The SWEET gene family in *Hevea brasiliensis* – its evolution and expression compared with four other plant species

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SWEET (Sugars Will Eventually be Exported Transporter) proteins, which feature up to seven transmembrane TM helix domains, selectively transport different kinds of sugar substrates, including sucrose, fructose, and glucose [1]. As a sugar efflux transporter, SWEET proteins play important roles in plant growth and development, stress responses, and plant–microbe interactions. Cellular sugar efflux is an essential function in many processes, such as phloem loading, nectar secretion, nourishing symbionts such as mycorrhiza, and in maternal efflux for filial tissue development [1]. Sugar efflux systems can be hijacked by pathogens for access to nutrition from hosts [2], and accordingly, mutations that block recruitment of the efflux mechanism by the pathogen facilitate plant resistance to their attack [3]. Previous studies on SWEETs have been focused mainly on two model plant species, namely *Arabidopsis thaliana* and *Oryza sativa* [4–6]. In *A. thaliana*, 17 SWEET family members have been characterized, and they fall into four phylogenetic

**Abbreviations**  
ABA, abscisic acid; GSDS, gene structure display server; SRA, sequence read archive; Suc, sucrose.
clades, in which AtSWEET1-3 are in Clade I, AtSWEET4-8 in Clade II, AtSWEET9-15 in Clade III, and AtSWEET16-17 in Clade IV. The SWEETs from most of the other plants are named following the nomenclature adopted in *A. thaliana*. SWEET genes, in their many isoforms, are versatile and their functions in plants are widely encompassing. For example, AtSWEET1 acts as a glucose transporter [7], with AtSWEET9 being a nectary-specific sugar transporter which is essential for nectar production [6]. AtSWEET11 and AtSWEET12 catalyze sucrose export from phloem parenchyma cells in source leaves and play a critical role in phloem loading [5]. AtSWEET16 and AtSWEET17, as vacuolar SWEET proteins, function as fructose-specific exporters, connecting the vacuolar lumen to the cytosol [8,9]. In *O. sativa*, OsSWEET11, located on the plasma membrane and expressed in the phloem of leaves, is presumably involved in phloem loading, as is the case with its Arabidopsis homologues, AtSWEET11 and AtSWEET12 [5]. OsSWEET11 and OsSWEET14 are specifically exploited by bacterial pathogens for virulence by means of direct binding of a bacterial effector to the SWEET promoter [4,10]. Recently, genome-wide expression patterns of SWEET genes have been characterized in *Brassica napus*, *Pyrus bretschneideri*, *Sorghum bicolor*, and *Glycine max* [11–14], all pointing to important roles of SWEETs in plant growth, development, and stress responses.

Natural rubber (*cis*-1,4-polysoprene, NR) is an important industrial and strategic raw material, the sole commercial source of which is *Hevea brasiliensis* (the Para rubber tree, Hevea hereafter), a perennial tropical tree species [15]. As sucrose is the precursor molecule for rubber biosynthesis and latex regeneration [16], understanding the mechanisms of its transport and metabolism in the rubber tree is of fundamental importance to improving rubber productivity [17]. Significant progress was made in the understanding of Hevea sucrose transport and metabolism with the cloning of six sucrose transporter (SUT) genes, among which *HbSUT3* (*HbSUT1B*) was found to be the key member responsible for sucrose loading into laticifers [18,19]. *HbNIN2* has also been identified as the key gene responsible for sucrose catabolism in rubber-producing laticifers [20]. Moreover, two Hevea sucrose synthase genes, *HbSUS2* and *HbSUS3*, were found to exert negative control over sucrose catabolism in the laticifers [21]. Hevea SWEET genes have not hitherto been investigated in detail, but their characterization has recently been facilitated by the Hevea genome and transcriptome having been independently sequenced by research groups from China [22], Malaysia [23,24], and Thailand [25], and by the availability of the proteome of Hevea latex [26].

We report here a genome-wide analysis of the SWEET gene family in Hevea where we compare the results with those from four other plant species, viz. *Manihot esculenta* and *Ricinus communis* belonging to the same family (Euphorbiaceae) as Hevea, and two model plants, *A. thaliana* and *Populus trichocarpa*. The study encompassed a total of 127 SWEET genes, the expression patterns of which were analyzed in different tissues in response to various treatments, and at several phases of tissue development. In addition, the gene structure and phylogeny of these genes were compared to help further understanding of the roles of SWEET genes in Hevea sugar transport. As a further objective in this investigation, data on SWEET genes and their expression in four other plant species were examined, along with the results from Hevea, to compare the structure of their respective gene families and appraise the functions of their members in the plant kingdom.

**Results and Discussion**

**Genome-wide identification of SWEET gene families in Hevea and four other plant species**

We identified all SWEET gene family members in five plant species (Hevea, *A. thaliana*, *P. trichocarpa*, *M. esculenta*, and *R. communis*) from their published genome sequences. In this exercise, the SWEET genes from the three Euphorbiaceae plants (Hevea, *M. esculenta*, and *R. communis*) were characterized for the first time. The most recent genome and protein sequences of these species were downloaded from Phytozone v10. Local BLAST searches of the genomes were performed using the published SWEET sequences of three model plants of *A. thaliana*, *O. sativa*, and *P. trichocarpa* as queries [1,5]. This analysis identified a total of 127 SWEET genes in the five selected plant species, comprising 36 SWEET genes in Hevea (Table 1a) [22], 28 in *M. esculenta* (Table 1b), 18 in *R. communis* (Table 1c), 17 in *A. thaliana* [10], and 28 in *P. trichocarpa* [6]. All the SWEET gene members newly identified this study were named according to the nomenclature of the *A. thaliana* SWEET gene family. The gene numbers of SWEET families identified here for the two model plants (*A. thaliana* and *P. trichocarpa*) matched those previously reported [5].

