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When SWEETs Turn Tweens: Updates and Perspectives

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Abstract

Sugar translocation between cells and between subcellular compartments in plants requires either plasmodesmata or a diverse array of sugar transporters. Interactions between plants and associated microorganisms also depend on sugar transporters. The sugars will eventually be exported transporter (SWEET) family is made up of conserved and essential transporters involved in many critical biological processes. The functional significance and small size of these proteins have motivated crystallographers to successfully capture several structures of SWEETs and their bacterial homologs in different conformations. These studies together with molecular dynamics simulations have provided unprecedented insights into sugar transport mechanisms in general and into substrate recognition of glucose and sucrose in particular. This review summarizes our current understanding of the SWEET family, from the atomic to the whole-plant level. We cover methods used for their characterization, theories about their evolutionary origins, biochemical properties, physiological functions, and regulation. We also include perspectives on the future work needed to translate basic research into higher crop yields.

Contents

Supplemental Material >

Uniporter: a carrier that allows the translocation of substrates across a membrane along the concentration gradients

Proton/sugar symporter: uses the electrochemical proton gradient to translocate sugars even against their concentration gradients, moving both in the same direction

Proton/sugar antiporter: uses the electrochemical proton gradient to translocate sugars even against their concentration gradients, moving sugars and protons in opposite directions

1. INTRODUCTION

It has been almost twelve years since sugars will eventually be exported transporters (SWEETs) were characterized (20), and progress in our understanding of these proteins has been rapid. Members of this family have been shown to function as uniporters (19, 20), facilitating the translocation of sugars across membranes along the substrate concentration gradient. This distinguishes them from other plant sugar carriers such as the sugar transport proteins (STPs), sucrose transporters/sucrose carriers (SUTs/SUCs), and tonoplast sugar transporters (TSTs), which transport sugars with the help of the electrochemical proton gradient in the same or opposite direction of sugar movement, as proton/sugar symporters or antiporters (19). Compared with the proton-dependent transporters, SWEETs are small transporters, typically consisting of seven transmembrane helixes (TMHs) (20, 37, 88). Their prokaryotic homologs, the SemiSWEETs, are even smaller and composed of only three TMHs—two SemiSWEETs are necessary to form a translocation pore (40, 54, 104). So far, no biological functions have been reported for SemiSWEETs.

Members of the SWEET family have been found in every sequenced plant genome. While SWEETs have been associated mainly with biological processes requiring sugar efflux in plants, such as nectar secretion (61), pollen nutrition (34, 55, 86), and seed filling (21, 82, 98, 99), experiments in heterogeneous systems (e.g., *Xenopus* oocytes and human cells) have shown that SWEETs can transport sugars bidirectionally. Therefore, potential roles in sugar uptake deserve more attention (**Supplemental Table 1**).

SWEETs have been cloned, crystallized, modeled, mutated, engineered, and genetically edited; yet, there is much that we still do not know about them. Thus, this is a good time to examine what we have learned about this family and to evaluate the avenues of research that are most needed going forward. To avoid redundancy with excellent reviews that have summarized the physiology of SWEETs in *Arabidopsis* (11, 14, 18, 19, 30, 46), here we focus instead on summarizing their molecular substrate recognition mechanisms and bridging research between model and crop plants.

2. EVOLUTION OF SWEETS AND SEMISWEETS

From an evolutionary perspective, the size of the SWEET family varies greatly across species. For example, in a study that characterized 3,249 7-TMH SWEETs and 3-TMH SemiSWEETs (43), 44.4% of these proteins were found in green plants, 24.6% in bacteria, 12.9% in Oomycota, 12.7% in Metazoa, and 1.2% in green algae, and less than 1% of these proteins were found in archaea, fungi, Protista, and other algae. Similarly, in a study that included 228 SWEETs and 58 SemiSWEETs (40), SWEETs were found in all eukaryotic supergroups and SemiSWEETs in archaea, bacteria, and to a much lesser extent in eukaryotes. Of course, this information may change with the release of newly annotated genomes or resequencing.

Two hypotheses for the origin of SWEETs have been proposed. Hu et al. (40) argued that eukaryotic SWEETs originated from the fusion of a SemiSWEET from archaea with another SemiSWEET from bacteria, accompanied by the appearance of an inversion helix (TMH4). This hypothesis was backed by the observation that the amino acid sequences of many bacterial MtN3 domain proteins preferentially aligned with the first 3 TMHs, while all of the archaeal SemiSWEETs aligned with the last 3 TMHs of SWEETs (40). On the other hand, Xuan et al. (106) proposed that SWEETs originated from gene duplication and fusion of SemiSWEETs. This hypothesis was backed by experimental evidence that the dimerization of SemiSWEETs can create a functional translocation pore (79, 104, 106) and then further supported by the discovery of SWEETs with 6 and 7 TMHs in bacteria and more than 100 SemiSWEETs with 3 or 4 TMHs in plants (43). Additionally, SuperSWEETs with more than 18 TMHs have been reported in oomycetes (43). It remains unclear what the roles of these additional TMHs are, but some possibilities include helping SuperSWEETs fold, changing their substrate selectivity, or facilitating their interaction with other proteins. Taken together, gene duplication and fusion were the major driving forces during the evolution of the SWEET family, which may lead to subfunctionalization or neofunctionalization in higher organisms (4, 58).

The number of *SWEET* genes in different species varies from 6 in *Physcomitrella patens*, a nonvascular moss species, to 55 in *Gossypium hirsutum*, a tetraploid dicot, and 108 in *Triticum aestivum*, a hexaploid monocot crop (14), indicating a significant expansion of the family in both monocot and dicot lineages (30, 58). The family can be divided into four phylogenetic clades based on protein sequences. The phylogenetic analysis by Li et al. (58) suggested that clade II is evolutionarily close to algae and thus considered the most ancient, with clade I and the common ancestor of clades III and IV diverging next. However, Zhang et al. (112) observed a discrepancy in the placement of clade II and clade I when a few more nonvascular species were included, intimating that more plant genomes are necessary to resolve the evolutionary history of the family. Interestingly, clade III has the highest number of genes (based on an analysis of 31 plant species) and no clade III SWEETs were identified after surveying 4 bryophyte species: 1 species each from hornworts and liverworts and 2 from mosses (58, 112). Therefore, the evidence suggests that clade III SWEETs did not appear until the speciation of vascular plants and that they expanded greatly afterward. Coincidently, cell wall invertases (CWINs), rather than vacuolar invertases (VINs) or cytosol invertases (CINs), were also not found in nonvascular species, and CWINs likely coevolved with the vascular development of higher plants (91). Thus, the evolutionary signatures of clade III SWEETs and CWINs in plants correspond well to their physiological functions in phloem loading and unloading.

Interestingly, positive selection sites were identified in only a few SWEETs from 31 surveyed species (58). Some of these SWEETs belong to clade III, including the soybean (*Glycine max*) GmSWEET10a that underwent a strong selection and GmSWEET10b that underwent an ongoing selection for seed size and oil content during domestication (58, 98). The identification of

positive selection sites may help predict which functions of SWEETs from clade III are under selection pressure. Overall, analysis of the evolutionary dynamics can trace the fate of SWEET genes and predict their potential functions.

Förster Resonance Energy Transfer (FRET) sensors: engineered probes composed of a substrate-binding protein and a pair of FRET donor and acceptor fluorescent proteins

Single-fluorophore biosensor: an engineered probe composed of a substrate-binding protein and a conformation-sensitive fluorescence protein

3. STRUCTURE AND SUBSTRATE SPECIFICITY

3.1. Methods Used for SWEET Characterization

Plasma membrane–localized SWEETs have been characterized primarily using mammalian cells, *Xenopus* oocytes, and yeast cells. The original system used was a Förster Resonance Energy Transfer (FRET)-sensor-based screen in HEK293T cells (which have very low endogenous sugar transport activity). Due to the high detection sensitivity with the relatively high affinities of glucose sensors such as FLIPglu600μΔ13V and FLII12Pglu700μδ6 or sucrose sensors such as FLIPsuc90μΔ1V (later renamed FLIPsuc90μδ3A) and FLIPsuc-2-10μ (**Table 1**), this system continues to serve as a powerful tool to detect SWEET transport activity (10, 22, 27, 61, 98). However, it is not a convenient system for measuring transport kinetics compared to *Xenopus* oocytes or yeast systems (**Table 1**). The functional characterization of SWEETs in oocytes is conducted using radiolabeled sugars (20, 22, 98), which is particularly useful in measuring sugar efflux compared with other systems. Finally, growth assays in yeast are the most accessible and popular methods of characterization. EBY4000 (*hxt1* through *-17D::loxP gal2D::loxP stl1D::loxP agt1D::loxP ydl247wD::loxP yjr160cD::loxP*) (102), the most commonly used yeast strain, lacks 17 hexose transporters and can be used to test the transport of a wide range of hexoses, such as glucose, fructose, galactose, and mannose. Nonetheless, yeast is not ideal for detecting sucrose transport, and FRET sensors or radiotracer experiments in oocytes must be used instead. Overall, yeast growth assays are not very sensitive, although yeast radiolabeled sugar uptake is much better (**Table 1**). For instance, the yeast growth assay failed to detect glucose accumulation mediated by the maize transporter ZmSWEET1b, but both yeast radiotracer uptake assays and the glucose sensor FLIPglu600μΔ13V detected it (93).

Beyond these three heterologous systems, other methods have been used, too, including a liposome isotope uptake assay (88), cyanobacterial sugar secretion assay (106), single-molecule FRET in insect cells (37), and SWEET-derived single-fluorophore biosensor (69). Plant cells have been used for functional studies, too. For example, the potato (*Solanum tuberosum*) transporter StSWEET11 was assayed with an interactor in protoplasts isolated from tobacco (2), while AtSWEET17 transport activity was detected using vacuoles isolated from *Arabidopsis* mesophyll cells (35), in addition to *Xenopus* oocytes (16). It is not unusual for multiple systems to be employed together, and, given the limitations of each system, care should be taken when interpreting results obtained from a single system (**Supplemental Table 1**).

Supplemental Material >

For SWEETs not localized in the plasma membrane, scientists may make artificial liposomes or isolate organelles (mostly vacuoles) for transport studies (**Table 1**). *Xenopus* oocytes and yeast are also options if the proteins can be modified and targeted to the plasma membrane. Another option would be to target sugar FRET sensors to the lumen of organelles. Ultimately, determining which system needs to be used depends on the SWEET of interest.

