
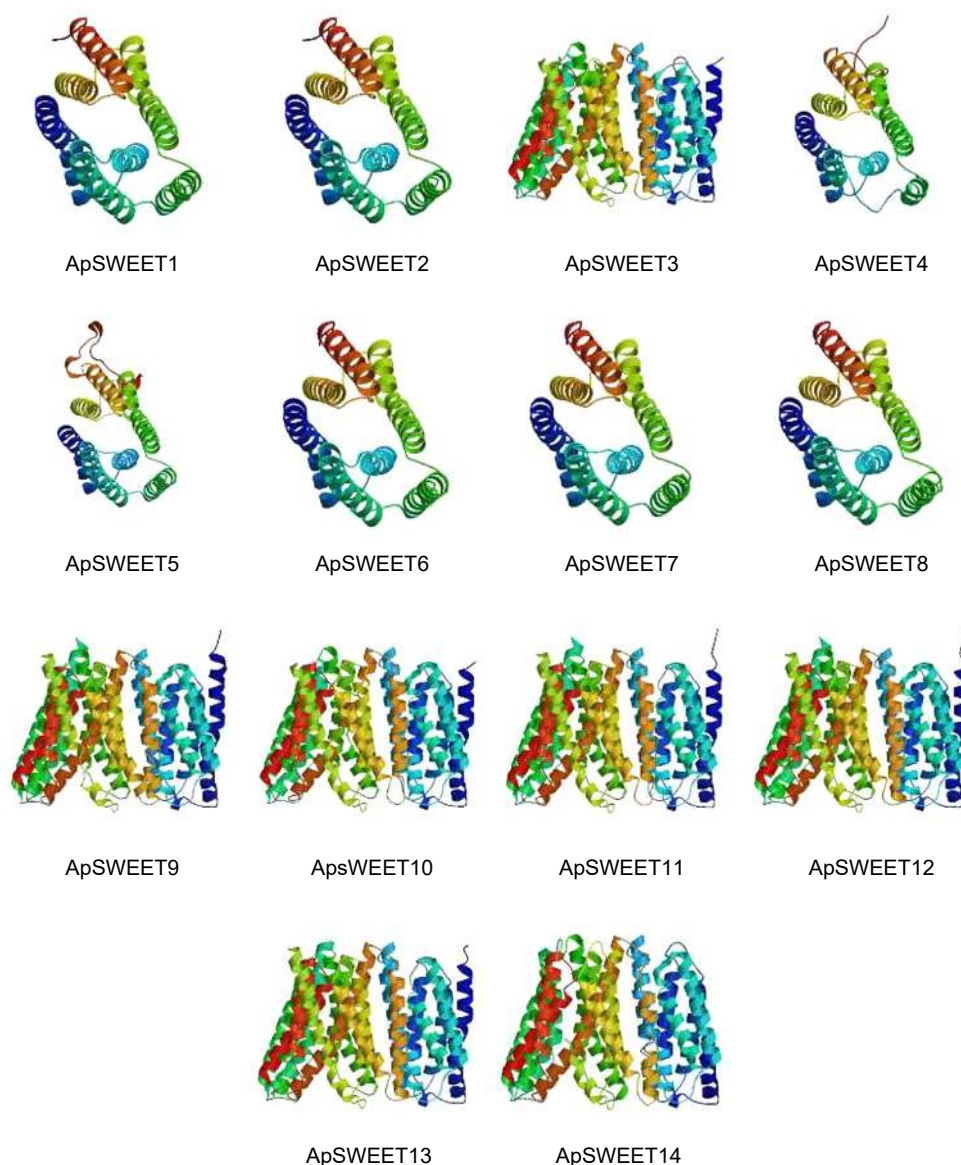
Fig. 5 Single protein structure of ApSWEETs.

gene family in *A. polygama* contained four highly conserved motifs: Motif1, Motif2, Motif4 and Motif5. These conserved motifs may play a key role in the biological function of the SWEET protein of *A. polygama*.

