Abstract: The Sugars Will Eventually be Exported Transporters (SWEET) family is a class of sugar transporters that play key roles in phloem loading, seed filling, pollen development and the stress response in plants. Here, a total of 18 JcSWEET genes were identified in physic nut (Jatropha curcas L.) and classified into four clades by phylogenetic analysis. These JcSWEET genes share similar gene structures, and alternative splicing of messenger RNAs was observed for five of the JcSWEET genes. Three (JcSWEET1/4/5) of the JcSWEETs were found to possess transport activity for hexose molecules in yeast. Real-time quantitative PCR analysis of JcSWEETs in different tissues under normal growth conditions and abiotic stresses revealed that most are tissue-specifically expressed, and 12 JcSWEETs responded to either drought or salinity. The JcSWEET16 gene responded to drought and salinity stress in leaves, and the protein it encodes is localized in both the plasma membrane and the vacuolar membrane. The overexpression of JcSWEET16 in Arabidopsis thaliana modified the flowering time and saline tolerance levels but not the drought tolerance of the transgenic plants. Together, these results provide insights into the characteristics of SWEET genes in physic nut and could serve as a basis for cloning and further functional analysis of these genes.

Keywords: SWEET gene family; sugar transporter; gene evolution; gene expression; abiotic stress; physic nut

1. Introduction

Sugars are the predominant product of plant photosynthesis; they not only participate in the storage and transportation of nutrients but also play important roles in signal transduction and stress responses [1–3]. Sugars are transported from the source tissue to the sink tissue through phloem in order to maintain normal plant growth and development. However, sugars cannot be transported independently across plant membranes; therefore, their transmembrane movement requires sugar transporters [4–6]. Currently, three families of transporters involved in intercellular sugar transport have been identified, including MSTs (monosaccharide transporters), SUTs (sucrose transporters) and SWEETs (Sugars Will Eventually be Exported Transporters) [7]. MSTs and SUTs contain 12 transmembrane domains (TMs) that belong to the major facilitator superfamily. However, SWEETs are structurally a different type of transporter, with seven TMs, and are classified with the MtN3/saliva family (PF03083) of transmembrane transporters [4]. Phylogenetic analysis has demonstrated that plant SWEET family genes can be divided into four clades. Different
family members have distinct patterns of tissue expression and participate in the transport of diverse sugar molecules. The subcellular localization of family members also varies, indicating that SWEET family genes have numerous biological functions that are extremely important for plant growth and development [8–10].

The number of SWEET genes varies across species, and the number present within a species does not correlate with the evolutionary complexity of that species; plants have larger numbers of SWEET gene family members than do other organisms [11]. To date, SWEET genes have been identified in a number of plants, including A. thaliana (Arabidopsis thaliana), rice (Oryza sativa), soybean (Glycine max) and maize (Zea mays). AtSWEET1 was the first plant SWEET protein to be identified; it transports glucose and is highly expressed during early flower development [4,12]. Chen et al. [13] found that AtSWEET11 and AtSWEET12 were localized to the plasma membrane of phloem parenchyma cells and are involved in sucrose transport. The double mutation of AtSWEET11 and AtSWEET12 affected sucroze phloem transport, indicating for the first time the key role of these plant SWEET proteins in phloem loading [14,15]. AtSWEET11, AtSWEET12 and AtSWEET15 are expressed during seed development, especially in maternal tissues; the triple mutant of ats11;12;15 showed severe seed defects, indicating that SWEET proteins are involved in the seed filling process in A. thaliana [16]. AtSWEET8 (Ruptured Pollen Grain 1, RGP1) was found to be strongly expressed in the microsporocyte (or microspores) and tapetum during male meiosis, and the rpg1 mutant exhibited severely reduced male fertility [17]. AtSWEET9 is a nectary-specific sugar transporter in this eudicot species and is essential for nectar production [18]. Recently, studies have shown that SWEET proteins are also involved in plant hormone transport. AtSWEET13 and AtSWEET14 participate in the transport of different gibberellins (GAs) and regulate GA-mediated physiological processes, including anther dehiscence and seed development [19]. OsSWEET11 and OsSWEET15 also play an important role in rice seed filling [20–22]. In soybean, most of the SWEET genes are expressed in seeds, and the mutation of GmSWEET15 results in retarded seed embryo development [23,24]. In maize, ZmSWEET13 paralogues (a, b, c) are among the most highly expressed genes in the leaf vasculature, and a triple mutant of the three ZmSWEET13 paralogues exhibited impaired phloem loading [25].

Soluble sugars are major osmolytes, and SWEET proteins can participate in plant stress responses by regulating the allocation of soluble sugars. In A. thaliana, AtSWEET16 and AtSWEET17 are involved in abiotic stress responses, and further, the overexpression of AtSWEET16 can increase the tolerance of cold stress [26,27]. AtSWEET15 (SAG29) is expressed primarily in senescing plant tissues; SAG29-overexpressing transgenic plants were hypersensitive to salinity stress, and the SAG29-deficient mutants were less sensitive to high salt levels [28]. The expression of AtSWEET11 and AtSWEET12 were down-regulated by cold treatment, and the double mutation of AtSWEET11 and AtSWEET12 exhibited greater freezing tolerance than the wild-type and both single mutants [29]. AtSWEET4 is a hexose facilitator, and the overexpression of AtSWEET4 in A. thaliana increased plant size and exhibited higher freezing tolerance [30]. In rice, OsSWEET13 and OsSWEET15 are major SWEET transporters regulating both sucroze transport and levels in response to drought and salinity stresses by the binding of an ABA-responsive transcription factor OsZIP72 to the promoters [31]. CsSWEET2 is a hexose transporter from Cucumber (Cucumis sativus), and it plays a vital role in improving plant cold tolerance by mediating sugar metabolism and compartmentation [32]. A total of 22 ClaSWEET genes were identified in the watermelon (Citullus lanatus) genome, and the expression patterns of ClaSWEET genes demonstrated that ClaSWEET proteins play key roles in responses to abiotic stresses, including drought, salt levels and low-temperature stresses [33]. In Camellia sinensis, CsSWEET16 contributes to sugar compartmentation across the vacuole and functions in modifying cold tolerance when overexpressed in A. thaliana [34].

Physic nut is a small perennial tree or large shrub of the Euphorbiaceae family; it has attracted wide attention due to its fast growth, ease of propagation, tolerance of poor-quality land, considerable adaptability and the high oil content of its seeds [35]. Genome
sequence and expression sequence tag (EST) libraries constructed by our team and others in recent years provide a solid basis for the analysis of physic nut gene families and their evolution [36–38]. In this study, a genome-wide analysis was conducted to identify the SWEET genes of physic nut. We analyzed the exon-intron structure and the phylogenetic relationships of JcSWEET genes in detail and examined the expression levels of JcSWEET genes in different tissues under normal growth conditions and abiotic stresses. The cDNA clones of 13 JcSWEETs were obtained and transferred to yeast to test sugar transport activities. Further study of the function of JcSWEET16 revealed that it is localized in the plasma membrane and vacuolar membrane and has roles in flowering time and saline tolerance when overexpressed in A. thaliana.