The lengths of SWEET-coding regions (CDS) were similar among the three Euphorbiaceae plants examined, ranging from 504 to 915 bp in Hevea, 513 to 906 bp in *M. esculenta*, and 504 to 891 bp in...
Table 1. Characteristics of SWEET genes in three Euphorbiaceae members, *H. brasiliensis*, *M. esculenta*, and *R. communis*.

| SWEETs | ID              | CDS length in bp | Predicted protein | Isoelectric point | Mol Wt  | No. of introns | Group |
|-------|-----------------|------------------|-------------------|-------------------|---------|----------------|-------|
| (A) *H. brasiliensis* | | | | | |
| HbSWEET1a | scaffold1368_1746 | 753 | 251 | 10.09 | 27668.01 | 5 | Clade I |
| HbSWEET1b | scaffold14412_5699 | 750 | 250 | 8.82 | 27885.95 | 5 | Clade I |
| HbSWEET1c | scaffold2014_38474 | 747 | 249 | 9.81 | 27486.73 | 5 | Clade I |
| HbSWEET2a | scaffold0663_726258 | 705 | 235 | 8.81 | 26322.47 | 5 | Clade I |
| HbSWEET2b | scaffold0291_2393 | 606 | 202 | 8.58 | 22646.22 | 4 | Clade I |
| HbSWEET2c | scaffold0649_515754 | 591 | 197 | 9.54 | 21862.14 | 4 | Clade I |
| HbSWEET2d | scaffold1397_73854 | 543 | 181 | 9.98 | 20036.78 | 5 | Clade I |
| HbSWEET2e | scaffold0991_115099 | 705 | 235 | 8.53 | 25981.78 | 5 | Clade I |
| HbSWEET2f | scaffold1207_75820 | 504 | 168 | 8.02 | 18705.26 | 3 | Clade I |
| HbSWEET3a | scaffold0047_2029699 | 747 | 249 | 9.76 | 27958.14 | 5 | Clade I |
| HbSWEET3b | scaffold0802_319652 | 747 | 249 | 9.93 | 28196.37 | 5 | Clade I |
| HbSWEET4a | scaffold0250_352964 | 726 | 242 | 8.69 | 26322.47 | 5 | Clade I |
| HbSWEET4b | scaffold0371_980268 | 771 | 257 | 6.76 | 28814.09 | 5 | Clade I |
| HbSWEET4c | scaffold0371_939664 | 561 | 187 | 9.54 | 21862.14 | 4 | Clade I |
| HbSWEET5a | scaffold0121_20098 | 714 | 238 | 9.60 | 26665.18 | 5 | Clade I |
| HbSWEET5b | scaffold0190_471668 | 543 | 181 | 9.93 | 20425.77 | 3 | Clade I |
| HbSWEET6 | scaffold1143_36139 | 783 | 261 | 9.96 | 28805.48 | 4 | Clade I |
| HbSWEET7 | scaffold1143_36139 | 783 | 261 | 9.96 | 28805.48 | 4 | Clade I |
| HbSWEET9a | scaffold1512_21440 | 768 | 256 | 10.20 | 28969.04 | 5 | Clade I |
| HbSWEET9b | scaffold0030_998488 | 813 | 271 | 9.11 | 30400.98 | 5 | Clade I |
| HbSWEET10a | scaffold1368_1746 | 753 | 251 | 10.09 | 27668.01 | 5 | Clade I |
| HbSWEET10b | scaffold0491_348730 | 828 | 276 | 9.10 | 31738.97 | 5 | Clade I |
| HbSWEET10c | scaffold0371_939664 | 561 | 187 | 9.54 | 21862.14 | 4 | Clade I |
| (B) *M. esculenta* | | | | | |
| MeSWEET1a | cassava4.1_014638 m | 750 | 250 | 9.63 | 27676.81 | 5 | Clade I |
| MeSWEET1b | cassava4.1_014650 m | 750 | 250 | 9.75 | 27920.23 | 5 | Clade I |
| MeSWEET2a | cassava4.1_015227 m | 702 | 234 | 8.55 | 26066.93 | 5 | Clade I |
| MeSWEET2b | cassava4.1_030719 m | 564 | 188 | 7.89 | 20898.87 | 3 | Clade I |
| MeSWEET3a | cassava4.1_028367 m | 741 | 247 | 9.63 | 27553.91 | 5 | Clade I |
| MeSWEET3b | cassava4.1_026477 m | 672 | 224 | 9.61 | 25566.19 | 3 | Clade I |
| MeSWEET4 | cassava4.1_016815 m | 582 | 194 | 9.37 | 21364.54 | 3 | Clade I |
| MeSWEET5 | cassava4.1_026390 m | 714 | 238 | 9.09 | 26997.57 | 5 | Clade I |
| MeSWEET6 | cassava4.1_014231 m | 780 | 260 | 10.12 | 28528.22 | 4 | Clade I |
| MeSWEET7 | cassava4.1_028141 m | 777 | 259 | 9.98 | 28488.95 | 4 | Clade I |
| MeSWEET9a | cassava4.1_032222 m | 678 | 226 | 9.40 | 25435.76 | 5 | Clade I |
| MeSWEET9b | cassava4.1_031208 m | 813 | 271 | 8.33 | 30264.89 | 5 | Clade I |
| MeSWEET10a | cassava4.1_013474 m | 840 | 280 | 8.18 | 31795.01 | 5 | Clade I |
| MeSWEET10b | cassava4.1_015602 m | 675 | 225 | 8.03 | 25795.72 | 3 | Clade I |
| MeSWEET10c | cassava4.1_021350 m | 843 | 281 | 9.05 | 31710.95 | 5 | Clade I |
R. communis (Table 1). The molecular weights of the SWEET proteins in three *Euphorbiaceae* species ranged from 18.7 to 33.9 kDa, while their isoelectric points (pIs) fell between 6.24 and 10.20 (Table 1).