The expression patterns in different organs are closely correlated with gene function and serve as a predictor of biological functions. Numerous studies have reported the involvement of *SWEET* genes in various physiological processes, which usually were associated with specific tissue expression patterns. Our results also revealed that some *SWEET* genes were ubiquitously expressed in the flower, fruit tissues, root, leaf and stem. For example, *ApSWEET5* and *ApSWEET11* were highly expressed in flower. Some studies have reported that *SWEET* genes may play an important role in reproductive development. For example, *AtSWEET13* and *AtSWEET14* were found to be expressed in the anther wall, responsible for facilitating sucrose efflux into locules to support pollen development and maturation[37], and mutations in *AtSWEET9* had been shown to impair nectar secretion[38,39]. These genes were specifically expressed in

pollen. Similarly, *OsSWEET11* and *SbSWEET9-3* were highly expressed in the panicle[40,41], suggesting that these genes may be essential for reproduction. Additionally, *ApSWEET1* and *ApSWEET5* exhibited specific expression in fruit. Similarly, high expression of *VvSWEET* transporters in flowers and berries highlighted a putative important role in sugar partitioning during flower and fruit development[4]. Furthermore, *ApSWEET3* and *ApSWEET10* displayed high expression in the roots (Fig. 5), while *ApSWEET2*, *ApSWEET9*, and *ApSWEET10* were relatively highly expressed in the leaves and stem, similar to the function of *AtSWEET17* as fructose transporter[42], these genes were proposed to participate in the phloem loading of photoassimilate in leaves of *A. polygama*. Our results suggested these genes may be involved in flower development, as well as the short and long-distance transportation and distribution of sugars. However, the regulatory mechanisms underlying the expression of these genes require further clarification.