2. Results

2.1. Identification and Phylogenetic Analysis of SWEET Family Genes in Physic Nut

Based on the domain sequences of A. thaliana and rice proteins, a total of 18 putative JcSWEET genes were identified from the published genome database [36,37] using a BLAST search analysis approach. The lengths of the coding sequences of JcSWEET genes ranged from 708 bp to 918 bp, and they encoded polypeptides containing 235 (JcSWEET2a/2b) to 305 (JcSWEET16) amino acids (Table S1). These JcSWEET genes were named based on their homologs in A. thaliana. All SWEET proteins were predicted to have seven transmembrane domains (TMs) (Figure S1). There are approximately 86 to 91 amino acids in the two MtN3/saliva domains, and their positions in all proteins are similar. Multiple sequence alignments of the 18 JcSWEETs and AtSWEET1 (AT1G21460) proteins revealed that all TMs were relatively conserved except for the fourth, which is characteristic of SWEET proteins (Figure S2).

To investigate the phylogenetic relationships among the SWEET genes in physic nut and other plant species, a phylogenetic tree was constructed by aligning 18 JcSWEET protein sequences, 17 AtSWEET protein sequences from A. thaliana and 21 OsSWEET protein sequences from rice using the program MEGA5.0. According to this phylogenetic tree, all JcSWEET proteins could be clustered into four clades, as previously reported for A. thaliana [4]. Clade I (JcSWEET1/2a/2b/3) contains four JcSWEET proteins, clade II (JcSWEET4/5/6) and clade IV (JcSWEET16/17a/17b) both contain three JcSWEET proteins, and the remaining eight JcSWEET proteins all belong to clade III (JcSWEET9a/9b/9c/10a/10b/11/12/15) (Figure 1).

2.2. Gene Structure and Chromosomal Distribution Analysis of SWEET Family Genes in Physic Nut

To analyze the structural characteristics of JcSWEET genes, we mapped their structures by submitting their full-length coding sequences and the corresponding genomic DNA sequences to the online Gene Structure Display Server (http://gsds.gao-lab.org/ (accessed on 19 January 2022)). All JcSWEET genes shared a similar exon-intron arrangement, with four to five introns in the coding region. The JcSWEET4 gene did not contain the third intron of other JcSWEET gene family members, while JcSWEET9b and JcSWEET10a did not contain the first intron of other family members. Alternative splicing of messenger RNAs was observed for five JcSWEET genes, including family members JcSWEET2a, JcSWEET2b, JcSWEET4, JcSWEET11 and JcSWEET15 (Figure 2).
Figure 1. Phylogenetic relationships of SWEET family genes in physic nut, rice, and A. thaliana. The sequences of the SWEET proteins from the above three plant species were aligned by CLUSTAL_X, and the phylogenetic tree was constructed using MEGA 5.0 and the neighbor-joining (NJ) method with default settings. Jc, Jatropha curcas; At, A. thaliana; Os, Oryza sativa. JcSWEET genes are identified by black triangles.

Figure 2. Structures of the 18 JcSWEET genes. The exons are shown as boxes (coding sequence (CDS) in black, untranslated region (UTR) in gray), while the introns are represented by lines.
Via the use of our previously constructed linkage map [36], 16 of the 18 JcSWEET genes were mapped to 8 of the 11 linkage groups (LGs), but the two remaining family members (JcSWEET1 and JcSWEET2b) were located on unmapped scaffolds. The genes were unevenly distributed on LGs, and no JcSWEET gene was found on LGs 2, 10 or 11. The highest concentration was located on LG 5, which contained six genes (JcSWEET3/5/10a/10b/11/12). Tandem duplication, defined as tandem repeats that are located within 50 kilobases (kb) of each other or are separated by <4 non-homologous spacer genes [39], was observed for SWEET genes in the physic nut genome. Tandem duplications were observed on LG 5, which contains four genes (JcSWEET10a, 10b, 11 and 12) that were grouped into the same clade (Clade III) of the phylogenetic tree (Figure S3).

In order to test whether these tandem duplicates arose from recent duplication events in physic nut, we constructed another phylogenetic tree using SWEET proteins from physic nut and a closely related species, the castor bean (Ricinus communis) (Figure S4). Based on this phylogenetic tree, these four tandem duplications are also present in the castor bean genome. Paralogs of JcSWEET10a/10b and JcSWEET11/12 were also observed as a tandem pair in the genomes of A. thaliana (AT5G50790/AT5G50800/AT5G50800/AT5G50813). These results indicate that these tandem repeats in physic nut have resulted from both ancient (in Dicotyledoneae) and recent (in Euphorbiaceae) gene duplication events.

2.3. Expression Profiles of SWEET Genes in Different Physic Nut Tissues

To study the expression patterns of JcSWEET genes and gain information about their roles in the growth and development of physic nut, quantitative RT-PCR (qRT-PCR) was performed to measure transcription levels in various tissues and organs, including the roots, stem cortex, leaves, female flowers, male flowers and developing seeds. Although the expression of almost all genes could be detected in the tissues tested, except for family members JcSWEET2a and JcSWEET9b, the expression of each family member varied greatly (Figure 3). Genes within the same clade also displayed considerable differences in their level of expression across the different tissues analyzed.

In clade I, JcSWEET1 was highly expressed in developing seeds (Figure 3), and its expression level was consistent with the seed filling process (Figure 4). JcSWEET2b was expressed in early developing seeds at low levels (Figure 4), while JcSWEET3 was expressed at a low level in developing seeds but highly in male flowers (Figure 3). In clade II, JcSWEET4 was expressed mainly in roots, flowers and seeds. JcSWEET5 was weakly expressed in all the tissues tested, while JcSWEET6 was expressed at a relatively higher level in flowers (Figure 3). In clade III, only a low level of expression was detected for JcSWEET9a across the assessed tissues, while a relatively high level of expression for JcSWEET9c was detected in flowers and seeds at the middle stage of development (Figures 3 and 4). JcSWEET15 was highly expressed in the stem cortex, male flower, and filling-stage seeds. For the tandem duplication JcSWEET10a/JcSWEET10b, transcripts of the JcSWEET10a gene were detected in seeds at the early and middle developmental stages, whereas only a low level of expression was observed for JcSWEET10b in developing seeds. Both JcSWEET11 and JcSWEET12 were expressed at low levels in roots, leaves, and the stem cortex, but these two family members were relatively highly expressed in seeds at the middle developmental stage (Figure 4). In addition, JcSWEET10a and JcSWEET11 were highly expressed in male flowers (Figure 3). In clade IV, the relative expression level of JcSWEET17a was higher than those of the others in the tested tissues under normal growth conditions.
Figure 3. Expression analysis of JcSWEET genes in physic nut plants under normal conditions. R, root; St, stem cortex; L, leaf; FF, female flower; MF, male flower; S, seed. Relative expression was normalized to that of the reference gene JcActin (internal control).