### Phylogenetic analysis of the SWEET gene families

In order to establish the phylogenetic relationships in the SWEET gene families among *Hevea* and the four other plant species, we aligned the 127 SWEET protein sequences in plants and constructed a phylogenetic tree as shown in Fig. 1 (Table S1). The plant SWEET proteins were clustered into four major groups with high bootstrap values, designated Clades I to IV. The 36 *Hevea* SWEET genes were dispersed among the four groups with 11, 7, 12, and 6 isoforms, respectively, in Clades I, II, III, and IV. Similarly, the SWEET family of genes in the other four species were also clustered into the above four groups, with 3, 5, 7, and 2 isoforms, respectively, in *A. thaliana*, 11, 3, 8, and 6 in *P. trichocarpa*, 6, 4, 12, and 6 in *M. esculenta*, and 3, 6, 6, and 3 in *R. communis* (Table 1, Fig. 1). Phylogenetic analysis as well as amino acid sequence comparison revealed universal existence of paralogous SWEET gene pairs and clusters in the five species. In *Hevea*, nine such SWEET gene pairs (*HbSWEET2a*/*2b* in Clade I, *HbSWEET2c*/*2d* in Clade I, *HbSWEET2e*/*2f* in Clade I, *HbSWEET4a*/*4b* in Clade II, *HbSWEET5a*/*5b* in Clade II, *HbSWEET10e*/*10f* in Clade III, *HbSWEET15a*/*15b* in Clade III, *HbSWEET16b*/*16c* in Clade IV, and *HbSWEET17a*/*17c* in Clade IV) and one gene cluster (*HbSWEET1a*, 1b, and 1c in Clade I) were identified.