3.2. Atomic Structures and Transport Mechanisms

The structures of four SemiSWEETs and two plant SWEETs have been resolved so far (37, 52, 54, 88, 94, 104). SemiSWEETs form dimers, and each protomer is composed of three TMHs that wrap into a 1-3-2 three-helix bundle (THB) conformation (104). The crystal structures of SemiSWEETs have been obtained in the inward-open state (EcSemiSWEET from *Escherichia*

Table 1 Comparison of different exogenous systems for SWEET characterization

System	Complexity	Sensitivity	Limitations	Main equipment	Representative references
Yeast growth assay	Simple procedure	Low	Only applicable to plasma membrane–localized transporters; mutant strains lack necessary endogenous transporters	No special needs	20, 88, 93
Radiotracer in yeast	Medium; ideal for influx	Medium	Only applicable to plasma membrane–localized transporters; mutant strains lack necessary endogenous transporters; restricted to commercially available radiolabeled substrates	Filtration system; liquid scintillation counter	20, 93
Radiotracer in oocytes	Medium to high; ideal for influx and efflux	High	Only applicable to plasma membrane–localized transporters; restricted to commercially available radiolabeled substrates	Microinjector; liquid scintillation counter	16, 20, 22
Förster Resonance Energy Transfer (FRET) sensors	Medium to high; ideal for influx and efflux	High	Limited by the availability of sugar FRET sensors; depends on the subcellular localization of a sensor	Fluorescence microscope; cell perfusion system	20, 22, 37, 61
Radiotracer in liposomes	Medium to high; not limited by protein localization	High	Restricted to commercially available radiolabeled substrates; requires the purification and reconstitution of the transporter	Ultracentrifuge; liquid scintillation counter	54, 88

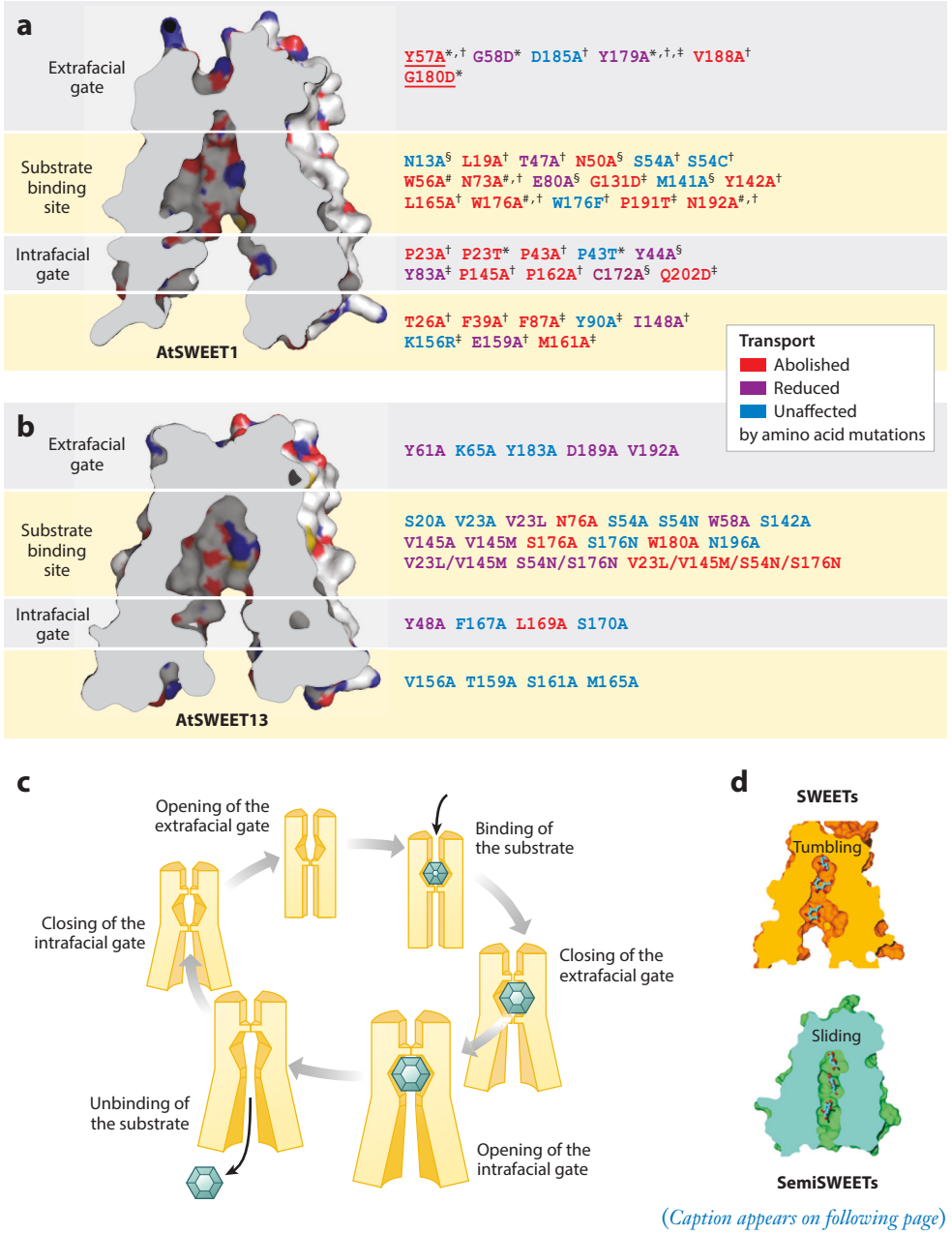
coli), occluded state (TySemiSWEET from *Thermodesulfovibrio yellowstonii* and LbSemiSWEET from *Leptospira biflexa*), and outward-open state (VsSemiSWEET from *Vibrio* sp. N418 and EcSemiSWEET). The existence of these different states has established that their transport cycles follow the alternating access model, where access to the substrate-binding site alternates between the two sides of the membrane (52). Similar to their bacterial counterparts, the first three TMHs of eukaryotic SWEETs form one THB while the last three form a second one (37, 88). The rice OsSWEET2b was crystalized in the inward (cytosolic)-open state, forming trimeric assembly (88), while the *Arabidopsis* AtSWEET13 was crystalized in the inward-open state with a 2′-deoxycytidine 5′-monophosphate that contains the hydroxyl moieties found in sugars bound to the putative sugar-binding sites (37). The topology of the THBs in SWEETs is similar to that in SemiSWEETs. Therefore, it was assumed that SWEETs also follow an alternating access mechanism.

Crystal structures of SWEETs and SemiSWEETs have provided vital insights into the transport mechanism, such as the location of the substrate-binding site and the identity of residues critical for transport activity. Two THBs are needed to form the substrate-binding pocket, which is located closer to the extrafacial side of the membrane. Within it, conserved Asn (N73 and N192 in AtSWEET1, N77 and N197 in OsSWEET2b, and N76 in AtSWEET13) and Trp/Phe (W176 in AtSWEET1, F181 in OsSWEET2b, and W180 in AtSWEET13) residues are essential for substrate recognition (37, 88) (**Figure 1*a*,*b***). The substrate-biding site is closed to the extrafacial side by a gate with conserved Asp and Tyr residues, and in SWEETs, the intrafacial gate contains several conserved Pro residues (88). The off-center position of the substrate-binding site raises questions about the number and functions of the additional THBs in SuperSWEETs, which may not be able to form functional substrate-binding sites. Resolving the structure of SuperSWEETs with cryo-electron microscopy could help clarify this issue (15).

The structures of SemiSWEETs have allowed researchers to formulate molecular dynamics simulations to understand the process of substrate translocation. For example, Latorraca et al. (52) performed long-timescale unbiased molecular simulations of LbSemiSWEET starting from the glucose-bound outward-open crystal structure. This study identified more gating residues on the extrafacial and intrafacial sides of the transporter that undergo a coordinated motion to keep the transporter closed at one of the ends to satisfy the requirements of the alternating access model (**Figure 1*c***). But the key observation of this study was that the conformational change from an outward-open state to an inward-open state remains the same with and without the substrate. In other words, the glucose molecule takes a free ride across the membrane as the transporter undergoes the conformational change between outward-open and inward-open states. Latorraca et al. (52) also performed simulations from the docked pose of glucose in the inward-open state of the EcSemiSWEET and observed the glucose release to the cytoplasmic side, helping identify critical residues that facilitate this process.

Molecular dynamics simulations have also been deployed to recreate the complete transport cycle of the eukaryotic OsSWEET2b (79). The key result from the study by Selvam et al. (79) was that glucose induces similar conformational changes as the transporter without glucose. In other words, SWEETs also follow the free-ride mechanism observed for LbSemiSWEET. Intriguingly, glucose molecules were found to actively remodel the free-energy landscape by lowering the energy barrier between the key conformational states of OsSWEET2b to facilitate their transport (79). However, while the conformational changes may be similar, the interaction of glucose with the residues in the translocation pore is markedly different between OsSWEET2b and the bacterial *Vibrio* sp. VsSemiSWEET (23). The structural origin of these differences is the presence of the inversion linker TMH4 in SWEETs. SemiSWEETs adopt a symmetric structure with a narrow translocation pore due to the homodimerization of the THBs, whereas SWEETs form an

asymmetric structure with a broader pore size. This structural difference leads to the sliding of glucose in the SemiSWEET pore, whereas glucose tumbles while interacting with pore residues in SWEETs (23) (**Figure 1*d***). This result has important implications for the substrate selectivity in SWEETs and SemiSWEETs, given that the pore size of the tunnel determines the size of the substrate that can be translocated. As a consequence, the physical mechanism of transport and the substrate preferences vary between SWEETs and SemiSWEETs.



(*Caption appears on following page*)

Figure 1 (*Figure appears on preceding page*)

Transport mechanisms in SWEETs and SemiSWEETs. (*a*) Homology model of AtSWEET1 generated from PDB ID 5CTG (the structure of OsSWEET2b) using SWISS-MODEL (100). Image was created with PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC. Glucose transport was abolished (*red*), reduced (*purple*), or unaffected (*blue*) by different amino acid mutations. Underlined mutations resulted in protein mislocalization. Superscripts in this panel correspond to reference citations as follows: *106; †88; ‡43; §79; #104. (*b*) Image of PDB ID 5XPD for the structure of AtSWEET13 (37) was created with PyMOL Molecular Graphics System, Version 2.0 Schrödinger, LLC. Sucrose transport was abolished (*red*), reduced (*purple*), or unaffected (*blue*) by different amino acid mutations. All mutations were reported by Han et al. (37). (*c*) Crystallographic studies and molecular dynamics simulations support an alternating access model in SemiSWEETs that includes opening and closing of extra- and intrafacial gates accompanied with changes in the conformation of individual protomers. Data from Reference 52. (*d*) Two different mechanisms of glucose transport are suggested by molecular dynamic simulations: the tumbling for SWEET transport and sliding for SemiSWEET transport. Panel *d* adapted with permission from Reference 23. Abbreviations: PDB ID, Protein Data Bank identification; SWEET, sugars will eventually be exported transporter.

SWEETs form functional homo- and heterooligomers. OsSWEET2b was captured as a tightly packed homotrimer in its crystal structure (88). Moreover, the split ubiquitin two-hybrid system in yeast, split GFP assays in plants, and single-molecule FRET experiments in Sf9 insect cells indicate that *Arabidopsis* SWEETs can both homo- and heterooligomerize (37, 106). Dimers seem to be favored over higher-order complexes of AtSWEET13 in the Sf9 expression system, and, interestingly, cytosolic C-terminal regions also dimerize in sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE) (37). As a consequence of these interactions, SWEETs can exhibit dominant-negative repression whereby nonfunctional proteins (usually mutated at residues in the intra- or extrafacial gates) can prevent the substrate transport of coexpressed wild-type proteins (37, 88, 106).

AtSWEET1, the founding member of the family (20), through homology-based prediction from crystal structures of SemiSWEETs and OsSWEET2b and molecular dynamics simulations, has served as a model to evaluate the functions of critical residues for glucose transport (79, 88, 104). In addition, many other residues have been selected and tested based on sequence alignments (43, 106). The effect of point mutations on the glucose transport activity of AtSWEET1 is shown in **Figure 1*a***, as well as those for AtSWEET13 in **Figure 1*b***. It is worth noting that some residues in the transport path in AtSWEET1 are different from those in AtSWEET13, which may be associated with their preferred substrates, as discussed below (37).