Figure 4. Patterns of expression of JcSWEET genes of seeds from 14 to 45 days after pollination (DAP). The relative expression was normalized to that of the reference gene JcActin (internal control).

2.4. Expression Profiles of JcSWEET Genes under Drought and Salinity Stress

To determine the roles of JcSWEET genes in abiotic stress responses, the expression patterns of JcSWEETs under drought and salinity stress were analyzed using our next-generation sequencing-based digital gene expression tag database [40,41]. Overall, a total of 12 out of the 18 JcSWEET genes showed differential expression levels in response to at least one stress in at least one tissue. JcSWEET3/10a exhibited similar expression patterns...
under drought and salinity stress and were significantly up-regulated in roots after 2 days of treatment, and the expression level of JcSWEET17b also increased markedly in leaves after 7 days of exposure to both imposed stresses. The expression level of JcSWEET15 increased dramatically in roots under drought stress but showed no significant response to salinity stress. The level of JcSWEET16 transcript increased in leaves but decreased in roots after salinity treatment for 2 days (Figure 5A).

![Figure 5](image)

**Figure 5.** Expression patterns of JcSWEET genes in response to drought and salinity stresses. (A) Heatmap showing the expression levels of JcSWEET genes under drought and salinity stresses. Values presented in the heatmap are the ratios of stress treatment to control. (B) Expression levels of selected JcSWEET genes in leaves under drought and salinity stress at the 7 day point measured using qRT-PCR. Relative expression was normalized to that of the reference gene JcActin (internal control).

To verify the expression profiles determined using the digital gene expression tag database, qRT-PCR was employed to analyze the expression levels of clade IV (JcSWEET16/17a/17b) genes in leaves under drought and salinity stresses at the day 7 time point. This analysis showed that JcSWEET17a/17b were up-regulated and JcSWEET16 was significantly down-regulated in leaves under drought and salinity stress treatments (Figure 5B); these findings were consistent with the corresponding transcript abundance changes obtained by Digital Gene Expression Profiling, indicating that the digital expression data were reliable.

### 2.5. Transport Activity of JcSWEETs in Yeast

To obtain cDNAs containing full-length coding sequences of JcSWEET genes, total RNA from three-week-old seedlings of physic nut cultivar GZQX0401 was used to perform reverse transcription. As a result, cDNA clones containing full-length coding sequences were obtained for 13 genes. For five genes (JcSWEET2a, JcSWEET6, JcSWEET9a, JcSWEET9b and JcSWEET11), cDNA clones were not obtained, most likely due to the low level of expression of each gene. Many plant SWEETs have been shown to transport hexoses or sucrose [9]. To examine which substrates could be transported by the JcSWEET proteins, 13
JcSWEET genes were expressed in the yeast mutant EBY.VW4000, which lacks endogenous hexose transporters. Accordingly, this mutant yeast can grow on media containing maltose but shows no or slow growth on media containing glucose, fructose, mannose, sucrose or galactose [42]. All transformants with empty vectors and constructs could grow well on synthetic-deficient (SD) media containing 2% maltose, indicating the presence of the expression vector or target gene (Figure 6). Yeast cells expressing either JcSWEET1 or JcSWEET4 could grow on media supplemented with mannose, glucose, galactose and sucrose, suggesting that both JcSWEET1 and JcSWEET4 can transport these four sugars in yeast. The expression of JcSWEET5 effectively restored the growth of EBY.VW4000 on media supplemented with mannose or glucose. However, transformants carrying the remaining genes showed no growth on any of the media, indicating that these ten JcSWEET proteins could not transport these five sugars in yeast using the assay method described here.

**Figure 6.** Complementation growth assay in the yeast EBY.VW4000 mutant. Yeast transformants expressing empty vector (negative control), AtSWEET1 and 13 JcSWEETs were grown on media containing 2% maltose, 2% mannose, 2% glucose, 2% galactose, 2% sucrose or 2% fructose. AtSWEET1 was used as positive controls for glucose, galactose and sucrose transport activities.
2.6. Overexpression of JcSWEET16 Causes Early Flowering and Increases Salt Stress Tolerance in A. thaliana

To investigate the functions of physic nut SWEET genes, several of the genes that demonstrated changes in expression as a result of drought and salinity stress were over-expressed in A. thaliana under the control of a CaMV (Capsicum Mottle Virus) 35S promoter. First, we analyzed the subcellular localization of the JcSWEET16 protein. To accomplish this, a JcSWEET16:GFP construct was generated and transiently co-transformed into A. thaliana mesophyll protoplasts with a vacuolar membrane marker (AtTPK1:mCherry) or a plasma membrane marker (AtPIP2A:mCherry). Confocal images showed that GFP fluorescence signals of JcSWEET16:GFP overlapped with red fluorescence derived from AtTPK1:mCherry and AtPIP2A:mCherry. To further examine localization, JcSWEET16:GFP was introduced into the epidermal cells of N. benthamiana leaves, and the GFP fluorescence was clearly observed on the plasma membrane (Figure 7). These results show that JcSWEET16 was localized not only on the vacuolar membrane but also on the plasma membrane, a pattern similar to the subcellular localization of SlSWEET15 in tomato (Solanum lycopersicum) [43].

Three independent T3 homozygous overexpressing JcSWEET16 transgenic lines (OE-3, OE-48 and OE-64) were obtained, and the expression in these lines was examined by semi-quantitative RT-PCR (Figure 8A). Under normal growth conditions, no significant differences were detected between wild-type and transgenic seedlings in terms of plant size or morphology. However, the transgenic lines displayed an early flowering phenotype. After 41 days of growth, all three OE lines had completed the flowering process, while the WT plants exhibited a flowering efficiency of 77.78% (Figure 8B,C). These results indicate that the overexpression of JcSWEET16 could accelerate flowering in A. thaliana.
At least 18 plants were used for each experiment. Scale bar = 2 cm.

Figure 8. Overexpression of JcSWEET16 (OE) promotes flowering in A. thaliana. (A) Relative expression levels of JcSWEET16 in different transgenic lines measured using semi-quantitative RT-PCR analysis. (B) WT plants and OE lines grown for 34 d. (C) Flowering time of WT plants and OE lines. At least 18 plants were used for each experiment. Scale bar = 2 cm.

To gain insight into the function of JcSWEET16 in response to salinity stress, the same three OE lines were used for salinity treatment. No differences were detected between WT and transgenic lines on a normal 1/2 MS medium, whereas the survival rate of the OE lines was significantly higher than those of WT seedlings when cultivated with 150 mM NaCl (Figure 9). However, there were no significant differences in seedling root length or chlorophyll content between WT and transgenic lines when cultivated with 300 mM Mannitol (Figure S5). These results suggest that the overexpression of JcSWEET16 in A. thaliana could improve saline tolerance.