### Table 1. (Continued).

| SWEETs   | ID                  | CDS length in bp | Predicted protein | Isoelectric point | Mol Wt     | No. of introns | Group |
|----------|---------------------|------------------|-------------------|------------------|------------|----------------|-------|
| MeSWEET10d | cassava4.1_013519 m | 837              | 279               | 7.81             | 31755.76   | 5              | Clade III |
| MeSWEET10e | cassava4.1_032927 m | 846              | 282               | 8.44             | 31954.92   | 5              | Clade III |
| MeSWEET11 | cassava4.1_028116 m | 852              | 284               | 8.09             | 31982.66   | 5              | Clade III |
| MeSWEET12a | cassava4.1_017557 m | 522              | 174               | 6.24             | 19568.11   | 2              | Clade III |
| MeSWEET13 | cassava4.1_026944 m | 834              | 278               | 8.88             | 31415.27   | 5              | Clade III |
| MeSWEET15a | cassava4.1_026251 m | 717              | 239               | 9.63             | 27175.79   | 5              | Clade III |
| MeSWEET15b | cassava4.1_014124 m | 789              | 263               | 9.64             | 29723.69   | 6              | Clade III |
| MeSWEET16a | cassava4.1_014996 m | 723              | 241               | 8.16             | 26331.94   | 5              | Clade IV  |
| MeSWEET16b | cassava4.1_015143 m | 711              | 237               | 7.24             | 25939.66   | 5              | Clade IV  |
| MeSWEET17 | cassava4.1_014640 m | 750              | 250               | 9.20             | 27984.99   | 5              | Clade IV  |
| MeSWEET17a | cassava4.1_032999 m | 513              | 171               | 9.59             | 18675.24   | 4              | Clade IV  |
| MeSWEET17b | cassava4.1_012690 m | 906              | 302               | 9.83             | 33200.06   | 5              | Clade IV  |
| MeSWEET17c | cassava4.1_014587 m | 753              | 251               | 9.76             | 27550.75   | 5              | Clade IV  |
| RcSWEET1 | 27985.m000892       | 744              | 248               | 10.08            | 27412.66   | 5              | Clade I   |
| RcSWEET2 | 30026.m001515       | 504              | 168               | 9.19             | 18787.52   | 3              | Clade I   |
| RcSWEET3 | 30169.m006529       | 753              | 251               | 9.27             | 28219.28   | 5              | Clade I   |
| RcSWEET4a | 29822.m003349       | 582              | 194               | 7.44             | 21739.97   | 3              | Clade II  |
| RcSWEET4b | 27613.m000628       | 699              | 233               | 6.64             | 25779.37   | 0              | Clade II  |
| RcSWEET4c | 29475.m000237       | 708              | 236               | 7.98             | 26033.96   | 0              | Clade II  |
| RcSWEET4d | 29822.m003348       | 726              | 242               | 8.80             | 27262.76   | 5              | Clade II  |
| RcSWEET5 | 30147.m013970       | 645              | 215               | 9.07             | 24416.23   | 3              | Clade II  |
| RcSWEET6 | 30068.m002528       | 783              | 261               | 9.98             | 28738.3    | 4              | Clade II  |
| RcSWEET9 | 29647.m002020       | 858              | 286               | 8.42             | 32111.69   | 5              | Clade III |
| RcSWEET10a | 30147.m014446      | 831              | 277               | 9.05             | 31790.91   | 5              | Clade III |
| RcSWEET10b | 30147.m014447      | 837              | 279               | 9.00             | 31743.64   | 5              | Clade III |
| RcSWEET11 | 30147.m014444       | 855              | 285               | 8.04             | 32313.45   | 5              | Clade III |
| RcSWEET12 | 30147.m014445       | 891              | 297               | 8.27             | 33206.31   | 5              | Clade III |
| RcSWEET15 | 29929.m004599       | 816              | 272               | 10.07            | 30647.06   | 6              | Clade III |
| RcSWEET16a | 29579.m000197       | 747              | 249               | 7.08             | 27723.79   | 5              | Clade IV  |
| RcSWEET16b | 29726.m004066      | 732              | 244               | 8.27             | 26803.66   | 5              | Clade IV  |
| RcSWEET17 | 30128.m008852       | 864              | 288               | 9.95             | 31371.19   | 5              | Clade IV  |
Except for the pairs of HbSWEET2c/2d, HbSWEET2e/2f, and HbSWEET10e/10f, the Ka/Ks values of the other paralogous gene pairs were less than 0.5, showing that these genes had undergone a purifying selection (Table 2). The different expression patterns exhibited by the two genes in most of the gene pairs suggested that a functional divergence had occurred after gene duplication (Fig. 4). In A. thaliana, there were two SWEET gene pairs (AtSWEET6/7 and AtSWEET16/17) and one paralogous gene cluster (AtSWEET11, 12, 13, 14). In P. trichocarpa, there were two SWEET gene pairs (PtSWEET15a/15b and PtSWEET17a/17b) and four paralogous gene clusters (PtSWEET1a, 1b, 1c, 1d; PtSWEET2a, 2b, 2c; PtSWEET3a, 3b, 3c; and PtSWEET10a, 10b, 10c, 10d). In M. esculenta and
### Structural organization of SWEET genes

The exon–intron structures of the 127 SWEET genes in five plant species were determined based on the predicted sequences. As shown in Fig. 2A, most Hevea SWEET members within the same groups share similar gene structures in terms of intron number, domain localization, and exon length. Although the lengths vary, introns are inserted into nearly the same locations of the gene ORFs. Most SWEET members contain 3–5 introns. Of the 36 members in Hevea, for example, 24 have 5 introns, 7 have 4 introns, and 5 have 3 introns (Fig. 2A, Table 1a). In the total of 127 SWEET genes among the five plant species, there were only three SWEET members with no introns, namely RcSWEET4b, RcSWEET4c, and AtSWEET6, all of which were clustered in Clade II (Fig. 2A-E). Some SWEET members lacked exons at the 5' end, such as HbSWEET2f, 4c, 5b, 10c, 10e, MeSWEET4, 3b, 2b, 12a, and 10b, RcSWEET2, 5, and 4a, and PtSWEET1c, 16c, and 15a (Fig. 2A-E). Most SWEET members contain 4–7 TM helix domains, and 25 of the 127 members lost one to three of the seven TM helix typical of plant SWEETs (Fig. 3). In addition, the lengths of most AtSWEET and RcSWEET genes are shorter than those of the other plant SWEET genes, perhaps reflecting a relationship between gene length and genome size of a given species.

### Tissue expression of SWEET genes

To investigate the functions of SWEET genes, gene expression profiles in different tissues were analyzed by using Solexa sequencing data in Hevea, M. esculenta, P. trichocarpa, and A. thaliana (Tables S2, S3). Analysis of gene expression from the Sequence Read Archive (SRA), adopted in the present study, has limitations as the data were compiled from different sources where genetic differences in the tested tissues and dissimilarities in the experimental conditions can make comparisons difficult. Nonetheless, such an analysis provides a broad overview of the functionalities of the various Hevea SWEETs relative to their counterparts in other plant species. The results provide useful indicators as to which SWEET genes are most commonly expressed from among the numerous isoforms. These results would serve as a guide for future follow-up research where more exacting methodologies can be employed.