In fruit, sugar (such as glucose, sucrose and fructose) is an important index that determines the quality of fruit. Many
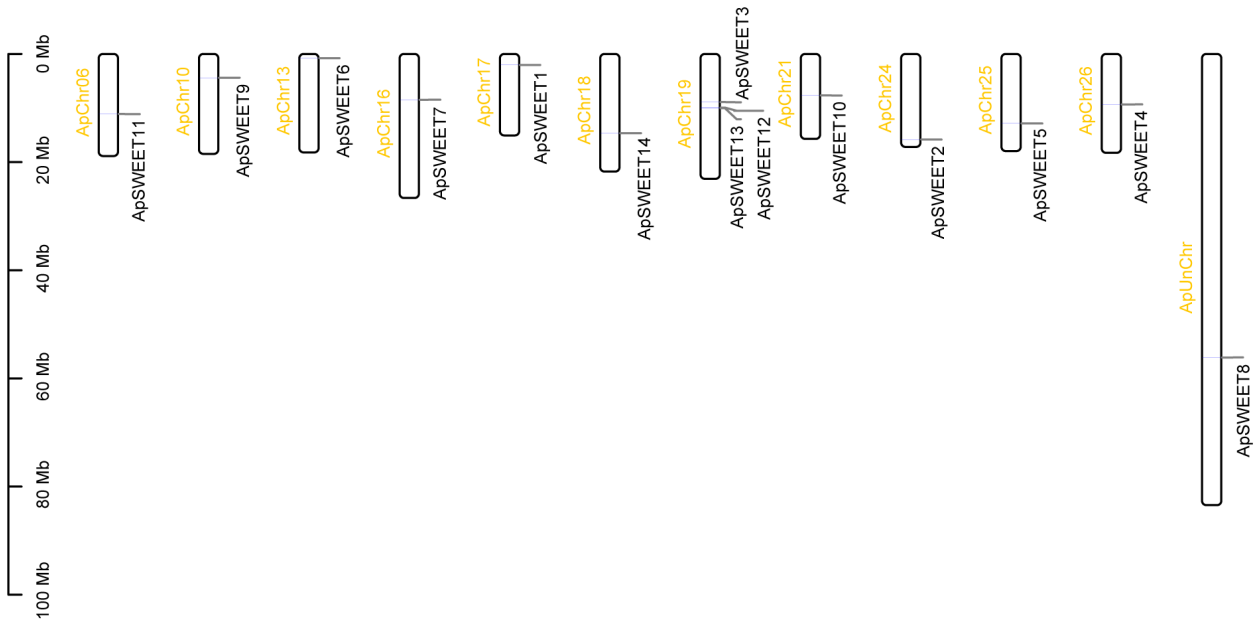
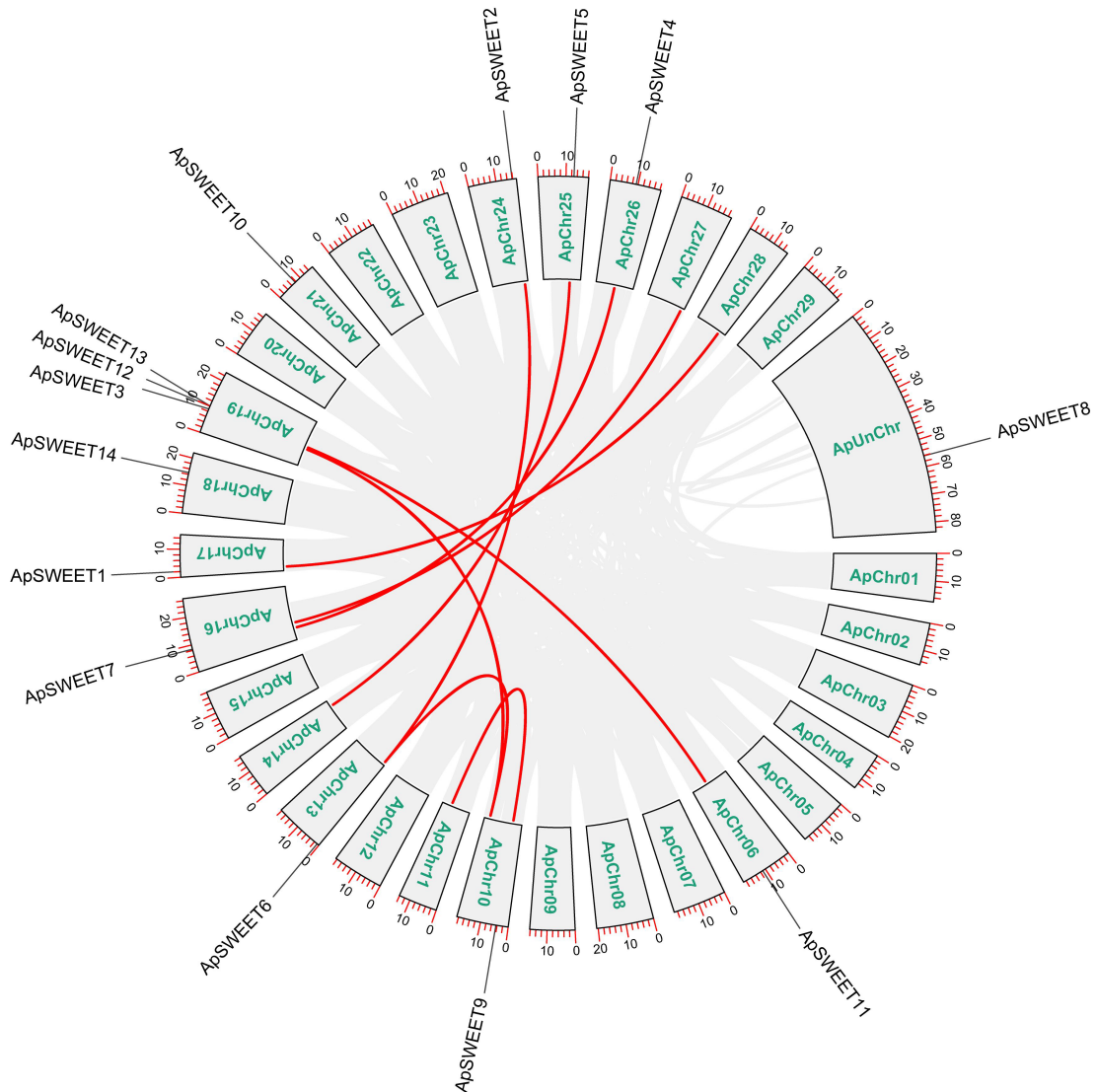**Fig. 6** Chromosome location of *ApSWEET* gene family.