3.3. Substrate Selectivity and Transport Affinity

Supplemental Material >

Generally, clade III SWEETs prefer to transport sucrose, while clades I, II, and IV favor hexoses (30) (**Supplemental Table 1**). Sequence comparison and the crystal structure of AtSWEET13, which can transport both glucose and sucrose (22, 37), revealed essential residues that, when mutated, shift substrate selectivity between sucrose and glucose. Specifically, Val23, Ser54, Val145, and Ser176 (numbered according to AtSWEET13) in disaccharide transporters correspond to larger Leu, Asn, Met, and Asn residues, respectively, in monosaccharide transporters, making the substrate-binding pocket of the latter too small to accommodate sucrose (37) (**Figure 1*a*,*b***).

When tested for uptake in yeast and *Xenopus* oocytes, the affinity constants of SWEETs have been found to range from five to a few hundred millimoles, depending on the substrate (20, 22, 38, 95) (**Supplemental Table 1**). Moreover, the ratio between their influx and efflux affinity constants is close to one. That is, SWEETs have been proposed to be symmetric transporters (69), unlike other well-characterized sugar carriers like the human GLUT1 (13).

Interestingly, the clade III AtSWEET13 and AtSWEET14 have also been shown to transport the plant hormone gibberellic acid (GA), whose structure (a five- plus a six-carbon ring) is similar to sucrose. The affinity constants for both transporters towards GA_3, the major bioactive GA produced in the fungus *Gibberella fujikuroi*, were found to be in the hundreds-of-micromoles range (45). This is a startling fact, considering that the affinity of clade III SWEETs for sucrose is tens of millimoles. Which residues in the transporters interact with GA and what recognition mechanism explains the high affinity are unknown, but the interaction seems physiologically relevant, as the *atsweet13 atsweet14* double mutant has a defect in anther dehiscence (45) and higher levels of GA in the elongation zone of roots compared with wild-type plants (72).

4. PHYSIOLOGICAL FUNCTIONS

SWEETs have been implicated in many cellular efflux steps of sugar allocation. The current evidence from *Arabidopsis*, where functional characterization of different family members is most complete (**Figure 2**), suggests that clade III SWEETs mediate phloem loading and unloading, while clade I and II SWEETs complete the final distribution of sucrose, glucose, and fructose within sink tissues (21, 22). Meanwhile, clade IV members are responsible for vacuole sugar storage (16). While most of these functions are conserved across different species, many exceptions exist, and analysis is complicated by the fact that closely related members are usually coexpressed and genetic knockout of any SWEET often results in compensatory upregulation of other family

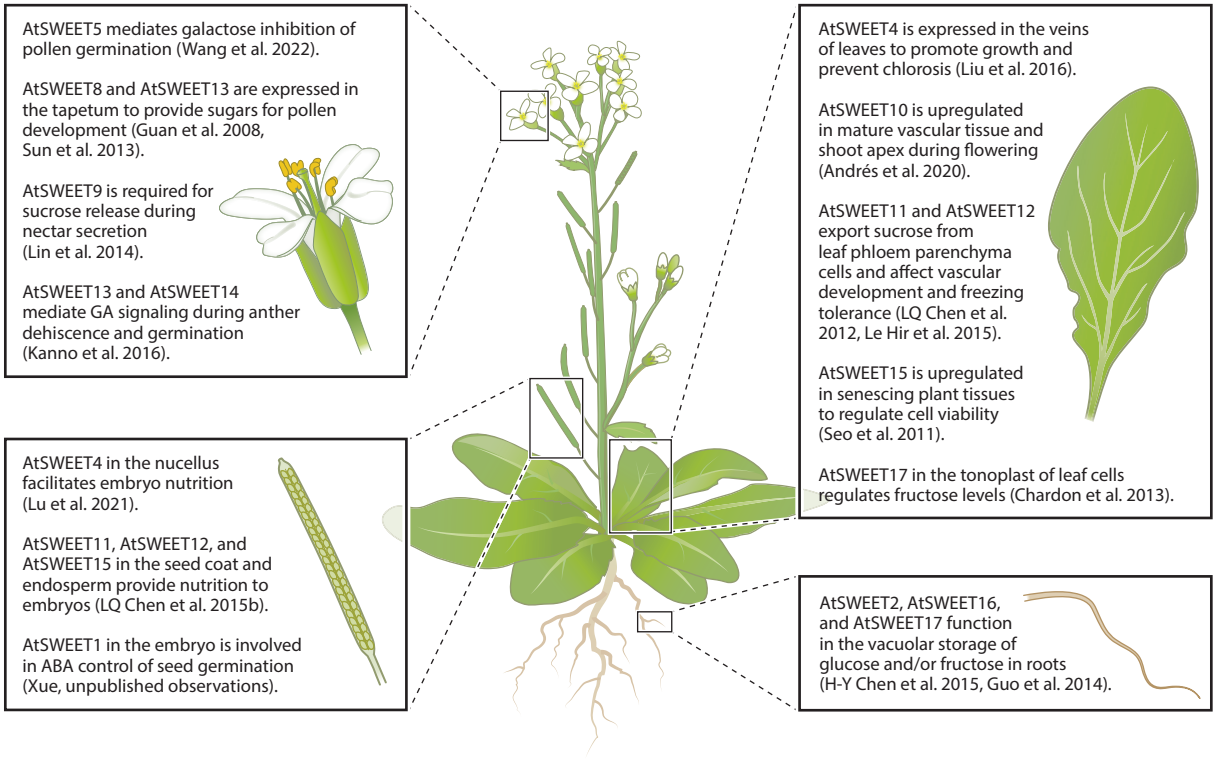


Figure 2

SWEETs in dicots. A review of the literature shows that *Arabidopsis thaliana* SWEETs participate in a diversity of biological processes, including phloem loading, pollen nutrition, nectar secretion, seed filling, senescence, and response to abiotic stress. Abbreviations: ABA, abscisic acid; GA, gibberellic acid; SWEET, sugars will eventually be exported transporter.

members. Changes in the expression pattern of SWEETs tend to precede critical developmental transitions that require extensive rerouting of sugar allocation, such as flowering (5) and the onset of senescence (41), further convoluting the interpretation of the evidence.

Apoplasmic phloem loading: the process by which sucrose is exported into the apoplasm from parenchyma cells and then imported into the sieve element/companion cell complex

Symplasmic sugar transport: diffusion of sugars through the plasmodesmata connections between cells

4.1. Phloem Loading and Unloading

Carbon is fixed in leaves via photosynthesis, and carbohydrates, mainly sucrose, are loaded into the phloem for transport to sink tissues in seeds, roots, stems, and young leaves that need to import sugars to support their development. Sugar transporters, including SWEETs, play central roles during allocation. In apoplasmic phloem–loading species like *Arabidopsis* and maize, some clade III SWEETs in leaf phloem parenchyma cells facilitate the release of sucrose into the apoplasmic space. Sucrose is then imported into the sieve element–companion cell complex (SE-CC) against the concentration gradient by SUT/SUC proton/sugar symporters (18), namely AtSUC2 in *Arabidopsis*. The *Arabidopsis* AtSWEET11 and AtSWEET12 are specifically expressed in phloem parenchyma cells proximal to the SE-CC in leaves, and the *atsweet11 atsweet12* double mutant shows stunted growth, compromised sucrose translocation into the phloem, and more sugar and starch retention in leaves (22). However, the phenotype of *atswseet11 atsweet12* is much less severe than that of the *atsuc2* mutant. This result was partially explained by the upregulation of *AtSWEET13* in *atsweet11 atsweet12* (22), which aligns with the recent finding that AtSWEET13 is expressed in phloem parenchyma cells, too (47). Indeed, the *atsweet11 atsweet12 atsweet13* triple mutant was more stunted than the *atsweet11 atsweet12* double mutant, although not as stunted as *atsuc2* (X. Xue, unpublished data). This indicates that other SWEETs may play a role in phloem loading beyond AtSWEET11, AtSWEET12, and AtSWEET13. Our sextuple mutant *atsweet10 atsweet11 atsweet12 atsweet13 atsweet14 atsweet15* was much smaller than *atsweet11 atsweet12 atsweet13*, comparable with the phenotype of the *atsuc2* mutant (X. Xue, unpublished data). This finding suggests that more SWEETs are involved in phloem loading or can be recruited to the process by yet-unknown feedback mechanisms, although the relative contributions of individual SWEETs await quantification. Similarly, Bezrutczyk et al. (12) reported that *ZmSWEET13a*, *ZmSWEET13b*, and *ZmSWEET13c* are highly expressed in leaf veins in maize, particularly in abaxial bundle sheath cells of rank-2 intermediate veins and phloem parenchyma cells. The *zmsweet13a zmsweet13b zmsweet13c* triple mutant is severely stunted with a high accumulation of starch and soluble sugar in leaves (10), similar to the *zmsut1* mutant (81) (**Figure 3**). These studies suggest that SWEET-SUT/SUC-mediated apoplasmic phloem loading is conserved throughout the plant kingdom.

The participation of SWEETs in the development of stem and root vegetables deserves particular attention. For example, the potato *StSWEET11* is expressed in the phloem of young swelling tubers during tuberization, which is involved in sucrose efflux into the cell wall space. StSWEET11 interacts with a FLOWERING LOCUS T (FT)-like protein, SELF-PRUNING 6A (StSP6A), to block sucrose leakage into apoplasm, therefore favoring symplasmic sucrose transport. Elevated sugar and starch in leaves and reduced final tuber yield were observed when *StSWEET11* was downregulated via RNA interference (RNAi) (2).

4.2. Seed Filling

Since the endosperm and embryo are symplasmically isolated from the maternal seed coat, apoplasmic seed filling requires at least two efflux steps. First, sugars must be released from maternal tissues into the apoplasmic space between the seed coat and the endosperm. Second, sugars must be released from the endosperm/aleurone into the apoplasm between the endosperm/aleurone and the embryo. Chen et al. (21) found that *AtSWEET11*, *AtSWEET12*, and

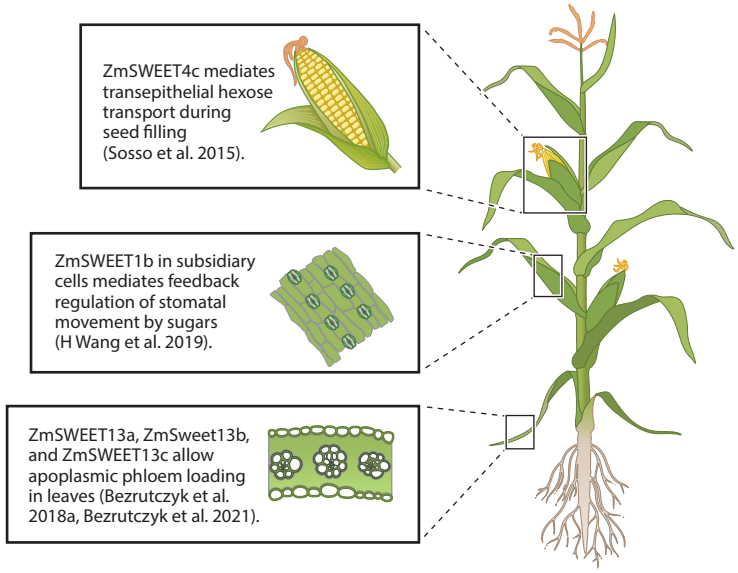


Figure 3

SWEETs in monocots. A review of the literature shows that *Zea mays* SWEETs participate in phloem loading, seed filling, and stomata opening. Abbreviation: SWEET, sugars will eventually be exported transporter.