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**Table 2.** Divergence between paralogous HbSWEET gene pairs in *H. brasiliensis*. The synonymous (Ks) and nonsynonymous (Ka) substitution rates between gene duplicate pairs were calculated by Ka/Ks-Calculator. MA, a model that averages parameters across 14 candidate models.

| Gene pairs            | Method | Ka   | Ks   | Ka/Ks | P-Value (Fisher) | Length | S-Sites | N-Sites |
|-----------------------|--------|------|------|-------|-----------------|--------|---------|---------|
| HbSWEET1b/1c          | MA     | 0.6391 | 1.2846 | 0.4975 | 4.17E-17         | 669    | 191.017 | 477.983 |
| HbSWEET2a/2b          | MA     | 0.6391 | 1.2846 | 0.4975 | 4.17E-17         | 669    | 191.017 | 477.983 |
| HbSWEET2c/2d          | MA     | 0.1047 | 0.1065 | 0.9835 | 0.8869          | 543    | 137.643 | 405.375 |
| HbSWEET2e/2f          | MA     | 0.0080 | 0.0085 | 0.9479 | 0.5795          | 504    | 98.1862 | 405.695 |
| HbSWEET4a/4b          | MA     | 0.1419 | 0.4445 | 0.3192 | 6.24E-18         | 684    | 186.783 | 505.775 |
| HbSWEET5a/5b          | MA     | 0.0750 | 0.1856 | 0.4042 | 4.17E-17         | 690    | 179.627 | 510.373 |
| HbSWEET7a/7b          | MA     | 0.1524 | 0.1124 | 1.3556 | 0.2410          | 696    | 185.281 | 510.719 |
| HbSWEET10a/10b        | MA     | 0.0584 | 0.3780 | 0.1544 | 6.24E-18         | 690    | 179.627 | 505.775 |
| HbSWEET10c/10d        | MA     | 0.1044 | 0.2728 | 0.3828 | 2.03E-05         | 543    | 142.305 | 400.695 |
| HbSWEET10e/10f        | MA     | 0.0750 | 0.1856 | 0.4042 | 6.24E-18         | 684    | 186.783 | 505.775 |
| HbSWEET15a/15b        | MA     | 0.0750 | 0.1856 | 0.4042 | 6.24E-18         | 684    | 186.783 | 505.775 |
| HbSWEET17a/17b        | MA     | 0.1524 | 0.1124 | 1.3556 | 0.2410          | 696    | 185.281 | 510.719 |

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R. communis, there was only one SWEET gene pair (MeSWEET10d/10e, RcSWEET4b/4c) in each species.

Upon further examining the genomic locations, we found that some SWEET genes in the same clade were located adjacent to each other. For example, in Hevea, HbSWEET4b and HbSWEET4c were located on scaffold0371, HbSWEET10a and HbSWEET10c on scaffold1273, HbSWEET10b, HbSWEET10d, and HbSWEET10f on scaffold0491, HbSWEET11 and HbSWEET12 on scaffold0807, and HbSWEET17a and HbSWEET17b on scaffold0340. In *P. trichocarpa*, PtSWEET1b, PtSWEET1c, and PtSWEET1d were located adjacent to each other on chromosome 2, PtSWEET3a and PtSWEET3c on chromosome 15, PtSWEET10a, PtSWEET10b, PtSWEET10c, and PtSWEET11 on chromosome 15, PtSWEET16b, PtSWEET16c, PtSWEET17a, and PtSWEET17b on chromosome 13. In *R. communis*, RcSWEET4a and RcSWEET4d were located on scaffold03922, and RcSWEET10a, RcSWEET10b, RcSWEET11, and RcSWEET12 on scaffold30147. These adjacent gene pairs and clusters had apparently been derived from tandem duplication events.
Fig. 2. Structural organization of SWEET genes from *H. brasiliensis* and four other plant species. (A) to (E), structural organization of SWEET genes in *H. brasiliensis*, *M. esculenta*, *R. communis*, *P. trichocarpa*, and *A. thaliana*, respectively. Exons and introns are represented by boxes and black lines, respectively. The TM helix domain is represented by pink boxes. The sizes of exons and introns are proportional to their sequence lengths. Background shading: Clade I, blue; Clade II, red; Clade III, Green; and Clade IV, Purple.
The SWEET gene family in *Hevea brasiliensis* J.-L. Sui et al.
source leaves were significantly higher than those of the other members, while three SWEET genes (HbSWEET1c, 10a, and 10b) were mainly expressed in sink leaves. In the other three plant species, MeSWEET17, MeSWEET2a, MeSWEET15b, PtSWEET2a, and PtSWEET16d were highly expressed in leaves and AtSWEET11 was mainly expressed in seedling plants (11 days old) (Fig. 4B-D). Some of the above-mentioned SWEET members might be involved in phloem loading and leaf development. In Hevea bark where rubber-producing laticifers reside, HbSWEET1a and HbSWEET16a showed a predominance expression, while in latex, the cytoplasm of laticifers, HbSWEET2a, HbSWEET10a, HbSWEET10b and HbSWEET16b were the predominant isoforms. These SWEET genes might play an important role in sugar transport between the laticifers and their neighboring bark tissues, and contribute to the regulation of sucrose concentrations in laticifers together with the sucrose transporter responsible for apoplastic sucrose uptake of laticifers, HbSUT3 [18,19]. In A. thaliana, AtSWEET9 has been identified as a nectary-specific sugar transporter [6]. In Hevea, HbSWEET9a exhibited a male flower-specific abundant expression and might have a similar function in nectary production as its A. thaliana orthologue, AtSWEET9. In addition, 14 other Hevea SWEET genes, viz. HbSWEET1a, 1c, 2a, 2e, 2f, 3a, 3b, 7, 10a, 10b, 10e, 11, 16b, and 17b, were