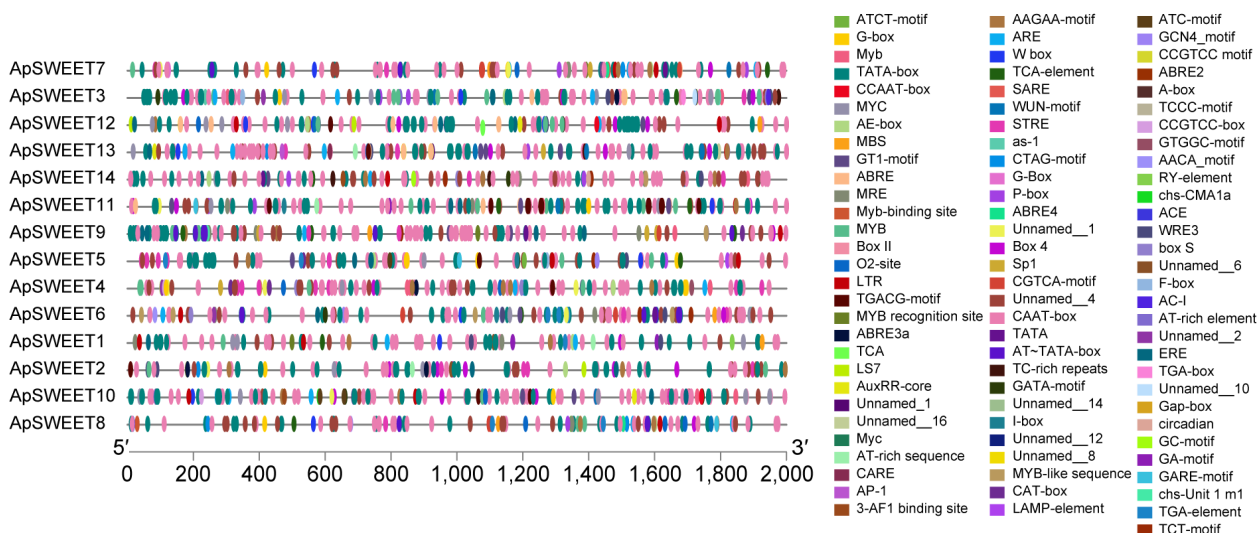**Fig. 7** Collinearity analysis of the *ApSWEET* gene family.

Fig. 8 The cis-elements in the promoter sequences of *ApSWEETs* gene in *A. polygama*.

studies have reported functions of *SWEET* genes in both sink and source organs, particularly in fruits like tomato, grape, apple and Chinese jujube. The different expression patterns of the *SWEET* gene during fruit development are closely related to their function and can be used to predict biological functions. For instance, in apples, the expression of *SWEET* genes at young (*MdSWEET1.1/2*, *MdSWEET2.4* and *MdSWEET3.5*) and ripe fruit development stages were different[23]. In grape, the expression of *VvSWEET10*, *VvSWEET12,* and *VvSWEET15* was higher in young fruit, but *VvSWEET15* was more abundant in mature fruit[28]. Furthermore, *SlSWEET7a*, *SlSWEET14* and *SlSWEET15* in tomato fruit are responsible the for transportation of glucose, fructose and sucrose[43], and *VvSWEET4* acted as a glucose transporter in grape[28]. In our study, seven *ApSWEET* genes were expressed at five different fruit development stage. Among them, three *ApSWEET* genes exhibited high expression at the early stage of fruit development, and two *ApSWEET* genes showed high expression in ripe fruit. Moreover, there is a certain correlation between gene expression and homology, with some genes belonging to the same clade as *ApSWEET1*, *VvSWEET10*, *VvSWEET11* and *VvSWEET15*[4,23]. On the basis of sugar content during fruit development, it was also speculated that *ApSWEET1* and two *ApSWEET* (*ApSWEET2* and *ApSWEET3*) may be involved in the transportation and distribution of sucrose and fructose during fruit ripening, while *ApSWEET10* and *ApSWEET11* may play a role in transportation and distribution of glucose during fruit ripening.