AtSWEET15 are sequentially expressed in the seed coat and endosperm. The *atsweet11 atsweet12 atsweet15* triple mutant retains more starch in the seed coat and results in wrinkled seeds with retarded embryo development and reduced starch and lipid content due to less sugar available in embryonic tissues (21). Similarly, two parallel studies in rice found that OsSWEET11 is necessary for sucrose release from maternal tissues during early seed filling (65, 109). However, Yang et al. (109) also showed that the *ossweet11 ossweet15* double mutants had more severe seed-filling defects with nonfunctional endosperm and more residual starch levels in the pericarp, intimating that OsSWEET11 and OsSWEET15 also participate in sucrose influx into the aleurone. Other OsSWEETs are also reported to participate in sugar transport and thereby impact starch metabolism during seed filling (55). In soybean, the glucose and sucrose transporters GmSWEET10a and GmSWEET10b are expressed in the seed coat for sugar export and underwent stepwise selection during domestication. The double mutant *gmsweet10a gmsweet10b* has a lower seed weight, accumulating significantly more sugars in the seed coat and less in the embryo (98), consistent with the results from *atsweet11 atsweet12 atsweet15*. In addition, GmSWEET15 expressed in the endosperm also plays a critical role in sucrose efflux in developing seeds to provide nutrients for filial tissue development (99).

During seed filling, the embryo takes up sugar at least in the form of hexoses, as suggested by the defects caused by the loss of CWINs (92). Thus, it is not surprising that clade II SWEETs, which transport hexoses, are also crucial in seed filling. In maize, *ZmSWEET4c*, a locus associated with domestication, is specifically expressed in the basal endosperm transfer layer (BETL), the route used by maternal nutrients to enter seeds. Loss of *ZmSWEET4c* causes a defect in seed filling and BETL differentiation (82). Similarly, the rice ortholog OsSWEET4 is also a target of domestication selection, and the *ossweet4* mutant shows a strong empty pericarp phenotype. Both ZmSWEET4c and OsSWEET4 transport glucose and fructose (82). Recently, Lu et al. (64) found that AtSWEET4 expressed in nucellus exports hexoses toward the endosperm in *Arabidopsis*.

Taken together, these results intimate a relay from clade III SWEETs in the seed coat to clade II in the embryo during seed filling and a conserved role of SWEET4 in the transport of sugars from maternal tissues to the endosperm.

Transcription activator-like (TAL) effector (TALE): a bacterial protein released via the type III secretion system during infection, regulating host gene expression

SWEETs are also crucial for fruit development. For instance, SlSWEET15 protein was detected in vascular tissues and seed coats in developing tomatoes (*Solanum lycopersicum*), where it is thought to mediate sucrose efflux from the phloem into the apoplasm surrounding parenchyma cells and from the seed coat into the apoplasm (49). In another example from cucumber (*Cucumis sativus* L.), the hexose transporter CsSWEET7 in companion cells of unloading vascular tissues is responsible for fruit development (59). The knockdown mutant of *cssweet7* exhibits smaller fruits with lower soluble sugar levels (59).

4.3. Response to Biotic Stresses and Symbiosis

Even before SWEETs were characterized as sugar transporters (20), the rice disease susceptibility gene *Xa13/Os8N3* (*OsSWEET11*) was already known to be involved in the interaction between plants and pathogens (24, 108). Many plant pathogenic bacteria inject type III transcription activator-like (TAL) effectors (TALEs) into host cells to cause virulence. Chu et al. (24) cloned the *xa13* recessive alleles and found that promoter mutations confer race-specific resistance to bacterial blight. At the same time, Yang et al. (108) found that *Os8N3* induction upon infection by *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) strain PXO99^{A} depends on the type III effector PthXo1. Os8N3 RNAi plants showed resistance to strain PXO99^{A} while remaining susceptible to other strains (108), but the pathogenesis mechanism was elusive until Xa13 was found to be a sugar transporter (20). We now know that PthXo1 directly binds to an effector binding element (EBE) in the promoter of *OsSWEET11* to activate its expression. This facilitates sugar release into the apoplasmic space (20, 75), where it is available for the bacteria to take up and reproduce. Both mutated PthXo1 and mutated EBE in the *OsSWEET11* promoter eliminate PthXo1-mediated induction, thus conferring disease resistance. Although *ossweet11* mutants are resistant to PXO99^{A}, rice carrying *xa13* alleles can still be infected by strains producing other type III effectors, such as AvrXa7, PthXo2, or PthXo3 (6, 20). Together, these studies suggest that the pathogen triggers disease susceptibility in a gene-for-gene manner.

Other clade III SWEETs are also susceptibility genes. The *Xoo* strain PXO339 induces *OsSWEET13/Xa25*, and downregulation of *OsSWEET13* leads to resistance (62). It is worth noting that PXO339 rapidly induces the expression of the dominant *Xa25* but not the recessive resistant allele *xa25*. Additionally, the TALE AvrXa7 binds to an EBE within the promoter of *OsSWEET14* and activates transcription (6, 75). Both AvrXa7 and PthXo3 induce the expression of *OsSWEET14*, and the knockout mutant of OsSWEET14 or knockdown mutant of *OsSWEET14* by RNAi results in resistance to *Xoo* strains relying on AvrXa7 or PthXo3 for virulence (6). Resistance to PXO339 may be due to the recessive allele failing to respond to transcriptional induction, altered sugar transport activity (since eight amino acids are different between the recessive and dominant alleles), or both (62). Later, Li et al. (56) proved that OsSWEET12 conferred susceptibility to *Xoo* strains by designing artificial TALEs that activated the expression of this transporter, while Streubel et al. (84) manipulated artificial TALEs to specifically target 20 *OsSWEETs* in rice to screen for other potential susceptibility genes. Consistent with the study by Li et al. (56), OsSWEET12 and OsSWEET15 were confirmed to confer susceptibility to *Xoo* (84), although no naturally occurring TALEs have been identified that can directly induce their transcription.

In dicot crops, SWEETs are also hijacked by bacterial pathogens to make hosts more susceptible. For example, in cassava (*Manihot esculenta*), TAL20Xam668 specifically induces the sucrose transporter *MeSWEET10a* to elevate the pathogenicity of *Xanthomonas axonopodis* pv. *manihotis*

(25). Furthermore, in cotton (*G. hirsutum*), *Xanthomonas citri* subsp. *malvacearum* specifically activates *GhSWEET10d* via Avrb6, an important TALE for virulence (27). Intriguingly, only the clade III sucrose facilitators are recruited to induce virulence by bacterial pathogens, indicating that clade III members and TALEs coevolved during the host and pathogen tug-of-war.

Furthermore, SWEETs can also be manipulated by protist and fungi pathogens. For example, expression of *AtSWEET11* and *AtSWEET12* is upregulated by the protist *Plasmodiophora brassicae* during gall formation in roots (commonly known as clubroot disease) and loss of *AtSWEET11* and *AtSWEET12* results in less sugar accumulation at the site of infection and a delay in pathogen development (90). The double mutant *atsweet11 atsweet12* also exhibits reduced susceptibility towards the fungal hemibiotroph *Colletotrichum higginsianum* (33), and *ZmSWEET4a*, *ZmSWEET4b*, and *ZmSWEET11a* were upregulated in infected seedlings and adult leaves by the basidiomycete fungus *Ustilago maydis* (83). Overexpression of the plasma membrane–localized *IbSWEET10* in sweet potato (*Ipomoea batatas* [L.] Lam) leads to decreased sucrose levels upon infection by *Fusarium oxysporum*, and, consequently, plants are more resistant, while the RNAi lines are more susceptible (60).

In addition to being hijacked by pathogens for nutrition, *SWEETs* are also upregulated to sequester sugars away from pathogens in response to pathogen infections. In *Arabidopsis*, AtSWEET2 localizes to the tonoplast and imports glucose into vacuoles, thus limiting cellular sugar efflux (17). *AtSWEET2* is dramatically induced during *Pythium irregulare* infection, and the *atsweet2* mutant shows reduced tolerance to *Pythium*. Therefore, sequestering carbon in vacuoles to reduce sugar efflux into the rhizosphere and inhibit pathogen growth is a strategy for plant disease resistance (17).

Expression profiling of different species, such as *Medicago truncatula* and *Lotus japonicus*, showed that SWEETs were transcriptionally regulated during symbiotic interaction and SWEET proteins were observed at the periarbuscular membranes. For example, the nodule-specific expression of *MtSWEET11* suggests that it has a role in sucrose distribution within nodules, but it was not found to be critical for symbiotic nitrogen fixation (50), whereas the periarbuscular membrane–localized MtSWEET1b is involved in arbuscular maintenance as arbuscule-specific overexpression of dominant-negative alleles of MtSWEET1b results in enhanced arbuscule collapse (3; for more detail, see also 14, 46). Thus, although there is a lack of direct evidence supporting the role of sugar transport mediated by SWEETs during symbiosis, it is not inconceivable, given the dependence of root microbiota on host-derived carbon.

4.4. Response to Abiotic Stresses

Sugars serve not only as sources of energy and carbon but also as osmolytes and signaling molecules (76–78). In addition, abiotic stresses such as cold, heat, drought, and nitrogen limitations alter sugar accumulation (78). These responses are often driven by changes in the expression of sugar transporters such as SWEETs.

Sugars are known as osmoprotectants. The expression of both the vacuolar transporter genes *CsSWEET16* in the tea plant *Camellia sinensis* and *AtSWEET16* in *Arabidopsis* is downregulated by cold stress, and overexpression of *AtSWEET16* confers plants increased tolerance to low temperatures (48, 96). RNAi downregulation of *AtSWEET4* expression leads to lower sugar content in leaves and chlorosis, while overexpression of *AtSWEET4*, *CsSWEET1a*, or *CsSWEET17*, encoding a plasma membrane–localized isoform, increases sugar accumulation and enhances freezing tolerance in *Arabidopsis* (63, 110). Overexpression of the senescence-upregulated sucrose transporter *AtSWEET15/SAG29* makes plants hypersensitive to salt stress by regulating cell viability (80). On the other hand, perturbations that result in a higher accumulation of soluble sugars in leaves, such

as that observed in the *atsweet11 atsweet12* double mutants, exhibit greater freezing tolerance (53). Together, these studies support the notion that the accumulation of soluble sugars preserves cell membrane integrity during cold stress (77).

SWEETs play a crucial role in response to drought stress. In *Arabidopsis*, water deficiency increases carbon translocation from shoots to roots to favor the development of the latter. This is accomplished by promoting phloem loading via upregulation of *AtSWEET11–AtSWEET13*, *AtSWEET15*, and *AtSUC2* in leaves, as well as phloem unloading via upregulation of *AtSWEET11–AtSWEET15* in roots (29). Furthermore, although not directly implicated with drought tolerance, *ZmSWEET1b* expressed in subsidiary cells positively regulates stomatal opening, likely affecting water use efficiency (93).

5. REGULATION

5.1. Transcriptional and Posttranscriptional Regulation

Many *SWEET* members often show very different spatial-temporal expression patterns over the developmental stages, which correlates with conserved functions among different species. For example, *AtSWEET9*, *Brassica rapa BrSWEET9*, and *Nicotiana attenuata NaSWEET9* are specifically expressed in the nectary tissue for nectar secretion during the reproductive stage (61); *AtSWEET11–AtSWEET13* are specifically expressed in phloem parenchyma cells and *ZmSWEET13a–ZmSWEET13c* are expressed in phloem parenchyma cells and abaxial bundle sheath cells of the leaf tissue for phloem loading (10, 12, 22, 47); and *ZmSWEET4* is expressed in the BETL and *OsSWEET4* is expressed at the base of the spikelet for seed filling (82). These similarities would suggest conserved regulation. However, unlike the extensive effort that has been put into the physiological characterization of SWEETs, only a handful of studies have attempted to provide evidence of their direct transcriptional and posttranscriptional regulation, including alternative splicing. For example, it has been reported that OsSWEET13 and OsSWEET14 from rice require their genomic DNA sequence for vascular-specific expression, as transcriptional β-glucuronidase (GUS) fusions under only their promoters lose specificity, indicating some regulatory domains reside in introns or coding sequences (31). A similar result was observed for AtSWEET11 and AtSWEET12 in *Arabidopsis*. A series of deletions fused with GUS showed that promoter regions alone could not recapitulate the expression patterns of *AtSWEET11*, but two duplicated exonic domains are individually essential for proper expression (112).