Fig. 3. Multiple sequence alignment for the predicted amino acid sequences of the SWEET genes from H. brasiliensis and four other plant species. Sequence alignment was performed using DNAMAN 6.0 software (http://www.lynnnon.com/). Identical amino acids are shaded, and gaps are indicated by dots.
Fig. 4. Expression analyses of the SWEET genes based on Solexa sequencing. (A), Hierarchical clustering and differential expression analysis of the HbSWEET genes in seven tissues (leaf, bark, latex, root, seed, female flower, and male flower), at four developmental stages of leaves (bronze, color change, pale-green and mature), during ethephon treatment (0 h, 3 h, 12 h, and 24 h, PRJNA310171), latex (RRIM600 and RY879, PRJNA257219), brown bast and tapping panel dryness (PRJNA262475), ET and JA treatment (PRJNA281775), Clone FX 3864 response to GCL012 (PRJNA259872), MeJA (PRJNA353743), Corynespora cassiicola tolerance (PRJNA179126), abiotic stress (drought, low temperature, PRJNA182078 and ethephon treatment, PRJNA182079), and tissues (leaf, bark, and latex, PRJNA201084); (B), Hierarchical clustering and differential expression analysis of the MeSWEET genes in different tissues (root, leaf, stem, PRJNA248260), infected by pathogenic Xanthomonas (PRJNA231851), CBSV virus (PRJNA232098), tissues (PRJNA324539), and bacterial blight pathogen (PRJNA257332); (C), Hierarchical clustering and differential expression analysis of the PtSWEET genes under ABA stimulation (0 h, 1 h, 4 h, 8 h, 12 h, and 24 h, PRJNA232098), methyl jasmonate stimulation (PRJNA244820), chilling, freezing, and heat shock (PRJNA207974, PRJNA215888), salinity stress (0 h, 6 h, 12 h, 24 h, and 72 h, PRJNA230867), tissues (PRJNA320431), drought stress (PRJNA227790); (D), Hierarchical clustering and differential expression analysis of the AtSWEET genes in different tissues (floral bud, root, seeding, PRJNA231088), MeJA or BTH (PRJNA354369), MeJA and CK (PRJNA318266), cold stress (PRJNA218632), salt stress (0 mM, 50 mM, 100 mM, 150 mM, PRJNA217812).
Fig. 4. Continued.
also expressed at high levels in flowers (Fig. 4A). No SRA expression data in flowers were found in the other three plant species. In Hevea roots, seven SWEET genes (HbSWEET1a, 2a, 2f, 3b, 4c, 10e, and 17c) were abundantly expressed. In the other plant species, MeSWEET10a, MeSWEET3a, and
meSWET15b were expressed at high levels in the storage roots of M. esculenta; their activities may be related to starch formation. Six P. trichocarpa SWEET genes (PtSWEET2a, 2c, 3a, 3c, 16b, and 16c) were expressed at high levels in the roots. On the other hand, most of A. thaliana SWEET genes showed low or no expression in the roots. PtSWEET16b and PtSWEET16d exhibited high expression in xylem fiber cells that may be related to xylem formation. There were many SWEET genes showing universal expressions in most tissues examined. These included HbSWEET1c, 10e, 2c, 3b, 17c, 2d, 2e, 2f, 1a, 16a, 6, 2a, 16b, 10b, and 10a, MeSWEET17, 1a, 1b, 16b, 17c, 10b, 10a, 2a, 2b, 17b, 15b, 10d, 16a, 3a, and 9a, PtSWEET2a, 16d, 15b, 16b, 2b, 17a, 2c, 16c, 3a, and 10c, and AtSWEET1, 2, 17, 11, 12, and 16. Interestingly, isoforms of SWEET2, 16, and 17 were observed among the universally expressed SWEET genes in all plant species examined, which might represent the main evolutionary direction of SWEET genes in plants. As shown in Fig. 4A, transcripts of 11 HbSWEET genes (HbSWEET4a, 4b, 5a, 5b, 9b, 10c, 10f, 12, 15a, 15b, and 17a) were barely detectable in almost all the tissues and all the treatments examined. Such genes comprise a large portion (~1/3) of the total HbSWEET gene family. This character seems to be shared by the SWEET gene families in other plant species. For example, similar expression patterns were observed for seven of 28 SWEET genes in M. esculenta (Fig. 4B), 12 of 28 in P. trichocarpa (Fig. 4C), and 4 of 17 in A. thaliana (Fig. 4D). This result suggests that the SWEET gene families in higher plants might have experienced an event of gene expansion followed by nonfunctionalization in the course of evolution. A similar phenomenon has been reported in our studies for the CDPK and CDPK-related kinase gene families in Hevea [27]. In addition, we found that most genes in Clade II have low or no expansion in all tissues examined in the four plant species.