Figure 9. Overexpression of JcSWEET16 improves saline tolerance in A. thaliana. (A) Four-day-old seedlings of WT and OE were transferred to 1/2 MS medium supplemented with 150 mM NaCl for 6 days. (B) Schematic representation of the seedling position. (C) Survival rate of the seedlings after salinity stress. The data shown are means ± SD from three biological experiments. Statistically significant differences were assessed using Student’s t-tests (* p < 0.05, ** p < 0.01).
3. Discussion

Over the past two decades, numerous sugar transporters have been discovered in humans, plants, bacteria and fungi and have been demonstrated to play key roles in growth and development, metabolism and homeostasis. The members of the recently identified family of SWEET sugar transporters in eukaryotes contain only seven TMs and can mediate both cellular uptake and efflux [4]. To date, SWEET gene family members have been identified in many plant species based on genome-wide analyses. In the present study, a total of 18 putative SWEET genes were identified in the physic nut genome; this number is close to those of A. thaliana (17) [4], rice (21) [11] and cucumber (17) [44], but fewer than in soybean (52) [24] and wheat (Triticum aestivum) (59) [45]. Polyploidy is an important contributor to plant genome evolution, and many angiosperms have undergone gene duplication within a gene family due to experiencing one or multiple polyploidization events [46,47], which is a genomic event that can explain the large number of SWEET genes in the soybean and wheat genomes.

Physic nut SWEET genes were classified into four clades according to their phylogenetic relationship (Figure 1), which was in agreement with the classification of SWEETs in A. thaliana and rice [4,11]. There are eight physic nut SWEET genes in clade III, four in clade I, and three in each of clades II and IV (Figure 1). The physic nut has more genes than A. thaliana in clades I, III and IV. Gene duplication plays a crucial role in the evolution of higher plants, as it not only expands genome content but also diversifies gene functions to help the organism to adapt to different environmental conditions [48]. A non-random pattern of introns indicates that they were acquired from a progenitor and stabilized through evolution [49]. Gene structure analysis indicates that all JcSWEET genes shared a similar exon-intron arrangement (Figure 2). This is similar to the SWEET gene structure in A. thaliana [4], indicating that angiosperm SWEET genes share a common origin and that there has been gene function divergence as gene expansion occurred later during evolution. In addition, tandem duplication events have been observed in the SWEET genes of soybean and rice [24]. Four JcSWEET genes (JcSWEET10a, 10b, 11 and 12) were considered to represent tandem duplication, and these duplications are also present in the genomes of castor bean and A. thaliana (Figures S3 and S4). These results suggest that the processes giving rise to the expansion of the JcSWEET genes in physic nut included both ancient and recent tandem duplication.

In A. thaliana, transport substrates for all SWEET family members have been identified, and different clade members have different transport activities: clade I members allow 2-deoxyglucose and sucrose transport, clade II members allow the transport of glucose and fructose [9]. In our study, we determined that both JcSWEET1 and JcSWEET4 proteins, from clades I and II, respectively, could mediate the uptake of mannose, sucrose, glucose and galactose in the yeast EBY.VW4000 mutant, while JcSWEET5, which also belongs to clade II, mediated the uptake of mannose and glucose in the assessed yeast mutant background (Figure 6). These results suggest that there may exist some differences in the functions of SWEET proteins among species. Furthermore, SWEET proteins have a broader substrate range in physic nut, indicating that these proteins may have multiple physiological functions and be involved in more complex biological processes. However, in the present study, JcSWEET2b/3/9c/10a/10b/12/15/16/17a/17b showed no sugar transport activity in yeast using the present assay method (Figure 6); this may be because the transport activity of these proteins is not located at the plasma membrane, and further studies are needed.

Plant leaves are the main source organ and play an important role in the synthesis of carbohydrates. Gene expression analysis revealed that JcSWEET17a had a relatively high expression level in physic nut leaves (Figure 3). AtSWEET17 of clade IV is a vacuolar transporter that controls fructose content in A. thaliana leaves and roots [50]. In addition, JcSWEET17a belongs to clade IV (Figure 1), indicating that it may play similar roles in balancing intracellular hexose homeostasis. Developing seeds are the strongest sink tissues
in many plants, and they need a substantial source of carbon for development, which implies that SWEET genes may direct a key role in seed development [24]. In *A. thaliana*, *AtSWEET15* is expressed in the seed coat and endosperm and functions in the transfer of sugars from the seed coat to the embryo [16]. The *AtSWEET15* paralog *JcSWEET15* was also found to be significantly expressed in filling-stage seeds (Figure 4), suggesting that this gene may play a similar role to that of *AtSWEET15*. In addition, the duplicates from clade III (*JcSWEET10a* and *10b*) show divergent expression patterns (Figures 3 and S3), suggesting the occurrence of subfunctionalization during the evolutionary process.

*AtSWEET9* is a nectary-specific sugar transporter and is essential for nectar production [18]. Both *JcSWEET9a* and *JcSWEET9c* are homologs of *AtSWEET9*, but they have significantly distinct expression patterns. The discrepancy between *AtSWEET9* and *JcSWEET9* is probably due to evolutionary differences at the genome level between these two species.

Recently, studies have shown that SWEET genes from clade IV are involved in abiotic stress tolerance and the overexpression of these genes in plants can enhance their tolerance of abiotic stress [26,27,51–53]. In our study, most of the *JcSWEET* genes showed changes in transcription levels under abiotic stress treatment, including drought and salinity stress (Figure 5A). Both *JcSWEET16* and *JcSWEET17b* are members of clade IV, but they showed opposite expression patterns under drought and salinity treatments. Further studies are needed to confirm whether and how these genes function in response to abiotic stresses.

The qRT-PCR analysis showed that the expression of *JcSWEET16* was down-regulated by salinity treatment (Figure 5B). Analysis of the survival rate of the seedlings showed that *JcSWEET16* overexpression could improve salinity tolerance in *A. thaliana*, which was in line with the modified expression levels (Figure 9C). In addition, *JcSWEET16* was localized not only at the vacuolar membrane but also at the plasma membrane (Figure 7), in contrast to tonoplast-localized *AtSWEET16* [27], indicating that it may function at different stages of growth and development in physic nut. Moreover, the overexpression of *JcSWEET16* in *A. thaliana* could accelerate flowering (Figure 8B,C). Soluble sugar is not only a source of carbon and energy; it also acts as an osmotic regulator [54,55], and transgenic plants that accumulate sucrose in the leaves show more rapid flowering in species such as tomato, potato (*Solanum tuberosum*) and *A. thaliana* [56–58]. Whether the overexpression of *JcSWEET16* promotes flowering in *A. thaliana* by affecting sugar metabolism remains to be studied.