We know too few direct regulators of SWEETs to discern conserved regulatory pathways, but a few studies deserve special mention. In rice, OsDOF11 regulates sugar transport in phloem by directly binding to the promoter region of *OsSWEET11* and *OsSWEET14* (103), while the abscisic acid (ABA)-responsive transcription factor OsbZIP72 binds to the promoter regions of *OsSWEET13* and *OsSWEET15* to activate their expression during drought and salinity stress (66). The cotton transcription factor GhMYB212 directly associates with binding sites in the promoter of *GhSWEET12* to regulate the carbon supply available for fiber elongation (87). Finally, the transcription activator MaRAP2-4 from *Mentha arvensis* is upregulated during waterlogging stress and can bind the promoter of *AtSWEET10* and upregulate its expression in transgenic *Arabidopsis* plants (70). This latter result suggests that not only SWEETs but also the regulatory networks that control their expression may be conserved across species.

SWEETs are also regulated at the posttranscriptional level. Many SWEETs have different isoforms, which may carry out different physiological functions. For example, the tea *CsSWEET17* is alternatively spliced into two isoforms: a plasma membrane–localized CsSWEET17 with transport activity and a cytoplasm-localized CsSWEET17-Ex (exclusion) that loses transport activity (110). Furthermore, their expressions are differentially regulated by cold acclimation (110),

likely at the posttranscriptional level, which is an exciting observation that deserves further investigation. The phloem parenchyma cell-specific expression of *AtSWEET11* is posttranscriptionally regulated too (112).

5.2. Posttranslational Regulation

SWEETs were detected to be phosphorylated in response to stresses (67, 97). Peptides of AtSWEET11 were found to be rapidly phosphorylated upon sucrose, H_2O_2, mannitol, ABA addition, cold, and low-K^+ treatment (67, 97). These data indicate that the function of SWEETs can be regulated at the posttranslational level, although molecular evidence demonstrating how transport activity is altered by phosphorylation is lacking. The tendency of their cytosolic C termini to form dimers may be involved in this process (37).

SWEET transport activity can also be regulated by interactors. For example, the interaction between StSP6A and StSWEET11 in potatoes impairs transport activity in protoplasts and yeast, which supports a mechanism where protein–protein binding blocks sucrose leakage to the apoplasm during tuber development (2). Also, the interaction between two copper transporters, OsCOPT1 and OsCOPT5, and OsSWEET11 was reported to modulate copper distribution during *Xoo* infection (111), although it is unclear whether this interaction will impact OsSWEET11 sugar transport. Finally, the dominant-negative effect displayed by SWEETs is a valuable way of inhibiting their transport activity in planta, primarily when the identity of the SWEETs expressed in a tissue is unknown or to circumvent the potential upregulation of compensatory SWEETs. Presumably, the mutated protein can form heterooligomers with any functional SWEET transporter present if they interact. This concept was demonstrated with a mutated OsSWEET11 in rice (32) and MtSWEET1b in *M. truncatula* (3).

Transcription activator-like effector nuclease (TALEN): engineered restriction enzyme that is generated to cut specific DNA sequences by fusing a TALE DNA-binding domain to a non-specific DNA-cleaving nuclease

6. SWEETS IN BIOTECHNOLOGY

The potential of manipulating SWEETs to enhance crop performance is best illustrated by the engineering of bacterial blight–resistant rice. Transcription activator-like effector nucleases (TALENs) have been used to edit EBEs in SWEET promoters. This was first demonstrated using AvrXa7 and PthXo3 to introduce indels in the promoter region of *OsSWEET14*, rendering plants resistant to *Xoo* strains that depend on this pair of effectors for virulence (57). Subsequent editing of Tal5 EBEs in *OsSWEET14* also resulted in disease resistance to the corresponding *Xoo* strains (42). As an alternative approach, ectopic overexpression of a dominant-negative form of OsSWEET11 in mesophyll cells can improve resistance to the sheath blight disease caused by *Rhizoctonia solani* (32).

Broad-spectrum resistance can be achieved by stacking EBE mutations in multiple *SWEETs*. Sequence analysis of TALE genes in 63 *Xoo* strains revealed 6 nonoverlapping EBEs in the promoters of *OsSWEET11*, *OsSWEET13*, and *OsSWEET14*. CRISPR-Cas9 editing of these EBEs in the Kitaake, IR64, and Ciherang-Sub1 cultivars resulted in resistance to *Xoo* strains that depend on the PthXo1, PthXo2B/$Tal7_{PXO61}$, PthXo2C, PthXo3, AvrXa7, TalC, or TalF effectors without yield penalties (31, 68). Similarly, stacking indels in EBEs recognized by PthXo1, PthXo2, PthXo2B/$Tal7_{PXO61}$, PthXo3, and a novel PthXo2C-like effector, $Tal5_{LN18}$, also resulted in broad-spectrum resistance (105) (**Figure 4**).

Compared with achievements in decreasing pathogen susceptibility, efforts toward improving carbon allocation using SWEETs have had limited success. Constitutive expression of *SWEETs* may not result in gains, depending on the specific tissue expression and subcellular localization of the target SWEETs. For example, overexpression of *AtSWEET11* and *AtSWEET12* using the *CaMV35S* promoter resulted in stunted phenotypes (30), and overexpression of *StSWEET11* in

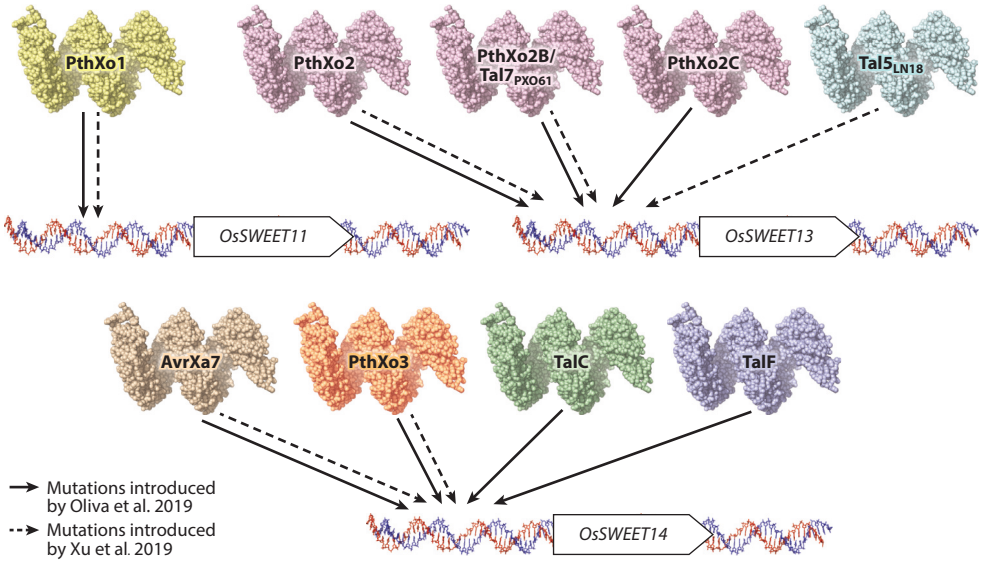


Figure 4

Engineering broad-spectrum *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) resistance in rice. Combined gene editing of the promoter regions of *OsSWEET11*, *OsSWEET13*, and *OsSWEET14* was demonstrated to decrease susceptibility to *Xoo* strains carrying the TALEs PthXo1, PthXo2, PthXo2B/Tal7$_{PXO61}$, PthXo2C, PthXo3, AvrXa7, TalC, TalF, and Tal5$_{LN18}$. Solid lines indicate mutations introduced by Oliva et al. (68), while dashed lines indicate mutations by Xu et al. (105).

potato decreased tuberization (2). Remarkably, tissue-specific overexpression of *GmSWEET10a* and *GmSWEET10b* in soybean leads to an increase in seed size and oil content (98), while overexpression of *MtSWEET1b* in *Medicago* roots enhanced growth of the intraradical mycelium during arbuscular mycorrhizal symbiosis (3).

Manipulating the expression of multiple *SWEETs* simultaneously with the help of master regulators could be an effective strategy to increase yields, albeit more research is necessary to identify such regulators. For example, the rice transcription factor *OsDOF11* is expressed in leaf vascular parenchyma cells and phloem cells, where it directly regulates the expression of *OsSWEET11*, *OsSWEET14*, and *OsSUT1* (103). Thus, targeted ectopic expression of regulators like OsDOF11 may be a better strategy to remove bottlenecks in sugar allocation, as it could result in the coactivation of other genes involved in the process.

Lastly, beyond crops, understanding the molecular function of SWEETs could also impact other biotechnology applications, such as biofuel production. For example, a recent study showed that a chimera of NcSWEET1 from the anaerobic fungi *Neocallimastigomycota* enhances glucose and D-xylose coutilization when expressed in *Saccharomyces cerevisiae* (71). Another more recent study showed that AtSWEET7 could cotransport glucose and xylose simultaneously without glucose inhibition on xylose transport in yeast for fermentation (51). The resulting strains could be valuable in improving the fermentation of lignocellulosic hydrolysates, which contain D-xylose, for efficient and rapid production of cellulosic biofuels and chemicals.

7. CONCLUSIONS AND PERSPECTIVES

Research on the SWEET family of sugar transporters has seen remarkable advances in the last few years. Nevertheless, many key questions remain about the relationship between their molecular

structures and functions, their gene and protein regulation, and the translation of basic research into improved crop yields.

At the molecular level, several residues involved in sugar recognition are conserved across the family, even in SemiSWEETs, while less conserved residues determine pore size and thus substrate selectivity of different clades. SemiSWEETs appear to have a narrow translocation pore, while SWEETs have a broader pore due to the presence of TMH4, suggesting differences in determining the size of favorable substrates (23). Researchers have proposed that SWEETs capable of transporting disaccharides have bigger substrate-binding pockets than those that favor monosaccharides, as demonstrated with amino acid replacements that made the sucrose transporter AtSWEET13 more like clade I and II glucose transporters (37), albeit some clade III SWEETs transport glucose and some clade I and II SWEETs transport sucrose.

Unfortunately, too few SWEET structures have been captured, compared to the numbers of SWEET members across different species and kingdoms, to give us an adequate understanding of substrate selectivity. Molecular dynamics simulations have shown success at predicting unseen states or the spontaneous transitions of SemiSWEETs and SWEETs. They have shown that glucose diffuses freely from the periplasmic to the cytoplasmic side to follow a free-ride mechanism and lowers the energy barrier between the key conformational states to facilitate their transport (79). Since a SWEET is able to transport different substrates (**Supplemental Table 1**), the questions are whether different sugars have various efficiencies in lowering this energy barrier and how we can improve the efficiency of sugar bound to SWEETs to favor sugar movement. In addition, the crystal structure of OsSWEET2b showed that TMH4 strongly interacts with THB1 but barely contacts THB2 in the inward open conformation (88). It is important, then, to identify whether this interaction is consistent in all conformational states and whether TMH4 fine-tunes transport activity. More crystallographic studies aided by molecular dynamics simulations could improve not only our understanding of the translocation pathway but also the evolution and physiological roles of different clades.