Expression profile of SWEET genes in response to hormone and stress treatments

Expression levels of SWEET genes in Hevea were also examined under various kinds of hormone and stress treatments. Ethephon, an ethylene generator, is widely used to stimulate latex production of the rubber tree, but the yield-stimulating mechanisms are still poorly understood [15,22]. As shown in Fig. 4A, expressions of HbSWEET10a were obviously upregulated by ethephon treatment in latex. In addition, HbSWEET10a was the predominant SWEET isoform in latex, the expression of which was higher than any of the other SWEET members, suggesting its important role in sugar transport of laticifers. Expressions of HbSWEET10a and HbSWEET2a appeared to be regulated by methyl jasmonate (MeJA) although in differing manners. Expressions of HbSWEET2c, HbSWEET2d, and HbSWEET3 were downregulated under drought treatment. Under low temperature treatment, expressions of HbSWEET1c were upregulated, whereas HbSWEET2c, 2d, 16a, and 17c were downregulated. Expressions of Hevea SWEET genes were also regulated by other kinds of stress treatments. For example, the expressions of HbSWEET1b, HbSWEET1c, and HbSWEET10e were affected by tapping panel dryness, a complex physiological disorder affecting latex production severely [28]; HbSWEET17b expressions were downregulated under the infection of Corynespora cassicicola, a fungal pathogen causing a leaf fall disease in Hevea [29].

The expression levels of SWEET genes in M. esculenta, P. trichocarpa, and A. thaliana were also examined when the plants were subjected to treatments of hormones and different stresses, including fungus infection, drought, and cold (Fig. 4B-D). The expressions of six MeSWEETs (MeSWEET1a, 10a, 10b, 15b, 17, and 17c) were affected by fungus infection. Expressions of PtSWEET2b and PtSWEET16d were induced by MeJA in roots. Expressions of PtSWEET15b were induced by drought and ABA (abscisic acid) treatments. In the model plant A. thaliana (Fig. 4D), the expressions of ATSWEET16 and ATSWEET17 were upregulated by MeJA in roots. In seedlings, expressions of ATSWEET12 were upregulated by MeJA, while those of ATSWEET16 were downregulated. Under cold treatment, expressions of ATSWEET1 and ATSWEET2 were upregulated, while those of ATSWEET16 and ATSWEET17 were downregulated.

Expression analyses of HbSWEET10a, HbSWEET16b, and HbSWEET1a based on qPCR

Rubber is synthesized and stored in the cytoplasm (latex) of highly specialized cells called laticifers that are differentiated from the cambium and arranged in rings. To further examine the expression of HbSWEET genes in latex and bark, quantitative RT-PCR (qPCR) analyses of HbSWEET10a, HbSWEET16b, and HbSWEET1a were performed. As shown in Fig. 5, the results from qPCR were in broad agreement with the sequencing-based expression analyses. HbSWEET10a and HbSWEET16b were mainly expressed in latex; HbSWEET1a was mainly expressed in bark and flower (Fig. 5A). HbSWEET10a was obviously upregulated by ethephon treatment in latex, while HbSWEET16b
was obviously downregulated after 24 hours of ethephon treatment in latex, which agrees well with the results based on RNA-seq (Fig. 4A, 5B, Table S3-1). We also further examined the expression of HbSWEET1a under ethephon treatment in bark, while HbSWEET1a was obviously upregulated (Fig. 5B).

The process of rubber harvesting, namely tapping, produces a conspicuous stimulating effect on latex production in virgin Hevea trees, and it has been partially ascribed to an enhanced sucrose uptake and sucrose catalolism in the laticifers [18,20]. As shown in Fig. 5C, HbSWEET10a and HbSWEET16b were obviously upregulated by tapping in three different clones PR107, Reyan7-33-97, and Reyan8-79. All above results revealed that HbSWEET10a, 16b, and 1a might play an important role in sugar transport in laticifer and bark.

Conclusion

In this study, a genome-wide analysis of SWEET gene families was undertaken for the first time in Hevea, M. esculenta, and R. communis. In silico analysis of the Hevea genome database facilitated the identification of 36 SWEET genes. The phylogenetic analysis of 127 SWEETs from Hevea and four other plant species (A. thaliana, P. trichocarpa, M. esculenta, and R. communis) classified all these SWEETs into four major groups. Members within each group might have had common evolutionary origins as seen from the sharing of similar protein motifs, exon–intron structures, and basic molecular functions. Solexa sequencing analyses revealed that SWEET2, 16, and 17 were universally expressed in different tissues of all the plant species examined, possibly representing the main evolutionary direction of plant SWEET gene families. Extensive expression analyses in different tissues and in response to various experimental treatments, including hormones, and biotic and abiotic stresses, identified multiple tissue-specific SWEET isoforms and isoforms showing striking responses to some of the treatments in Hevea and three other plant species (A. thaliana, P. trichocarpa, and M. esculenta). These results indicate versatile roles of SWEETs in plant growth, development, and stress responses and provide a foundation for further functional investigation of the SWEET gene families in the plant kingdom.

Materials and methods

Database search for SWEET genes in H. brasiliensis and four other plant species

Sequences of A. thaliana and P. trichocarpa SWEET genes were downloaded from the A. thaliana Information Resource (http://www.Arabidopsis.org) and GenBank (http://www.ncbi.nlm.nih.gov/genbank). The genome and protein sequences of A. thaliana [30], P. trichocarpa [31], M. esculenta [32], and R. communis [33] were downloaded from Phytozome v10 (http://www.phytozome.net/). The H. brasiliensis genome and transcriptome data were obtained from GenBank (http://www.ncbi.nlm.nih.gov/nuccore/448814761) [22]. Local BLAST alignment was performed using published SWEET sequences from A. thaliana and P. trichocarpa as queries to search against the deduced proteome of each species for the candidate SWEETs from H. brasiliensis, A. thaliana, P. trichocarpa, M. esculenta, and R. communis. All putative candidates were manually verified with the InterProScan server (http://www.ebi.ac.uk/Tools/pfa/iprscan/) to confirm the presence of protein kinase and TM helix domains.