### 4. Materials and Methods

#### 4.1. Preparation of Plant Materials

Physic nut (*Jatropha curcas* L.) cultivar GZQX0401 was used in this study. After disinfecting with 1:5000 KMnO₄ solution for 30 min, the seeds were germinated in sand and grown in trays containing a 3:1 mixture of sand and soil in a greenhouse illuminated with natural sunlight in Guangzhou (113.3 °E, 23.1 °N). The trays were irrigated with 1.0 L (L) of Hoagland nutrient solution (pH 6.0) once every two days at dusk after the emergence of the first true leaf. Roots, stem cortex, and leaves were sampled at the six-leaf stage (eight weeks after germination). Male and female flowers were sampled in the summer (June 2019), and fresh seeds were sampled in the autumn (September 2020). Sampling at the different stages of seed development was in accordance with previous methods [59].

Exposure to 100 mM NaCl can induce a moderate stress response but is not acutely lethal in physic nut [60]. Indeed, in our previous study, we observed visible signs of leaf chlorosis and defoliation after the treatment [61]. Therefore, stress treatment was begun at the six-leaf stage (eight weeks after germination). For salinity treatment, the seedlings were irrigated with Hoagland solution plus 100 mM NaCl every day. For drought treatment, irrigation was withheld. Leaf samples were collected 7 days after the onset of drought stress and salinity stress. The details of salinity and drought treatment were in accordance with previous methods [40,41]. All samples were frozen immediately in liquid nitrogen and stored at −80°C before qRT-PCR analysis. Three independent biological replicates were performed for each analysis.
4.2. Sequence Database Searches and Gene Cloning

To identify physic nut SWEET genes, we searched for SWEET genes in the physic nut genome database of the Kazusa DNA Research Institute [37] and our genome database [36] using A. thaliana and rice SWEET protein sequences as queries. SWEET protein sequences from A. thaliana and rice were obtained from the A. thaliana genome database TAIR (https://www.arabidopsis.org/ (accessed on 13 February 2020)) and the rice genome annotation database (http://rice.uga.edu/ (accessed on 23 February 2020)), respectively. Sequences giving E values of less than $1 \times 10^{-10}$ were selected for further analysis, and all JcSWEET sequences were revised based on information in the expressed sequence tag (EST) database (http://www.ncbi.nlm.nih.gov/ (accessed on 9 January 2022)) and our physic nut and Jatropha integerrima EST datasets (SRA197144 and SRA197148 in GenBank). The Pfam program (http://pfam.xfam.org/ (accessed on 9 January 2022)) was used to confirm the presence of the MtN3 domain of all putative SWEET proteins. All target sequences were subsequently used to clone the full-length JcSWEET genes. The cDNA from three-week-old seedlings of physic nut was used as a template for amplifying the JcSWEET genes with the specific primers listed in Table S2. The PCR products were cloned into the pMD18-T vector (TaKaRa) and then sequenced.

4.3. Sequence Analysis and Phylogenetic Tree Construction

Transmembrane domains in JcSWEET proteins were predicted using TMHMM Server v. 2.0 [62]. Information, including accession number and MtN3/saliva (PQ-loop repeat) domain position in the JcSWEET genes, was acquired from NCBI. Multiple sequence alignments of protein sequences were performed by DNAMAN. The exon/intron structures of JcSWEET genes were analyzed using Gene Structure Display Server (GSDS, http://gsds.gao-lab.org/ (accessed on 19 January 2022)) [63] by comparing the coding sequences and the corresponding genomic sequences. Chromosome localization was performed using MapChart 2.32, based on the linkage map constructed in our previous study [36,64].

To analyze the relationships of the SWEET genes in physic nut, the full-length JcSWEET protein sequences and SWEET protein sequences from A. thaliana and rice were used to generate a phylogenetic tree. The tree was constructed using MEGA 5.0 by the neighbor-joining (NJ) method with default settings, and the results were displayed with iTOL (http://itol.embl.de/ (accessed on 8 January 2022)). Amino acid sequences of the SWEET proteins used for the analysis are listed in Table S3.

4.4. RNA Isolation and qRT-PCR

Total RNA was extracted from the samples, and the first strand cDNA was synthesized as previously described [65]. All qRT-PCR experiments were run on a LightCycler® 480 Real-Time PCR System (Roche, Basel, Switzerland). The reference gene JcActin was used as the internal control, and the expression levels were calculated using the $2^{-\Delta\Delta CT}$ method. All specific primer sequences are listed in Table S2.

4.5. Plasmid Construction and Complementation Assays in Yeast

pMD18-T-JcSWEET clones were used as templates for amplifying the coding regions of the JcSWEET genes with XhoI and BamHI cleavage sites, and the amplified fragments were cloned into the yeast expression vector pDR195. The specific primers are listed in Table S2. Then, all resulting constructs were transformed into the hexose transport-deficient yeast strain EBY.VW4000. For the yeast complementation growth assay, serial dilutions (1, 0.1, 0.01 and 0.001) of all desired transformants were spotted on synthetic deficient media containing 2% maltose (as the control), glucose, galactose, mannose, fructose or sucrose. The plates were photographed after 2–4 days of growth at 30 °C.

4.6. Subcellular Localization of JcSWEET16

The coding sequence of the JcSWEET gene (without stop codons) was amplified with the specific primer pair 5'-GGTACCATGGCTAGCTTAAGCTTC-3' and 5'-GTCGACAA
-GATCATTATCAACTTT-3′ and then cloned into the 35S:GFP vector. *A. thaliana* mesophyll protoplasts were isolated and transformed with the resulting construct as previously described [66]. For transient expression in *N. benthamiana* leaves, the resulting binary vector was transformed into *A. tumefaciens* strain GV3101 and used to infect *N. benthamiana* epidermal cells. To determine the positions of inner membranes, AtPIP2A:mCherry and AtTPK1:mCherry were used as markers for the plasma membrane and vacuolar membrane, respectively. For FM4-64 staining, tobacco leaves were incubated in 4 µM FM4-64 for 15 min before observation. Fluorescence was observed on a Leica TCS SP8 confocal laser scanning microscope.

### 4.7. Plant Transformation and Salinity Treatment of Transgenic *A. thaliana*

In order to get transgenic *A. thaliana*, the expression vector 35S:JcSWEET16:GFP was constructed and was transformed into *A. tumefaciens* plants (Col-0 ecotype) as described previously [67]. Seeds from single insertion homozygous transgenic lines were chosen for the subsequent analysis. The expression levels in the transgenic lines were determined by semi-quantitative RT-PCR with the specific primers 5′-GCACCGTCTTCCAATTCGTT-3′ and 5′-AGCCTCCATTGAGAAACAG-3′, and the reference gene *AtActin* (AT3G18780) was used as the internal control (*AtActin*-F: AGATGCCCAGAAGTCTTGTTCC, *AtActin*-R: TTTGCTCATACGGTCAGCGATA).