Materials and methods

Plant materials

Sampled plants were grown outdoors on the campus of Jilin Agricultural University, Jilin, China (43°48'48'' N, 125°24'15'' E). The annual precipitation is 867 mm, and the annual highest and lowest temperatures are 35 and −40 °C, respectively. Each field plot was divided into three subplots, and the seedlings were planted in each plot (3 m × 4 m). The phosphorous (4.12 ± 0.31 g/Kg), nitrogen (32.17 ± 1.98 g/Kg) and potassium (3.51 ± 0.19 g/Kg) concentrations were sufficient. Seedlings in all experiments were of uniform size. The expression of *ApSWEET*

genes was detected in *A. polygama* has different tissues and fruits at different development stages. Leaf, flower, root, stem, and fruit were collected. Fruit collected at 10, 40, 60, 80, and 85 d after full bloom respectively. After the mature fruits are harvested, fruit were stored for 1, 2, 3, 4, and 5 d. All fresh plant samples were collected with three independent replicates and immediately frozen in liquid nitrogen, then stored at −80 °C.

Identification of gene family members

Actinidia polygama SWEET gene family was identified by protein Blast of the 17 Arabidopsis SWEET proteins against the *Actinidia polygama* genome database (https://figshare.com/s/f46aea0009a54a6a0528).

The NCBI CDD (www.ncbi.nlm.nih.gov/Structure/cdd/wrpsb.cgi) and PFAM (http://pfam.sanger.ac.uk/) website were used to search for the conserved domains of the candidate members.

Physicochemical properties and structural analysis

For the protein sequences encoded by the gene family members obtained above, Expasy (http://web.expasy.org/) was used to predict their molecular weight, isoelectric point, stability and other physicochemical properties, respectively. WoLFP-SORT (www.genscript.com/wolf-psort.html) was used to predict protein subcellular localization, and TMHMM (https://services.healthtech.dtu.dk/service.php?TMHMM-2.0) used to predict transmembrane structure. The gene structure of SWEET gene family members of *Actinidia polygama* was analyzed according to the location information of introns and exons on chromosomes. MEME was used to predict the motif of the protein-conserved domain. The analysis results were visualized using TBtools and modified by AI. The online software SOPMA (http://npsa-pbil.ibcp.fr/cgi-bin/npsa_automat.pl?page=npsa_sopma.html) was used to predict the secondary structure of SWEET protein, including random curling, chain extension structure, alpha helix and beta-turn. Using the SWISS-MODEL to analyze homology modeling (www.swissmodel.expasy.org).