Phylogenetic and gene structure analyses

Multiple alignments of the amino acid sequences of SWEETs from five species were performed using the Clustal X (version 1.83) program. The phylogenetic tree was constructed with MEGA6.0 [34] by employing the neighbor-joining (NJ) method with a bootstrap test for 1000 replicates. Exon–intron structures of the six species SWEET genes were analyzed by comparing the cDNA and their genomic DNA sequences through the web server GSDS 2.0 (http://gsds.cbi.pku.edu.cn/). The KaKs-Calculator program (https://sourceforge.net/projects/kaksclulator/) was used to calculate the nonsynonymous (Ka) and synonymous (Ks) substitutions in coding regions.

Expression analysis based on Solexa sequencing

For Solexa sequencing-based expression analyses, Sequence Read Archive (SRA) data were downloaded from the NCBI database (Table S2) [27]. The sequences included those for H. brasiliensis (C. cassicola tolerance, PRJNA179126; abiotic stress, PRJNA182078 and PRJNA182079; tissues, PRJNA201084 [35]; tissues, leaf development and ethephon treatment, PRJNA310171 [22,27]; Clone FX3864 response to GCL012, PRJNA259872; ET and JA treatment, PRJNA281775 [36]; brown bast and tapping panel dryness, PRJNA262475 [28]; Hevea clones PR107 and RY879, PRJNA257219 [37]; RRIM600 and RY7-20-59, PRJNA254411; MeJa, PRJNA353743; M. esculenta (Xanthomonas tolerance, PRJNA231851; CCSV virus infected, PRJNA243380; tissue, PRJNA248260; bacterial blight pathogen infected, PRJNA257322; tissue, PRJNA324539); A. thaliana (salt stress, PRJNA217812; tissues, PRJNA231088; cold stress, PRJNA218632; MeJa or BTH, PRJNA354369; MeJa and Ck, PRJNA318266); P. trichocarpa (ABA stimulation, PRJNA232098; methyl jasmonate treatment, PRJNA244820; chilling, freezing and heat shock, PRJNA207974 and PRJNA215888; salinity
stress, PRJNA230867; tissue, PRJNA320431; drought stress, PRJNA227790). Raw RNA-seq reads were processed to trim terminal low-quality bases and adapter sequences via an in-house custom pipeline. The clean reads were then mapped to the genome using Bowtie2, and RSEM software was used for quantifying transcript abundance with default parameters [38].

Quantitative reverse transcriptase PCR (qPCR)

To examine the expression of HsSWEET1a, HsSWEET16b, and HhSWEET1a in latex and bark, quantitative RT-PCR (qPCR) was performed. Unless otherwise noted, Reyan7-33–97 (synonym for CATAS7–33–97 or RY3–33–97), Reyan8–79 ( synonym for CATAS8–79 or RY8–79), and PR107 rubber trees (H. brasiliensis) selected for QPCR in this study were cultivated at the experimental plantation of the Rubber Research Institute of the Chinese Academy of Tropical Agricultural Sciences (Danzhou, Hainan, China). These trees were regularly tapped for latex collection in a half spiral pattern, every 3 days, without Ethrel stimulation (S/2, d/3). To study the tissue-specific expression of HsSWEET genes, different tissues were collected from 10-year-old mature trees of clone Reyan7-33-97 that had been tapped for the last 2 years. The same type of tree was also used to examine the effect of Ethrel on expression. To analyze the effect of tapping on HhSWEET genes expression, 8-year-old mature virgin (never tapped) trees (Roche Diagnostics, Penzberg, Germany) using SYBR Green premix kit (TaKaRa) according to the manufacturer’s instructions. The primer pairs used for the HsSWEET genes were 5’-CTGACACATC AACTCACTTCA-3’ (F) and 5’-CATCG GGTGGTTAATGCTCT-3’ (R) (HhSWEET1a), 5’-GT CCGCTTTTGCTGCA-3’ (F) and 5’-AAGTCCAAATC CTTGCTTGCA-3’ (R) (HhSWEET16b), 5’-TCTCCTTTCC GCCTGTATG-3’ (F) and 5’-GGCTGTTCTTCCTTGG GTGC-3’ (R) (HhSWEET1a). For genes as internal control, YLS8 was used in gene expression analyses in the latex responding to tapping and Ethrel treatment, RH2b was used for tissue expression as recommended by Li et al. (2011) [39]. The details for experimental manipulations and data analysis were as described by Tang et al. (2010) [18].

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Authors contributions

CRT conceived and designed the experiments. JLS, XHX, and JYQ performed the experiments. XHX and YJF analyzed the data. JLS, XHX, and CRT wrote the manuscript. All authors read and approved the final manuscript.

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The SWEET gene family in *Hevea brasiliensis*

J.-L. Sui et al.

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Supporting information

Additional Supporting Information may be found online in the supporting information tab for this article:

Table S1. SWEET Accessions.

Table S2. Basic information for Solexa sequencing data of *Hevea brasiliensis* and three other plant species.

Table S3. RNA-seq analysis of the expressions of SWEET genes in *Hevea brasiliensis* and three other plant species.