Supplemental Material >

It is worth noting that, although SWEETs have been widely reported to transport sugars, at least two members from *Arabidopsis*, AtSWEET13 and AtSWEET14, have been demonstrated to transport GAs (45). The SWEET family is not the only one reported to transport very different substrates. For example, NITRATE TRANSPORTER 1 (NRT1)/PEPTIDE TRANSPORTER (PTR) family (NPF) members transport a huge variety of substrates, including nitrate, chloride, glucosinolates, ABA, jasmonates, auxin, and GAs. In addition, many NPF proteins transport more than one substrate (26). We need to explore whether SWEETs recognize such a diversity of substrates as well, and how and why this promiscuity evolved.

Lipids play a critical role in maintaining the oligomeric state of membrane proteins via direct binding combined with changes in bulk membrane properties, as demonstrated for VsSemiSWEET (36), or with alteration of the stability of different conformational states, as indicated for OsSWEET2b (101). However, the structures of SemiSWEETs and SWEETs have not revealed specific lipid-binding sites. Therefore, investigating the impact of membrane composition, such as the plasma versus vacuole membrane, on their substrate selectivity and regulation could provide new avenues for engineering SWEETs and enhancing abiotic stress tolerance in crops. This is particularly relevant because sugar accumulation offers protection against cold stress, and SWEETs may be involved in water use efficiency (93).

It is still critical to develop new yeast strains or systems for the convenient analysis of SWEET transport activity. Mainly, generating a yeast strain with a low background signal to detect sucrose transport would substantially facilitate characterization. In addition, most of the existing heterologous systems are ideal for plasma membrane–localized SWEETs but not endomembrane-localized SWEETs. Identifying the mechanism underlying different subcellular localizations of SWEETs

may help target some SWEETs to the plasma membrane for functional studies in addition to shedding light on the relationship between structures and localization. Moreover, the frequently used FRET sensors can only detect glucose and sucrose (20, 22). Thus, new FRET sensors with higher affinities and optimized dynamic ranges for other sugars are needed. So far, only a few SWEETs have been reported to transport GA. It is also worth exploring whether the recently developed GA FRET sensor can be used for SWEET mediating GA transport tests (73).

The C termini of SWEETs can be modulated by phosphorylation (97). We need to find the regulatory kinases and bring new insights into how these modifications alter transport activities, whether phosphorylation can shift the kinetics of SWEETs, and what conditions trigger these modifications. Furthermore, since SWEETs can form dimers and oligomers (37, 88, 106), we also need to explore whether the transport activities of individual units are fine-tuned when the numbers of interacting monomers change or when oligomerization of SWEETs with different substrate selectivities occur. Some SWEETs have been reported to functionally interact with StSP6A in potato and OsCOPT1 and OsCOPT5 in rice (2, 111). In addition, membrane protein–protein interaction assays in yeast have shown that SWEETs physically interact with many other proteins (44). It would be valuable to address whether these interactions also happen in planta and what their effect may be on transport activities and biological functions.

Additionally, we do not yet know whether there is an interplay between phosphorylation and oligomerization of SWEETs. Structural and computational studies have not provided a basis for the potential interplay between different regulatory modes of SWEETs. For reference, the plant nitrate transporter NRT1.1 undergoes a phosphorylation-induced dimer breakdown to allow transition between low- and high-affinity states (85). Finding out whether SWEETs can undergo similar transitions would require capturing the structure of SWEETs with their C-terminal tails in a phosphorylated and oligomeric state. Alternatively, an integrated computational investigation of different phosphorylation and oligomerization states could also reveal potential mechanisms of interplay between regulatory modes.

At the organismal level, extensive research has focused on functional studies of SWEETs in different species. In comparison, we know relatively little about their regulation. We know that the specific expression of SWEETs is associated with their cell-type/tissue-specific functions and that their genomic sequences seem to be required for their vein tissue–specific accumulation in rice and *Arabidopsis* (31, 112). However, specific patterns of SWEET expression are highly diverse. For example, *ZmSWEET4* is specifically expressed in cells of the BETL for sugar import into the corn endosperm for seed filling (82), while *ZmSWEET1b* is specifically expressed in subsidiary cells for guard cell movement (93). However, we do not know the mechanism controlling their specificity. More effort should be made to identify responsible regulatory domains and decode underlying mechanisms, as understanding the precise control of sugar flux is critical for optimizing sugar allocation (9). Yeast one-hybrid screening combined with single-cell sequencing (12, 47) and other high-throughput methods like ribosome sequencing (Ribo-Seq) or translating ribosome affinity purification sequencing (TRAP-Seq) could prove valuable to support this effort (7, 39).

Many abiotic stresses, such as drought and heat stress (1, 74), and nutrient deficiencies, such as magnesium and phosphate (28, 89), alter carbon allocation among different tissues (9). SWEETs respond to these stresses to varying degrees at the transcriptional level. We need to determine how these responses affect sugar allocation and what other mechanisms regulate and modify the activity of SWEETs under these different conditions. Genetically engineered FRET sugar sensors turned out to be a powerful tool to monitor sugar dynamic changes in vivo under ABA treatment (107) that can mimic stress responses. Thus, expressing FRET sensors in *sweet* mutants will facilitate a better understanding of the roles of SWEET in stress responses at the cellular or even subcellular

levels. Alternatively, the activity of SWEETs can be monitored directly with transport activity biosensors (69).

Lastly, increasing crop yields requires not only carbon, in the form of sugars, but also water and nitrogen. It would be interesting to design a SWEET with broad substrate selectivity and high transport capacity to explore how this will affect yields. At present, multiscale in silico models that integrate carbon-, water-, and nitrogen-associated transporters at the subcellular and whole-plant levels are lacking, limiting our ability to predict how modifying *SWEET* expression patterns could improve yields (8). Given our rudimentary understanding of SWEET regulation, iterating between the generation of transgenic lines and high-throughput phenotyping may be necessary to sequentially remove pathway bottlenecks under different environmental conditions (9).

SUMMARY POINTS

1. The crystal structures of plant SWEETs and bacterial SemiSWEETs have revealed key amino acids forming the sugar-binding sites and gates, while molecular dynamics simulations illustrate the conformational state changes taking place during sugar translocation.
2. The SWEET family is phylogenetically divided into four clades. Commonly, clade III SWEETs prefer to transport sucrose, and clades I, II, and IV favor the transport of hexoses.
3. Current evidence suggests that cooperation between SWEETs and sucrose transporters/sucrose carriers (SUT/SUC) during apoplasmic phloem loading is conserved among monocot and dicot plants.
4. Phosphorylation and protein–protein interactions between family members or other proteins have been reported and may be important for the regulation of transport activity.
5. SWEETs can be hijacked for sugars by pathogenic bacteria and fungi, and gene edits in the promoters of multiple *SWEETs* have been shown to confer broad-spectrum resistance to *Xanthomonas oryzae* infection in rice.
6. In a few studies, tissue-specific overexpression of SWEETs has resulted in improved freezing tolerance, increased seed size and quality, or enhanced symbiosis with soil microorganisms.

FUTURE ISSUES

1. More crystal structures of SWEETs (including C terminus) of interest should be captured.
2. The molecular mechanism underlying substrate specificity should be elucidated.
3. The modifiers regulating SWEET activities need to be identified.
4. The mechanism responsible for the tissue-specific expression of SWEETs should be determined.
5. Further research is needed to address the evolutionary relationship among different clades.

6. More studies should be focused to understand the roles of SWEETs in response to stresses.
7. The impact of membrane composition on SWEET activities requires further investigation.
8. SWEET-medicated sugar flux should be engineered for crop yield production, biofuel production, or stress resistance.

DISCLOSURE STATEMENT

The authors are not aware of any affiliations, memberships, funding, or financial holdings that might be perceived as affecting the objectivity of this review.

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LITERATURE CITED

1. Abdelrahman M, Burritt DJ, Gupta A, Tsujimoto H, Tran L-SP. 2020. Heat stress effects on source–sink relationships and metabolome dynamics in wheat. *J. Exp. Bot.* 71(2):543–54
2. **Abelenda JA, Bergonzi S, Oortwijn M, Sonnewald S, Du M, et al. 2019. Source-sink regulation is mediated by interaction of an FT homolog with a SWEET protein in potato. *Curr. Biol.* 29(7):1178–86.e6**
3. An J, Zeng T, Ji C, de Graaf S, Zheng Z, et al. 2019. A *Medicago truncatula* SWEET transporter implicated in arbuscule maintenance during arbuscular mycorrhizal symbiosis. *New Phytol.* 224(1):396–408
4. Andersson DI, Jerlström-Hultqvist J, Näsvall J. 2015. Evolution of new functions de novo and from preexisting genes. *Cold Spring Harb. Perspect. Biol.* 7(6):a017996
5. Andrés F, Kinoshita A, Kalluri N, Fernández V, Falavigna VS, et al. 2020. The sugar transporter SWEET10 acts downstream of *FLOWERING LOCUS T* during floral transition of *Arabidopsis thaliana*. *BMC Plant Biol.* 20(1):53
6. Antony G, Zhou J, Huang S, Li T, Liu B, et al. 2010. Rice *xa13* recessive resistance to bacterial blight is defeated by induction of the disease susceptibility gene Os-*11N3*. *Plant Cell* 22(11):3864–76
7. Bazin J, Baerenfaller K, Gosai SJ, Gregory BD, Crespi M, Bailey-Serres J. 2017. Global analysis of ribosome-associated noncoding RNAs unveils new modes of translational regulation. *PNAS* 114(46):E10018–27
8. Benes B, Guan K, Lang M, Long SP, Lynch JP, et al. 2020. Multiscale computational models can guide experimentation and targeted measurements for crop improvement. *Plant J.* 103(1):21–31
9. Beuchat G, Xue X, Chen L-Q. 2020. Review: The next steps in crop improvement: adoption of emerging strategies to identify bottlenecks in sugar flux. *Plant Sci.* 301:110675
10. Bezrutczyk M, Hartwig T, Horschman M, Char SN, Yang J, et al. 2018a. Impaired phloem loading in *zmsweet13a,b,c* sucrose transporter triple knock-out mutants in *Zea mays*. *New Phytol.* 218(2):594–603
11. Bezrutczyk M, Yang J, Eom J-S, Prior M, Sosso D, et al. 2018b. Sugar flux and signaling in plant–microbe interactions. *Plant J.* 93(4):675–85
12. Bezrutczyk M, Zöllner NR, Kruse CPS, Hartwig T, Lautwein T, et al. 2021. Evidence for phloem loading via the abaxial bundle sheath cells in maize leaves. *Plant Cell* 33(3):531–47
13. Bloch R. 1974. Human erythrocyte sugar transport. *J. Biol. Chem.* 249(11):3543–50
14. Breia R, Conde A, Badim H, Fortes AM, Gerós H, Granell A. 2021. Plant SWEETs: from sugar transport to plant–pathogen interaction and more unexpected physiological roles. *Plant Physiol.* 186(2):836–52