Construction of molecular evolutionary tree

The MUSCLE was used for sequence comparison of the gene family members and was beautified with GENEDOC. The
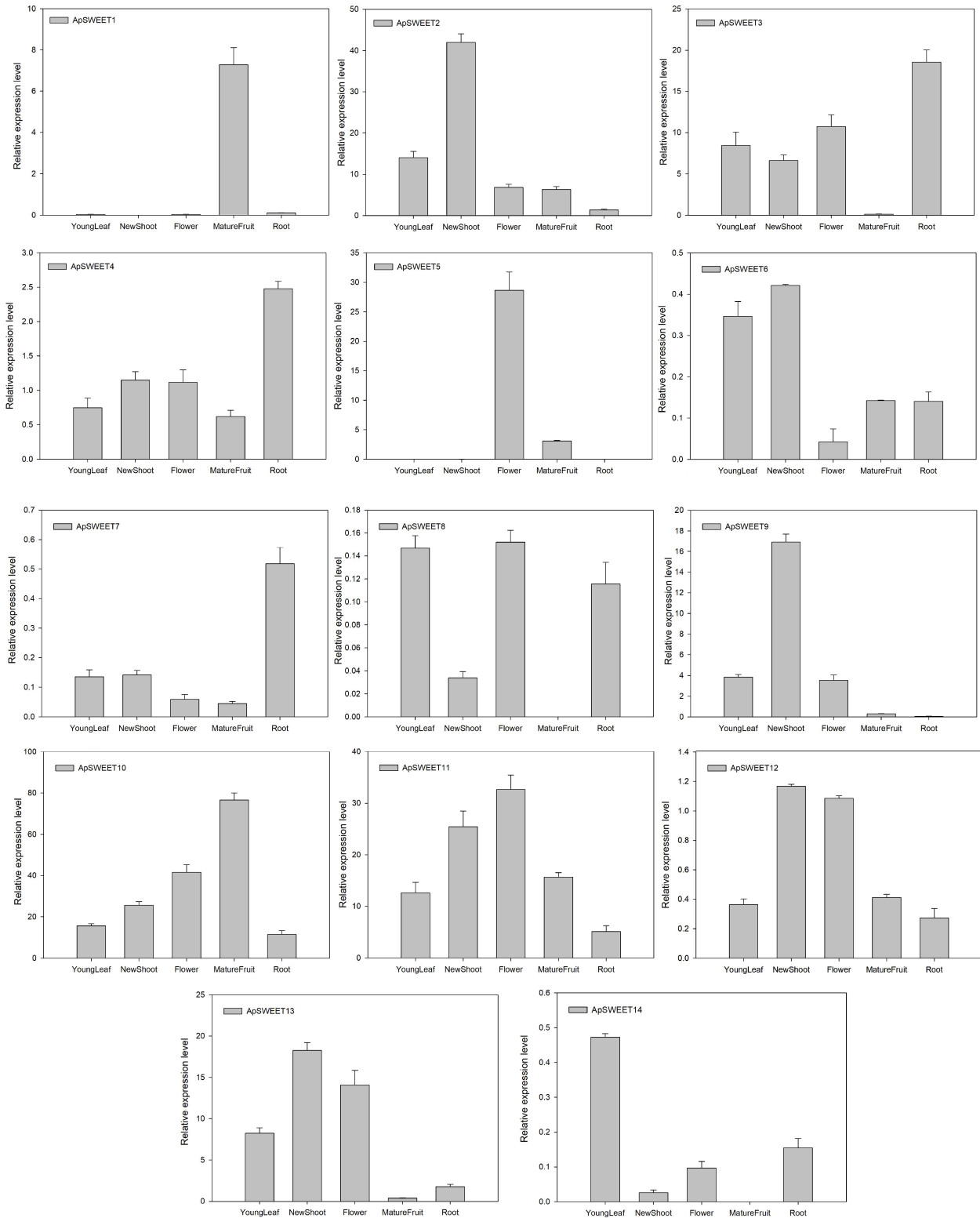
Fig. 9 *ApSWEET* expression of different tissues.

comparison results were clipped with trim AI and analyzed with IQ-TREE evolutionary tree. Finally, the exhibited (http://tree.bio.ed.ac.uk/software/figtree/) for the beautification of the illustration.