After surface disinfection, the seeds of transgenic and wild-type lines were incubated for 2 days in the dark at 4 °C. Plants were grown under a long-day photoperiod (16 h light/8 h dark) at 22 ± 2 °C in a growth chamber. Flowering time was scored by observing the bolting ratio, and a bolting height of 0.5 cm was taken to indicate bolting. For salinity treatment, transgenic and wild-type lines were surface-sterilized and sown on one half-strength Murashige and Skoog (MS) medium (pH 5.7, KOH) containing 1.0% (w/v) sucrose and 1.0% agar (w/v). The seeds were incubated in the dark at 4 °C for 2 days and then grown in a growth chamber with a long-day photoperiod (16 h light/8 h dark) at 22 ± 2 °C. After growth for 4 days, similarly sized seedlings were transferred to new 1/2 MS medium supplemented with 150 mM NaCl as described previously [68]. The survival rate of the seedling was counted, and photos were taken after 6 days of treatments.

### Supplementary Materials:
The following supporting information can be downloaded at: [https://www.mdpi.com/article/10.3390/ijms23105391/s1](https://www.mdpi.com/article/10.3390/ijms23105391/s1).

### Author Contributions:
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The authors declare no conflict of interest.

### References

1. Ruan, Y.-L. Sucrose Metabolism: Gateway to Diverse Carbon Use and Sugar Signaling. *Annu. Rev. Plant Biol.* 2014, 65, 33–67. [CrossRef] [PubMed]
2. Rolland, F.; Moore, B.; Sheen, J. Sugar Sensing and Signaling in Plants. *Plant Cell* 2002, 14, 185–205. [CrossRef] [PubMed]
3. Walmsley, A.R.; Barrett, M.P.; Bringaud, F.; Gould, G.W. Sugar transporters from bacteria, parasites and mammals: Structure–activity relationships. *Trends Biochem. Sci.* 1998, 23, 476–481. [CrossRef]
4. 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]
5. Patrick, J.W.; Offler, C.E. Compartmentation of transport and transfer events in developing seeds. *J. Exp. Bot.* **2001**, *52*, 551–564. [CrossRef]

6. Patrick, J.W. Phloem Unloading: Sieve Element Unloading and Post-Sieve Element Transport. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* **1997**, *48*, 191–222. [CrossRef]

7. Eom, J.-S.; Chen, L.-Q.; Sozzo, D.; Julius, B.T.; Lin, L.; 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. Jeena, G.S.; Kumar, S.; Shukla, R.K. Structure, evolution and diverse physiological roles of SWEET sugar transporters in plants. *Plant Mol. Biol.* **2019**, *100*, 351–365. [CrossRef]

9. Chen, L.-Q.; Cheung, L.S.; Feng, L.; Tanner, W.; Frommer, W.B. Transport of Sugars. *Annu. Rev. Biochem.* **2015**, *84*, 865–894. [CrossRef]

10. Chandran, D. Co-option of developmentally regulated plant SWEET transporters for pathogen nutrition and abiotic stress tolerance. *IUBMB Life* **2015**, *67*, 461–471. [CrossRef]

11. Yuan, M.; Wang, S. Rice MtN33/Saliva/SWEET Family Genes and Their Homologs in Cellular Organisms. *Mol. Plant* **2013**, *6*, 665–674. [CrossRef] [PubMed]

12. Wellmer, F.; Alves-Ferreira, M.; Dubois, A.; Riechmann, J.L.; Meyerowitz, E.M. Genome-Wide Analysis of Gene Expression during Early Arabidopsis Flower Development. *PLoS Genet.* **2006**, *2*, e117. [CrossRef] [PubMed]

13. Chen, L.-Q.; Qu, X.-Q.; Hou, B.-H.; Sozzo, 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] [PubMed]

14. Braun, D.M. Plant science. SWEET! The pathway is complete. *Science* **2012**, *335*, 173–174. [CrossRef]

15. Baker, R.F.; Leach, K.A.; Braun, D.M. *DMRT as Sugar: New Sucrose Effluxers in Plants*. *Mol. Plant* **2012**, *5*, 766–768. [CrossRef]

16. Chen, L.-Q.; Lin, I.W.; Qu, X.-Q.; Sozzo, D.; McFarlane, H.E.; Londono, A.; Samuels, A.L.; Frommer, W.B. A Cascade of Sequentially Expressed Sugar Transporters in the Seed Coat and Endosperm Provides Nutrition for the Arabidopsis Embryo. *Plant Cell* **2015**, *27*, 607–619. [CrossRef]

17. Guan, Y.-F.; Huang, X.-Y.; Zhu, J.; Gao, J.-F.; Zhang, H.-X.; Yang, Z.-N. RUPTURED POLLEN GRAIN1, a Member of the MtN33/saliva Gene Family, Is Crucial for Exine Pattern Formation and Cell Integrity of Microspores in Arabidopsis. *Plant Physiol.* **2008**, *147*, 852–863. [CrossRef] [PubMed]

18. Lin, I.W.; Sozzo, 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]

19. Kanno, Y.; Oikawa, T.; Chiba, Y.; Ishimaru, Y.; Shimizu, T.; Sano, N.; Koshiba, T.; Kamiya, Y.; Ueda, M.; Seo, M. AtSWEET13 and AtSWEET14 regulate gibberellin-mediated physiological processes. *Nat. Commun.* **2016**, *7*, 13245. [CrossRef]

20. Yang, J.; Luo, D.; Yang, B.; Frommer, W.B.; Eom, J.S. SWEET 11 and 15 as key players in seed filling in rice. *New Phytol.* **2018**, *218*, 604–615. [CrossRef]

21. Gao, Y.; Zhang, C.; Han, X.; Wang, Z.Y.; Ma, L.; Yuan, P.; Wu, J.N.; Zhu, X.F.; Liu, J.M.; Li, L.P.; et al. Inhibition of OsSWEET11 function in mesophyll cells improves resistance of rice to sheath blight disease. *Mol. Plant Pathol.* **2018**, *19*, 2149–2161. [CrossRef] [PubMed]

22. Ma, L.; Zhang, D.; Miao, Q.; Yang, J.; Xuan, Y.; Hu, Y. Essential Role of Sugar Transporter OsSWEET11 During the Early Stage of Rice Grain Filling. *Plant Cell Physiol.* **2017**, *58*, 863–873. [CrossRef] [PubMed]

23. Wang, S.; Yokosho, K.; Guo, R.; Whelan, J.; Ruan, Y.-L.; Ma, J.F.; Shou, H. The Soybean Sugar Transporter GmSWEET15 Mediates Sucrose Export from Endosperm to Early Embryo. *Plant Physiol.* **2019**, *180*, 2133–2141. [CrossRef] [PubMed]

24. 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-sequencing analysis. *BMC Genom.* **2015**, *16*, 520. [CrossRef] [PubMed]

25. Bezrutczyk, M.; Hartwig, T.; Horschman, M.; Char, S.N.; Yang, J.; Yang, B.; Frommer, W.B.; Sozzo, D. Impaired phloem loading in *zmSwee13ab, b* sucrose transporter triple knock-out mutants in Zea mays. *New Phytol.* **2018**, *218*, 594–603. [CrossRef]