2. Describes how StSWEET11 and StSP6A interact to block sucrose leakage into apoplasmic space during tuberization.

15. Brown CJ, Trieber C, Overduin M. 2021. Structural biology of endogenous membrane protein assemblies in native nanodiscs. *Curr. Opin. Struct. Biol.* 69:70–77
16. Chardon F, Bedu M, Calenge F, Klemens PAW, Spinner L, et al. 2013. Leaf fructose content is controlled by the vacuolar transporter SWEET17 in *Arabidopsis*. *Curr. Biol.* 23(8):697–702
17. Chen H-Y, Huh J-H, Yu Y-C, Ho L-H, Chen L-Q, et al. 2015. The Arabidopsis vacuolar sugar transporter SWEET2 limits carbon sequestration from roots and restricts *Pythium* infection. *Plant J.* 83(6):1046–58
18. Chen LQ. 2014. SWEET sugar transporters for phloem transport and pathogen nutrition. *New Phytol.* 201(4):1150–55
19. Chen LQ, Cheung LS, Feng L, Tanner W, Frommer WB. 2015a. Transport of sugars. *Annu. Rev. Biochem.* 84:865–94
20. Chen LQ, Hou BH, Lalonde S, Takanaga H, Hartung ML, et al. 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. *Nature* 468(7323):527–32
21. Chen LQ, Lin IW, Qu XQ, Sosso D, McFarlane HE, et al. 2015b. A cascade of sequentially expressed sucrose transporters in the seed coat and endosperm provides nutrition for the Arabidopsis embryo. *Plant Cell* 27(3):607–19
22. Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, et al. 2012. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. *Science* 335(6065):207–11
23. Cheng KJ, Selvam B, Chen L-Q, Shukla D. 2019. Distinct substrate transport mechanism identified in homologous sugar transporters. *J. Phys. Chem. B* 123(40):8411–18
24. Chu Z, Yuan M, Yao L, Ge X, Yuan B, et al. 2006. Promoter mutations of an essential gene for pollen development result in disease resistance in rice. *Genes Dev.* 20(10):1250–55
25. Cohn M, Bart RS, Shybut M, Dahlbeck D, Gomez M, et al. 2014. *Xanthomonas axonopodis* virulence is promoted by a transcription activator-like effector-mediated induction of a SWEET sugar transporter in cassava. *Mol. Plant Microbe Interact.* 27(11):1186–98
26. Corratgé-Faillie C, Lacombe B. 2017. Substrate (un)specificity of Arabidopsis NRT1/PTR FAMILY (NPF) proteins. *J. Exp. Bot.* 68(12):3107–13
27. Cox KL, Meng F, Wilkins KE, Li F, Wang P, et al. 2017. TAL effector driven induction of a *SWEET* gene confers susceptibility to bacterial blight of cotton. *Nat. Commun.* 8(1):15588
28. Dasgupta K, Khadilkar AS, Sulpice R, Pant B, Scheible W-R, et al. 2014. Expression of sucrose transporter cDNAs specifically in companion cells enhances phloem loading and long-distance transport of sucrose but leads to an inhibition of growth and the perception of a phosphate limitation. *Plant Physiol.* 165(2):715–31
29. Durand M, Porcheron B, Hennion N, Maurousset L, Lemoine R, Pourtau N. 2016. Water deficit enhances C export to the roots in *Arabidopsis thaliana* plants with contribution of sucrose transporters in both shoot and roots. *Plant Physiol.* 170(3):1460–79
30. Eom J-S, Chen L-Q, Sosso D, Julius BT, Lin I, et al. 2015. SWEETs, transporters for intracellular and intercellular sugar translocation. *Curr. Opin. Plant Biol.* 25:53–62
31. Eom J-S, Luo D, Atienza-Grande G, Yang J, Ji C, et al. 2019. Diagnostic kit for rice blight resistance. *Nat. Biotechnol.* 37(11):1372–79
32. Gao Y, Zhang C, Han X, Wang ZY, Ma L, et al. 2018. Inhibition of *OsSWEET11* function in mesophyll cells improves resistance of rice to sheath blight disease. *Mol. Plant Pathol.* 19(9):2149–61
33. Gebauer P, Korn M, Engelsdorf T, Sonnewald U, Koch C, Voll LM. 2017. Sugar accumulation in leaves of Arabidopsis *sweet11/sweet12* double mutants enhances priming of the salicylic acid-mediated defense response. *Front. Plant Sci.* 8:1378
34. Guan Y-F, Huang X-Y, Zhu J, Gao J-F, Zhang H-X, Yang Z-N. 2008. *RUPTURED POLLEN GRAIN1*, a member of the MtN3/saliva gene family, is crucial for exine pattern formation and cell integrity of microspores in Arabidopsis. *Plant Physiol.* 147(2):852–63
35. Guo W-J, Nagy R, Chen H-Y, Pfrunder S, Yu Y-C, et al. 2014. SWEET17, a facilitative transporter, mediates fructose transport across the tonoplast of Arabidopsis roots and leaves. *Plant Physiol.* 164(2):777–89
36. Gupta K, Donlan JAC, Hopper JTS, Uzdavinys P, Landreh M, et al. 2017. The role of interfacial lipids in stabilizing membrane protein oligomers. *Nature* 541(7637):421–24

20. Describes the initial characterization of SWEETs as sugar uniporters and for pathogen nutrition.

21. First evidence of the function of SWEETs in seed filling in *Arabidopsis*.

22. First characterization of SWEETs as the sugar effluxers in the phloem parenchyma cells during apoplasmic phloem loading.

37. Han L, Zhu Y, Liu M, Zhou Y, Lu G, et al. 2017. Molecular mechanism of substrate recognition and transport by the AtSWEET13 sugar transporter. *PNAS* 114(38):10089–94

37. Reports the first crystal structure of a clade III SWEET to interpret how SWEET transports sucrose versus glucose.

38. Ho L-H, Klemens PAW, Neuhaus HE, Ko H-Y, Hsieh S-Y, Guo W-J. 2019. *Sl*SWEET1a is involved in glucose import to young leaves in tomato plants. *J. Exp. Bot.* 70(12):3241–54
39. Hsu PY, Calviello L, Wu H-YL, Li F-W, Rothfels CJ, et al. 2016. Super-resolution ribosome profiling reveals unannotated translation events in *Arabidopsis*. *PNAS* 113(45):E7126–35
40. Hu Y-B, Sosso D, Qu X-Q, Chen L-Q, Ma L, et al. 2016. Phylogenetic evidence for a fusion of archaeal and bacterial SemiSWEETs to form eukaryotic SWEETs and identification of SWEET hexose transporters in the amphibian chytrid pathogen *Batrachochytrium dendrobatidis*. *FASEB J.* 30(10):3644–54
41. Huang C, Yu J, Cai Q, Chen Y, Li Y, et al. 2020. Triple-localized WHIRLY2 influences leaf senescence and silique development via carbon allocation. *Plant Physiol.* 184(3):1348–62
42. Hutin M, Sabot F, Ghesquière A, Koebnik R, Szurek B. 2015. A knowledge-based molecular screen uncovers a broad-spectrum *OsSWEET14* resistance allele to bacterial blight from wild rice. *Plant J.* 84(4):694–703
43. Jia B, Zhu XF, Pu ZJ, Duan YX, Hao LJ, et al. 2017. Integrative view of the diversity and evolution of SWEET and SemiSWEET sugar transporters. *Front. Plant Sci.* 8:2178
44. Jones AM, Xuan Y, Xu M, Wang R-S, Ho C-H, et al. 2014. Border control—a membrane-linked interactome of *Arabidopsis*. *Science* 344(6185):711–16

45. First report that SWEETs transport more than sugars.

45. Kanno Y, Oikawa T, Chiba Y, Ishimaru Y, Shimizu T, et al. 2016. AtSWEET13 and AtSWEET14 regulate gibberellin-mediated physiological processes. *Nat. Commun.* 7:13245

46. Kim J-Y, Loo EP-I, Pang TY, Lercher M, Frommer WB, Wudick MM. 2021. Cellular export of sugars and amino acids: role in feeding other cells and organisms. *Plant Physiol.* 187(4):1893–914
47. Kim J-Y, Symeonidi E, Pang TY, Denyer T, Weidauer D, et al. 2021. Distinct identities of leaf phloem cells revealed by single cell transcriptomics. *Plant Cell* 33(3):511–30
48. Klemens PA, Patzke K, Deitmer J, Spinner L, Le Hir R, et al. 2013. Overexpression of the vacuolar sugar carrier *AtSWEET16* modifies germination, growth, and stress tolerance in Arabidopsis. *Plant Physiol.* 163(3):1338–52
49. Ko H-Y, Ho L-H, Neuhaus HE, Guo W-J. 2021. Transporter SlSWEET15 unloads sucrose from phloem and seed coat for fruit and seed development in tomato. *Plant Physiol.* 187(4):2230–45
50. Kryvoruchko IS, Sinharoy S, Torres-Jerez I, Sosso D, Pislariu CI, et al. 2016. MtSWEET11, a nodule-specific sucrose transporter of *Medicago truncatula*. *Plant Physiol.* 171(1):554–65
51. Kuanyshev N, Deewan A, Jagtap SS, Liu J, Selvam B, et al. 2021. Identification and analysis of sugar transporters capable of co-transporting glucose and xylose simultaneously. *Biotechnol. J.* 16:2100238

52. Describes the mechanism of sugar transport switch by a combination of crystal structures and molecular dynamics simulations.

52. Latorraca NR, Fastman NM, Venkatakrishnan AJ, Frommer WB, Dror RO, Feng L. 2017. Mechanism of substrate translocation in an alternating access transporter. *Cell* 169(1):96–107.e12

53. Le Hir R, Spinner L, Klemens PAW, Chakraborti D, de Marco F, et al. 2015. Disruption of the sugar transporters *AtSWEET11* and *AtSWEET12* affects vascular development and freezing tolerance in *Arabidopsis*. *Mol. Plant* 8(11):1687–90
54. Lee Y, Nishizawa T, Yamashita K, Ishitani R, Nureki O. 2015. Structural basis for the facilitative diffusion mechanism by SemiSWEET transporter. *Nat. Commun.* 6(1):6112
55. Li P, Wang L, Liu H, Yuan M. 2022. Impaired *SWEET*-mediated sugar transportation impacts starch metabolism in developing rice seeds. *Crop J.* 10(1):98–108
56. Li T, Huang S, Zhou J, Yang B. 2013. Designer TAL effectors induce disease susceptibility and resistance to *Xanthomonas oryzae* pv. oryzae in rice. *Mol. Plant* 6(3):781–89
57. Li T, Liu B, Spalding MH, Weeks DP, Yang B. 2012. High-efficiency TALEN-based gene editing produces disease-resistant rice. *Nat. Biotechnol.* 30(5):390–92
58. Li X, Si W, Qin Q, Wu H, Jiang H. 2018. Deciphering evolutionary dynamics of *SWEET* genes in diverse plant lineages. *Sci. Rep.* 8(1):13440
59. Li Y, Liu H, Yao X, Wang J, Feng S, et al. 2021. Hexose transporter CsSWEET7a in cucumber mediates phloem unloading in companion cells for fruit development. *Plant Physiol.* 186(1):640–54
60. Li Y, Wang Y, Zhang H, Zhang Q, Zhai H, et al. 2017. The plasma membrane-localized sucrose transporter IbSWEET10 contributes to the resistance of sweet potato to *Fusarium oxysporum*. *Front. Plant Sci.* 8:197