Chromosome localization and collinearity analysis of gene family

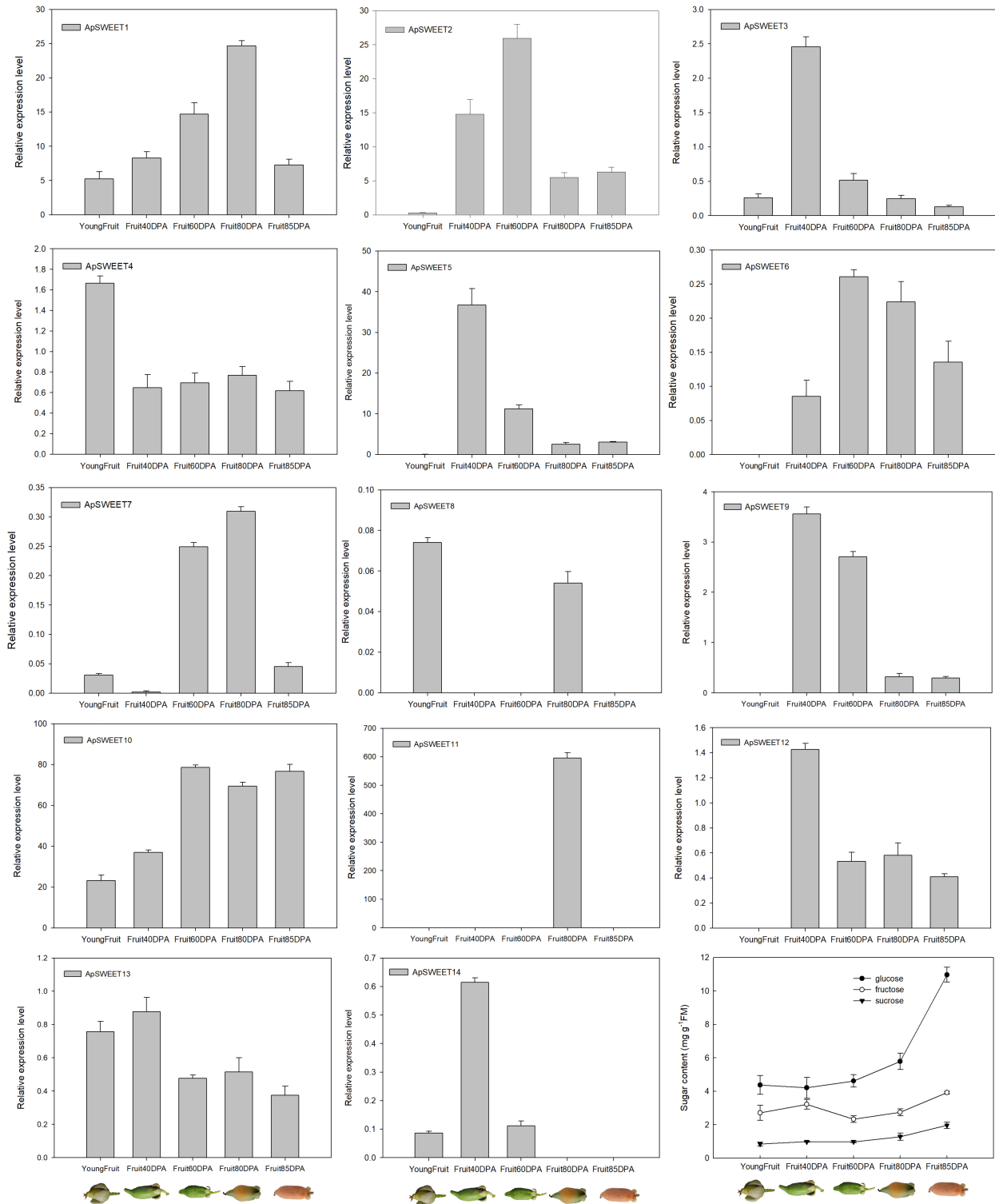Based on the whole genome and location information downloaded from the *Actinidia polygama* genome database

Fig. 10   *ApSWEET* expression of different fruit development stage.

(https://figshare.com/s/f46aea0009a54a6a0528), TBtools was used to conduct chromosome localization, collinearity, and gene tandem repeat event analysis for all SWEET gene family members of *Actinidia polygama*, and the results were visualized. MCScan was used to compare the whole genome sequence of *Actinidia polygama*, and the collinearity relationship was obtained. The homologous gene map was drawn with TB tools.