26. Valifard, M.; Le Hir, R.; Müller, J.; Scheuring, D.; Neuhaus, H.E.; Pommerenig, B. Vacuolar fructose transporter SWEET17 is critical for root development and drought tolerance. *Plant Physiol.* **2021**, *187*, 2716–2730. [CrossRef]

27. Klemens, P.A.; Patzke, K.; Deitmer, J.; Spinner, L.; Le Hir, R.; Bellini, C.; Bedu, M.; Chardon, F.; Krapp, A.; Neuhaus, H.E. Overexpression of the Vacular Sugar Carrier AtSWEET16 Modifies Germination, Growth, and Stress Tolerance in Arabidopsis. *Plant Physiol.* **2013**, *163*, 1338–1352. [CrossRef]

28. Seo, P.J.; Park, J.-M.; Kang, S.K.; Kim, S.-G.; Park, C.-M. An Arabidopsis senescence-associated protein SAG29 regulates cell viability under high salinity. *Planta* **2011**, *233*, 189–200. [CrossRef]

29. Le Hir, R.; Spinner, L.; Klemens, P.A.W.; Chakraborti, D.; de Marco, F.; Vilaine, E.; Wolff, N.; Lemoine, R.; Porcheron, B.; Géry, C.; et al. Disruption of the Sugar Transporters AtSWEET11 and AtSWEET12 Affects Vascular Development and Freezing Tolerance in Arabidopsis. *Mol. Plant* **2015**, *8*, 1687–1690. [CrossRef]

30. Liu, X.; Zhang, Y.; Yang, C.; Tian, Z.; Li, J. AtSWEET4, a hexose facilitator, mediates sugar transport to axial sinks and affects plant development. *Sci. Rep.* **2016**, *6*, 24563. [CrossRef] [PubMed]

31. Mathan, J.; Singh, A.; Ranjan, A. Sucrose transport in response to drought and salt stress involves ABA-mediated induction of OsSWEET13 and OsSWEET15 in rice. *Physiol. Plant.* **2020**, *171*, 620–637. [CrossRef] [PubMed]
32. Hu, L.; Zhang, F.; Song, S.; Yu, X.; Ren, Y.; Zhao, X.; Liu, H.; Liu, G.; Wang, Y.; He, H. CsSWEET2, a Hexose Transporter from Cucumber (*Cucumis sativus* L.), Affects Sugar Metabolism and Improves Cold Tolerance in *Arabidopsis*. *Int. J. Mol. Sci.* 2022, 23, 3886. [CrossRef] [PubMed]

33. Xuan, C.; Lan, G.; Si, F.; Zeng, Z.; Wang, C.; Yadav, V.; Wei, C.; Zhang, X. Systematic Genome-Wide Study and Expression Analysis of SWEET Gene Family: Sugar Transporter Family Contributes to Biotic and Abiotic Stimuli in Watermelon. *Int. J. Mol. Sci.* 2021, 22, 8407. [CrossRef] [PubMed]

34. Wang, L.; Yao, L.; Hao, X.; Li, N.; Qian, W.; Yue, C.; Ding, C.; Zeng, J.; Yang, Y.; Wang, X. Tea plant SWEET transporters: Expression profiling, sugar transport, and the involvement of CsSWEET16 in modifying cold tolerance in *Arabidopsis*. *Plant Mol. Biol.* 2018, 96, 577–592. [CrossRef] [PubMed]

35. Zhou, A.; Ma, H.; Feng, S.; Gong, S.; Wang, J. *DdSWEET17*, a Tonoplast-Localized Sugar Transporter from *Dianthus spiculifolius*, Affects Sugar Metabolism and Confers Multiple Stress Tolerance in *Arabidopsis*. *Int. J. Mol. Sci.* 2022, 23, 3886. [CrossRef] [PubMed]

36. Soltis, D.E.; Visger, C.J.; Soltis, P.S. The polyploidy revolution then...and now: Stebbins revisited. *Am. J. Bot.* 2021, 98, 238–250. [CrossRef] [PubMed]

37. Durand, M.; Mainson, D.; Porcheron, B.; Maurousset, L.; Lemoine, R.; La Camera, S.; Ferrand, M.; Lacombe, B.; et al. Carbohydrate Transport Mediates Cold Tolerance of *Arabidopsis*. *Curr. Biol.* 2021, 31, 697–702. [CrossRef] [PubMed]

38. Hu, L.-P.; Zhang, F.; Song, S.-H.; Tang, X.-W.; Xu, H.; Liu, G.-M.; Wang, Y.; He, H.-J. Genome-wide identification, characterization, and phylogenetic analysis of the SWEET gene family in *Arabidopsis thaliana*. *BMC Gen. Genomics* 2017, 18, 76. [CrossRef] [PubMed]

39. Ko, H.-Y.; Ho, L.-H.; Neuhaus, H.E.; Guo, W.-J. Transporter SlSWEET15 unloads sucrose from phloem and seed coat for fruit and seed development in *Arabidopsis*. *Agron. Sci. China* 2021, 20, 117–126. [CrossRef] [PubMed]

40. Zhang, L.; Zhang, C.; Wu, P.; Chen, Y.; Li, M.; Jiang, H.; Wu, G. Global Analysis of Gene Expression Profiles in Physic Nut (*Jatropha curcas L.*). *PLoS ONE* 2014, 9, 96798. [CrossRef] [PubMed]

41. Zhang, L.; Zhang, C.; Wu, P.; Chen, Y.; Li, M.; Jiang, H.; Wu, G. Global Analysis of Gene Expression Profiles in Physic Nut (*Jatropha curcas L.*) Seedlings Exposed to Salt Stress. *PLoS ONE* 2014, 9, 96798. [CrossRef] [PubMed]

42. Wieczorke, R.; Krampe, S.; Weierstall, T.; Freidel, K.; Hollenberg, C.P.; Boles, E. Concurrent knock-out of at least 20 transporter genes is required to block uptake of hexoses in *Saccharomyces cerevisiae*. *FEBS Lett.* 1999, 464, 123–128. [CrossRef]

43. Ko, H.-Y.; Ho, L.-H.; Neuhaus, H.E.; Guo, W.-J. Transporter SlSWEET15 unloads sucrose from phloem and seed coat for fruit and seed development in *Arabidopsis*. *Plant Physiol.* 2021, 187, 2230–2245. [CrossRef] [PubMed]

44. Hu, L.-P.; Zhang, F.; Song, S.-H.; Tang, X.-W.; Xu, H.; Liu, G.-M.; Wang, Y.; He, H.-J. Genome-wide identification, characterization, and expression analysis of the SWEET gene family in cucumber. *J. Integr. Agric.* 2017, 16, 1486–1501. [CrossRef]

45. Gao, Y.; Wang, Z.Y.; Kumar, V.; Xu, X.F.; Yuan, D.P.; Zhu, X.F.; Li, T.Y.; Jia, B.; Xuan, Y.H. Genome-wide identification of the SWEET gene family in wheat. *Genes* 2018, 622, 284–292. [CrossRef]