61. Lin IW, Sosso D, Chen L-Q, Gase K, Kim S-G, et al. 2014. Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. *Nature* 508(7497):546–49
62. Liu Q, Yuan M, Zhou Y, Li X, Xiao J, Wang S. 2011. A paralog of the MtN3/saliva family recessively confers race-specific resistance to *Xanthomonas oryzae* in rice. *Plant Cell Environ*. 34(11):1958–69
63. Liu X, Zhang Y, Yang C, Tian Z, Li J. 2016. AtSWEET4, a hexose facilitator, mediates sugar transport to axial sinks and affects plant development. *Sci. Rep.* 6(1):24563
64. Lu J, Le Hir R, Gómez-Páez D-M, Coen O, Péchoux C, et al. 2021. The nucellus: between cell elimination and sugar transport. *Plant Physiol*. 185(2):478–90
65. Ma L, Zhang D, Miao Q, Yang J, Xuan Y, Hu Y. 2017. Essential role of sugar transporter OsSWEET11 during the early stage of rice grain filling. *Plant Cell Physiol*. 58(5):863–73
66. Mathan J, Singh A, Ranjan A. 2021. Sucrose transport in response to drought and salt stress involves ABA-mediated induction of *OsSWEET13* and *OsSWEET15* in rice. *Physiol. Plant.* 171(4):620–37
67. Niittylä T, Fuglsang AT, Palmgren MG, Frommer WB, Schulze WX. 2007. Temporal analysis of sucrose-induced phosphorylation changes in plasma membrane proteins of *Arabidopsis*. *Mol. Cell. Proteom.* 6(10):1711–26
68. Oliva R, Ji C, Atienza-Grande G, Huguet-Tapia JC, Perez-Quintero A, et al. 2019. Broad-spectrum resistance to bacterial blight in rice using genome editing. *Nat. Biotechnol.* 37(11):1344–50
69. Park J, Chavez TM, Guistwhite JA, Gwon S, Frommer WB, Cheung LS. 2022. Development and quantitative analysis of a biosensor based on the Arabidopsis SWEET1 sugar transporter. *PNAS* 119:e2119183119
70. Phukan UJ, Jeena GS, Tripathi V, Shukla RK. 2018. MaRAP2-4, a waterlogging-responsive ERF from *Mentha*, regulates bidirectional sugar transporter *AtSWEET10* to modulate stress response in *Arabidopsis*. *Plant Biotechnol. J.* 16(1):221–33
71. Podolsky IA, Seppälä S, Xu H, Jin Y-S, O'Malley MA. 2021. A SWEET surprise: Anaerobic fungal sugar transporters and chimeras enhance sugar uptake in yeast. *Metab. Eng.* 66:137–47
72. Rizza A, Tang B, Stanley CE, Grossmann G, Owen MR, et al. 2021. Differential biosynthesis and cellular permeability explain longitudinal gibberellin gradients in growing roots. *PNAS* 118(8):e1921960118
73. Rizza A, Walia A, Lanquar V, Frommer WB, Jones AM. 2017. In vivo gibberellin gradients visualized in rapidly elongating tissues. *Nat. Plants* 3(10):803–13
74. Rodrigues J, Inzé D, Nelissen H, Saibo NJM. 2019. Source–sink regulation in crops under water deficit. *Trends Plant Sci*. 24(7):652–63
75. Römer P, Recht S, Strauß T, Elsaesser J, Schornack S, et al. 2010. Promoter elements of rice susceptibility genes are bound and activated by specific TAL effectors from the bacterial blight pathogen, *Xanthomonas oryzae* pv. *oryzae*. *New Phytol*. 187(4):1048–57
76. Rosa M, Prado C, Podazza G, Interdonato R, González JA, et al. 2009. Soluble sugars—metabolism, sensing and abiotic stress: a complex network in the life of plants. *Plant Signal. Behav*. 4(5):388–93
77. Ruan Y-L, Jin Y, Yang Y-J, Li G-J, Boyer JS. 2010. Sugar input, metabolism, and signaling mediated by invertase: roles in development, yield potential, and response to drought and heat. *Mol. Plant* 3(6):942–55
78. Sami F, Yusuf M, Faizan M, Faraz A, Hayat S. 2016. Role of sugars under abiotic stress. *Plant Physiol. Biochem*. 109:54–61
79. Selvam B, Yu Y-C, Chen L-Q, Shukla D. 2019. Molecular basis of the glucose transport mechanism in plants. *ACS Cent. Sci.* 5(6):1085–96
80. Seo PJ, Park JM, Kang SK, Kim SG, Park CM. 2011. An *Arabidopsis* senescence-associated protein SAG29 regulates cell viability under high salinity. *Planta* 233(1):189–200
81. Slewinski TL, Meeley R, Braun DM. 2009. *Sucrose transporter1* functions in phloem loading in maize leaves. *J. Exp. Bot.* 60(3):881–92
82. Sosso D, Luo D, Li Q-B, Sasse J, Yang J, et al. 2015. Seed filling in domesticated maize and rice depends on SWEET-mediated hexose transport. *Nat. Genet.* 47(12):1489–93
83. Sosso D, van der Linde K, Bezrutczyk M, Schuler D, Schneider K, et al. 2019. Sugar partitioning between *Ustilago maydis* and its host *Zea mays* L during infection. *Plant Physiol*. 179(4):1373–85

68. Application of CRISPR-Cas9 to edit multiple TALE binding elements in the promoters of *SWEETs* for broad-spectrum resistance.

79. Describes mechanistic prediction of a complete transport cycle of glucose in a eukaryotic SWEET using molecular dynamics simulations.

84. Streubel J, Pesce C, Hutin M, Koebnik R, Boch J, Szurek B. 2013. Five phylogenetically close rice *SWEET* genes confer TAL effector-mediated susceptibility to *Xanthomonas oryzae* pv. *oryzae*. *New Phytol*. 200(3):808–19
85. Sun J, Zheng N. 2015. Molecular mechanism underlying the plant NRT1.1 dual-affinity nitrate transporter. *Front. Physiol.* 6:386
86. Sun M-X, Huang X-Y, Yang J, Guan Y-F, Yang Z-N. 2013. Arabidopsis RPG1 is important for primexine deposition and functions redundantly with RPG2 for plant fertility at the late reproductive stage. *Plant Reprod*. 26(2):83–91
87. Sun W, Gao Z, Wang J, Huang Y, Chen Y, et al. 2019. Cotton fiber elongation requires the transcription factor GhMYB212 to regulate sucrose transportation into expanding fibers. *New Phytol*. 222(2):864–81
88. Tao Y, Cheung LS, Li S, Eom J-S, Chen L-Q, et al. 2015. Structure of a eukaryotic SWEET transporter in a homotrimeric complex. *Nature* 527(7577):259–63
89. Tränkner M, Tavakol E, Jákli B. 2018. Functioning of potassium and magnesium in photosynthesis, photosynthate translocation and photoprotection. *Physiol. Plant.* 163(3):414–31
90. Walerowski P, Gündel A, Yahaya N, Truman W, Sobczak M, et al. 2018. Clubroot disease stimulates early steps of phloem differentiation and recruits SWEET sucrose transporters within developing galls. *Plant Cell* 30(12):3058–73
91. Wan H, Wu L, Yang Y, Zhou G, Ruan Y-L. 2018. Evolution of sucrose metabolism: the dichotomy of invertases and beyond. *Trends Plant Sci*. 23(2):163–77
92. Wang E, Wang J, Zhu X, Hao W, Wang L, et al. 2008. Control of rice grain-filling and yield by a gene with a potential signature of domestication. *Nat. Genet*. 40(11):1370–74
93. Wang H, Yan S, Xin H, Huang W, Zhang H, et al. 2019. A subsidiary cell-localized glucose transporter promotes stomatal conductance and photosynthesis. *Plant Cell* 31(6):1328–43
94. Wang J, Yan C, Li Y, Hirata K, Yamamoto M, et al. 2014. Crystal structure of a bacterial homologue of SWEET transporters. *Cell Res*. 24(12):1486–89
95. Wang J, Yu Y-C, Li Y, Chen L-Q. 2022. Hexose transporter SWEET5 confers galactose sensitivity to Arabidopsis pollen germination via a galactokinase. *Plant Physiol*. In press. **https://doi.org/10.1093/plphys/kiac068**
96. Wang L, Yao L, Hao X, Li N, Qian W, et al. 2018. Tea plant SWEET transporters: expression profiling, sugar transport, and the involvement of CsSWEET16 in modifying cold tolerance in *Arabidopsis*. *Plant Mol. Biol.* 96(6):577–92
97. Wang P, Hsu C-C, Du Y, Zhu P, Zhao C, et al. 2020. Mapping proteome-wide targets of protein kinases in plant stress responses. *PNAS* 117(6):3270–80
98. Wang S, Liu S, Wang J, Yokosho K, Zhou B, et al. 2020. Simultaneous changes in seed size, oil content and protein content driven by selection of *SWEET* homologues during soybean domestication. *Natl. Sci. Rev*. 7(11):1776–86
99. Wang S, Yokosho K, Guo R, Whelan J, Ruan Y-L, et al. 2019. The soybean sugar transporter GmSWEET15 mediates sucrose export from endosperm to early embryo. *Plant Physiol*. 180(4):2133–41
100. Waterhouse A, Bertoni M, Bienert S, Studer G, Tauriello G, et al. 2018. SWISS-MODEL: homology modelling of protein structures and complexes. *Nucleic Acids Res*. 46:W296–303
101. Weigle AT, Carr M, Shukla D. 2021. Impact of increased membrane realism on conformational sampling of proteins. *J. Chem. Theory Comput.* 17(8):5342–57
102. Wieczorke R, Krampe S, Weierstall T, Freidel K, Hollenberg CP, Boles E. 1999. Concurrent knock-out of at least 20 transporter genes is required to block uptake of hexoses in *Saccharomyces cerevisiae*. *FEBS Lett*. 464(3):123–28
103. Wu Y, Lee S-K, Yoo Y, Wei J, Kwon S-Y, et al. 2018. Rice transcription factor OsDOF11 modulates sugar transport by promoting expression of *Sucrose Transporter* and *SWEET* genes. *Mol. Plant* 11(6):833–45
104. Xu Y, Tao Y, Cheung LS, Fan C, Chen L-Q, et al. 2014. Structures of bacterial homologues of SWEET transporters in two distinct conformations. *Nature* 515(7527):448–52
105. Xu Z, Xu X, Gong Q, Li Z, Li Y, et al. 2019. Engineering broad-spectrum bacterial blight resistance by simultaneously disrupting variable TALE-binding elements of multiple susceptibility genes in rice. *Mol. Plant* 12(11):1434–46

88. Describes the crystal structure of the first eukaryotic SWEET transporter in a homomeric trimer.

106. Xuan YH, Hu YB, Chen L-Q, Sosso D, Ducat DC, et al. 2013. Functional role of oligomerization for bacterial and plant SWEET sugar transporter family. *PNAS* 110(39):E3685–94
107. Xue X, Yu Y-C, Wu Y, Xue H, Chen L-Q. 2021. Locally restricted glucose availability in the embryonic hypocotyl determines seed germination under abscisic acid treatment. *New Phytol*. 231(5):1832–44
108. Yang B, Sugio A, White FF. 2006. *Os8N3* is a host disease-susceptibility gene for bacterial blight of rice. *PNAS* 103(27):10503–8
109. Yang J, Luo D, Yang B, Frommer WB, Eom J-S. 2018. SWEET11 and 15 as key players in seed filling in rice. *New Phytol*. 218(2):604–15
110. Yao L, Ding C, Hao X, Zeng J, Yang Y, et al. 2020. CsSWEET1a and CsSWEET17 mediate growth and freezing tolerance by promoting sugar transport across the plasma membrane. *Plant Cell Physiol*. 61(9):1669–82
111. Yuan M, Chu Z, Li X, Xu C, Wang S. 2010. The bacterial pathogen *Xanthomonas oryzae* overcomes rice defenses by regulating host copper redistribution. *Plant Cell* 22(9):3164–76
112. Zhang C, Li Y, Wang J, Xue X, Beuchat G, Chen L-Q. 2021. Two evolutionarily duplicated domains individually and post-transcriptionally control *SWEET* expression for phloem transport. *New Phytol*. 232(4):1793–807

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Contents

Errata

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