Gene family promoter analysis

The 2 kb nucleotide sequences upstream of the transcription starting points of 14 genes of the gene family were predicted
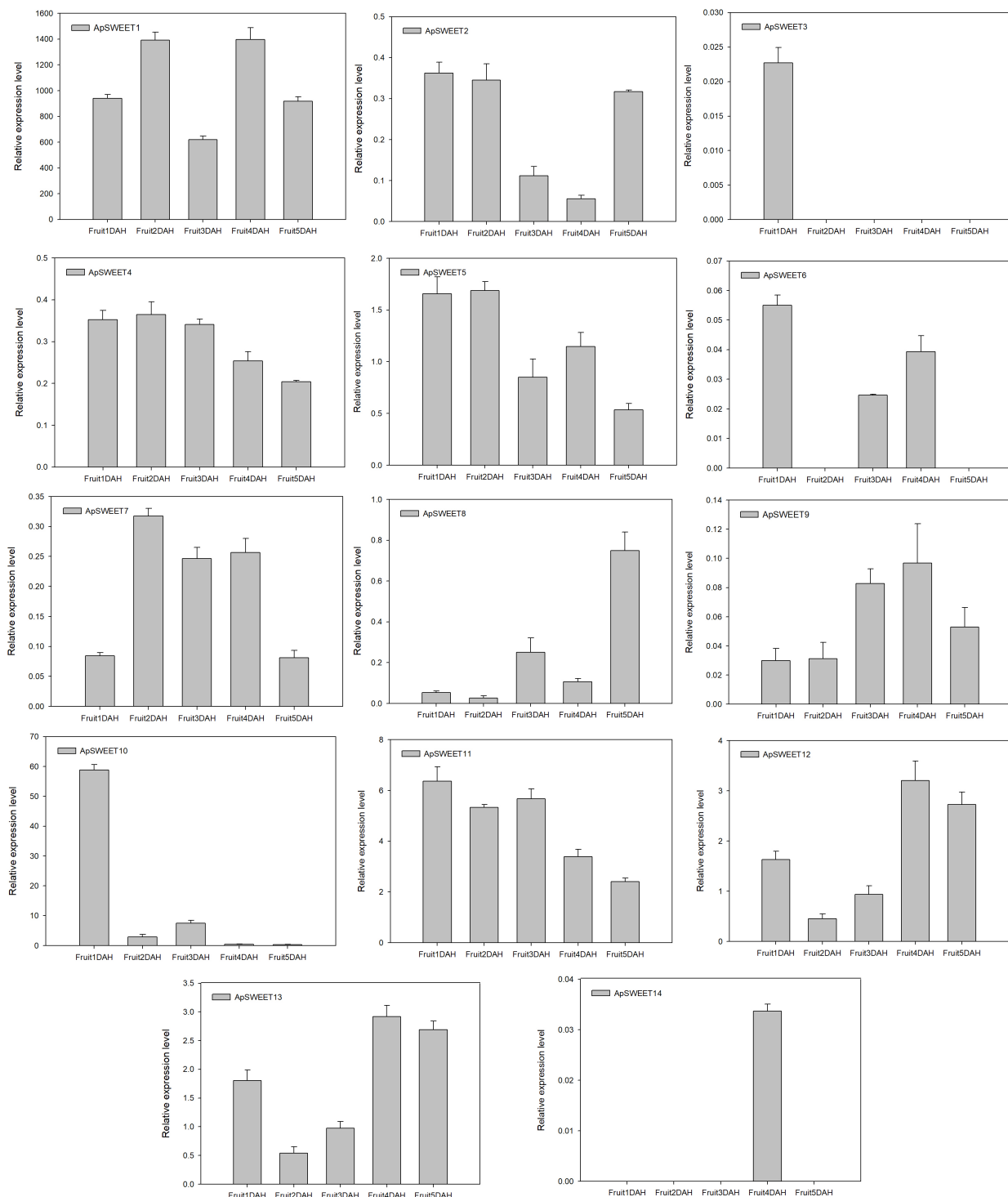
Fig. 11 *ApSWEET* expression of different storage periods.

using PlantCARE, and TBtools software was used for visualization.

RNA extraction and real-time PCR

To validate the reliability of RNA-Seq, qRT-PCR for transcripts was carried out as described by Yu et al.[44]. Total RNA was extracted from leaves, flowers, root, stem, and fruit sampled simultaneously. For each experiment, 1 μg of clean RNA was converted to cDNA using the PrimeScript™ RT reagent Kit (TaKaRa Bio., Dalian, China) according to the manufacturer's protocol. Gene-specific primers were designed using Primer5.0 software (Premier Biosoft). Gene expression was performed using the SYBR Green Real-time PCR kit (TaKaRa Bio). *ACTIN* was used as a housekeeping gene after examining its constitutive expression pattern from the RNA-seq results. Relative gene expression levels were calculated with the $2^{-\Delta\Delta C_T}$ method[45].

The sequences of the primers used for qRT-PCR are listed in Supplemental Table S4.

Data availability

All data generated or analyzed during this study are included in this published article and its supplementary information files.

Conflict of interest

The authors declare that they have no conflict of interest.

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