46. Solitis, D.E.; Visger, C.J.; Solitis, P.S. The polyploidy revolution then...and now: Stebbins revisited. *Am. J. Bot.* 2014, 101, 1057–1078. [CrossRef]

47. Severin, A.J.; Cannon, S.B.; Graham, M.M.; Grant, D.; Shoemaker, R.C. Changes in Twelve Homoeologous Genomic Regions in Soybean following Three Rounds of Polyploidy. *Plant Cell* 2011, 23, 3129–3136. [CrossRef]

48. Vision, T.J.; Brown, D.G.; Tanksley, S.D. The Origins of Genomic Duplications in *Arabidopsis*. *Science* 2000, 290, 2114–2117. [CrossRef]

49. Long, M.; Rosenberg, C.; Gilbert, W. Intron phase correlations and the evolution of the intron/exon structure of genes. *Proc. Natl. Acad. Sci. USA* 1995, 92, 12495–12499. [CrossRef]

50. Chardon, F.; Bedu, M.; Calenge, F.; Klemens, P.A.; Spinner, L.; Clement, G.; Chietera, G.; Léran, S.; Ferrand, M.; Lacombe, B.; et al. Leaf Fructose Content Is Controlled by the Vacuolar Transporter SWEET17 in *Arabidopsis*. *BMC Plant Biol.* 2014, 14, 284. [CrossRef] [PubMed]

51. Yao, L.; Ding, C.; Hao, X.; Zeng, J.; Yang, Y.; Wang, X.; Wang, L. CsSWEET1a and CsSWEET17 Mediate Growth and Freezing Tolerance by Promoting Sugar Transport across the Plasma Membrane. *Plant Cell Physiol.* 2020, 61, 1669–1682. [CrossRef] [PubMed]

52. Lu, J.; Sun, M.-H.; Ma, Q.-J.; Kang, H.; Liu, Y.-J.; Hao, Y.-J.; You, C.-X. MdSWEET17, a sugar transporter in apple, enhances drought tolerance in tomato. *J. Integr. Agric.* 2019, 18, 2041–2051. [CrossRef]

53. Zhou, A.; Ma, H.; Feng, S.; Gong, S.; Wang, J. DeSWEET17, a Tonoplast-Localized Sugar Transporter from Dianthus scipulifolius, Affects Sugar Metabolism and Confers Multiple Stress Tolerance in *Arabidopsis*. *Int. J. Mol. Sci.* 2018, 19, 1564. [CrossRef] [PubMed]

54. Durand, M.; Mainsion, D.; Porcheron, B.; Maurousset, L.; Lemoine, R.; Pourtau, N. Carbon source–sink relationship in *Arabidopsis thaliana*: The role of sucrose transporters. *Planta* 2018, 247, 587–611. [CrossRef] [PubMed]

55. Lemoine, R.; La Camera, S.; Atanassova, R.; Dédalessché, F.; Allario, T.; Pourtau, N.; Bonnemain, J.L.; Laloi, M.; Coutos-Thévenot, P.; Maurousset, L.; et al. Source-to-sink transport of sugar and regulation by environmental factors. *Front. Plant Sci.* 2013, 4, 272. [CrossRef]

56. Corbesier, L.; Lejeune, P.; Bernier, G. The role of carbohydrates in the induction of flowering in *Arabidopsis thaliana*: Comparison between the wild type and a starchless mutant. *Plant Sci.* 1998, 136, 195–206. [CrossRef] [PubMed]

57. Micallef, B.J.; Haskins, K.A.; Vanderveer, P.J.; Roh, K.-S.; Shevmaker, C.K.; Sharkey, T.D. Altered photosynthesis, flowering, and fruiting in transgenic tomato plants that have an increased capacity for sucrose synthesis. *Plant Sci.* 1995, 136, 327–334. [CrossRef]
58. Müller-Röber, B.; Sonnewald, U.; Willmitzer, L. Inhibition of the ADP-glucose pyrophosphorylase in transgenic potatoes leads to sugar-storing tubers and influences tuber formation and expression of tuber storage protein genes. *EMBO J.* 1992, 11, 1229–1238. [CrossRef]

59. Jiang, H.; Wu, P.; Zhang, S.; Song, C.; Chen, Y.; Li, M.; Jia, Y.; Fang, X.; Chen, F.; Wu, G. Global Analysis of Gene Expression Profiles in Developing Physic Nut (*Jatropha curcas* L.) Seeds. *PLoS ONE* 2012, 7, e36522. [CrossRef]

60. da Silva, E.N.; Gomes Silveira, J.A.; Rodrigues Fernandes, C.R.; Batista Dutra, A.T.; de Aragao, R.M. Ion uptake and growth of physic nut under different salinity levels. *Rev. Cienc. Agron.* 2009, 40, 240–246. [CrossRef]

61. Zhao, Q.; Zhang, L.; Zhu, S.; Zhang, S.; Wu, P.; Chen, Y.; Jiang, H.; Wu, G.; Li, M. Effects of several abiotic stresses on photosynthetic rate and other physiological indexes in *Jatropha curcas* L. seedlings. *J. Trop. Subtrop. Bot.* 2012, 20, 432–438. [CrossRef]

62. Krogh, A.; Larsson, B.; von Heijne, G.; Sonnhammer, E.L. Predicting transmembrane protein topology with a hidden markov model: Application to complete genomes. *J. Mol. Biol.* 2001, 305, 567–580. [CrossRef] [PubMed]

63. Guo, A.-Y.; Zhu, Q.H.; Chen, X.; Luo, J.C. GSDS: A gene structure display server. *Yi Chuan Hered.* 2007, 29, 1023–1026. [CrossRef]

64. Voorrips, R.E. MapChart: Software for the graphical presentation of linkage maps and QTLs. *J. Hered.* 2002, 93, 77–78. [CrossRef] [PubMed]

65. Xiong, W.; Xu, X.; Zhang, L.; Wu, P.; Chen, Y.; Li, M.; Jiang, H.; Wu, G. Genome-wide analysis of the WRKY gene family in physic nut (*Jatropha curcas* L.). *Gene* 2013, 524, 124–132. [CrossRef] [PubMed]

66. Yoo, S.-D.; Cho, Y.-H.; Sheen, J. Arabidopsis mesophyll protoplasts: A versatile cell system for transient gene expression analysis. *Nat. Protoc.* 2007, 2, 1565–1572. [CrossRef]

67. Clough, S.J.; Bent, A.F. Floral dip: A simplified method for Agrobacterium-mediated transformation of *Arabidopsis thaliana*. *Plant J.* 1998, 16, 735–743. [CrossRef]

68. Huang, Y.; Zhao, H.; Gao, F.; Yao, P.; Deng, R.; Li, C.; Chen, H.; Wu, Q. A R2R3-MYB transcription factor gene, FtMYB13, from Tartary buckwheat improves salt/drought tolerance in *Arabidopsis*. *Plant Physiol. Biochem.* 2018, 132, 238–248. [CrossRef]