Plant Physiology and Biochemistry 156 (2020) 1–6

Contents lists available at ScienceDirect

Plant Physiology and Biochemistry

journal homepage: www.elsevier.com/locate/plaphy

Review

Structure and regulation of SWEET transporters in plants: An update

Anjali Anjali [a,1], Urooj Fatima [a,1], M.S. Manu [b], Sureshkumar Ramasamy [b], Muthappa Senthil-Kumar [a,*]

[a] National Institute of Plant Genome Research, Aruna Asaf Ali Marg, New Delhi, 110067, India
[b] Biochemical Science Division, CSIR-National Chemical Laboratory, Pune, 411008, India

ARTICLE INFO

Keywords:
Oligomerization
Post-translational modification
Phosphorylation
Sugar transporters
Sugar will eventually be exported transporters

ABSTRACT

Sugar will eventually be exported transporters (SWEETs), a novel family of sugar transporters found in both eukaryotes and prokaryotes, facilitate sugar flux across the cell membrane. Although these transporters were first discovered in plants, their homologs have been reported in different organisms. SWEETs have critical roles in various developmental processes, including phloem loading, nectar secretion, and pathogen nutrition. The structure of bacterial homologs, called SemiSWEETs, has been well studied thus far. Here, we provide an overview of SWEET protein structure and dynamic function by analyzing the solved crystal structures and predicted models that are available for a few SWEETs in a monocot plant (rice) and dicot plant (*Arabidopsis thaliana*). Despite the advancement in structure-related studies, the regulation of SWEETs remains unknown. In light of reported regulatory mechanisms of a few other sugar transporters, we propose the regulation of SWEETs at the post-translational level. We then enumerate the potential post-translational modification sites in SWEETs using computational tools. Overall, in this review, we critically analyze SWEET protein structure in plants to predict the post-translational regulation of SWEETs. Such findings have a direct bearing on plant nutrition and defense and targeting the regulation at these levels will be important in crop improvement.

1. Introduction

SWEETs, sugars will eventually be exported transporters, are among the major transporters found in both eukaryotes and prokaryotes for facilitating sugar flux across the cellular membrane. SWEET genes and their homologs are widely distributed across almost all kingdoms of life (Chen et al., 2010, Chen et al., 2015; Lin et al., 2014), including bacteria and archaea. The plant genome contains approximately 20 SWEET paralogs. The model plant *Arabidopsis thaliana* itself has genes for 17 SWEET members, while only one gene has been reported in animals; *Caenorhabditis elegans* is an exception, containing seven paralogs of SWEET. All eukaryotic SWEETs are constituted by seven predicted transmembrane (7TM) domains, in which 3TM repeats are connected by a linker helix. This entire structure is arranged in a 3-1-3 topology in the cell membrane (Chen et al., 2010). Breakthrough findings via bioinformatics analyses have revealed the presence of SWEET homologs in bacteria. Since these homologs are composed of only a single 3TM, they were named SemiSWEETs. The structure of SemiSWEETs indicates the possibility of duplication of the basic 3TM unit in eukaryotes, which might have led to the emergence of eukaryotic SWEETs during evolution.

In plants, SWEETs are mainly localized to the plasma membrane of the cells and also present on different cell organelles including, endoplasmic reticulum, Golgi membrane and tonoplast (Feng and Frommer, 2015) Plasma membrane-localized SWEET transporters efflux sugars from cytosol into the apoplast and perform specific roles, including nectar secretion (Eom et al., 2015), apoplastic phloem loading (Chen et al., 2015), seed filling, and pollen nutrition (Guan et al., 2008; Sun et al., 2013). Moreover, SWEET proteins are major players in governing the long-distance distribution of sugars throughout the plant; therefore, SWEETs must be tightly regulated by the plant to ensure the proper distribution of sugar to all sink tissues (Chen et al., 2012). Besides their role in plant growth and development, SWEETs also contribute to pathogen susceptibility (Chen et al., 2010). A prominent example is rice

Abbreviations: **PTM**, **post-translational modification**; **RMSD**, **root mean squared deviation**; **SWEET**, **sugar will eventually be exported transporter**; **THB**, **triple-helix bundle**; **TM**, **transmembrane domain**.

* Corresponding author. National Institute of Plant Genome Research, Aruna Asaf Ali Marg , PO Box Number 10531, 110067, New Delhi.
E-mail address: skmuthappa@nipgr.ac.in (M. Senthil-Kumar).

[1] These authors contributed equally.

https://doi.org/10.1016/j.plaphy.2020.08.043
Received 13 July 2020; Received in revised form 16 August 2020; Accepted 25 August 2020
Available online 29 August 2020

blight, where the TAL effector produced by the pathogen *Xanthomonas oryzae* targets the SWEET gene to drive sugars from the host to provide nutrition to the pathogen (Chen et al., 2010). Several reports in other plant species have suggested that SWEETs are targeted by pathogens for their survival and multiplication (Asai et al., 2015; Chong et al., 2014; Cohn et al., 2014). Therefore, the effective modulation of SWEETs by plants is crucial to regulate the sugar flow at the site of infection to prevent pathogen multiplication.

Despite the considerable advancement in structural and molecular studies related to SWEETs, the underlying regulatory mechanisms of these transporters from transcriptional to post-translational levels are still unexplored. Several studies elucidating the modulation of various sugar transporters in plants have hinted at the regulation of transporters at the post-translational level. A recent report showed the regulatory mechanism of sugar transporter protein 13 (STP13) via phosphorylation for antibacterial defense in *A. thaliana* (Yamada et al., 2016). It may accordingly be inferred that SWEET proteins may also undergo several post-translational modifications (PTMs) for their regulation. Another report showed the regulation of plant sucrose transporter 2 (SUC2) via ubiquitination and phosphorylation (Xu et al., 2020). These findings point to phosphorylation as one of the most common regulatory gears for these transporters, and it is therefore plausible that SWEETs also undergo phosphorylation. This highlights the need to gain an in-depth understanding of the SWEET protein structure to predict the possible regulatory mechanisms. We therefore focused our analyses on identifying the potential sites for amino acid modification through *in silico* prediction. In this review, we describe the structure of SWEETs, their organization in the membrane, and their roles in recognition and substrate binding. In addition, we speculate the possible regulatory mechanisms for SWEETs based on reports on the regulation of different sugar transporters.

2. Structure of SWEETs

To date, the crystal structures of many sugar transporters have been solved. Different families of membrane transporters share a common topology required for the proper functioning and stability of membrane transporters (Jaehme et al., 2015). Structure analysis of SWEETs revealed the 3-1-3 topology (Chen et al., 2010), with 3TM repeats containing related amino acid sequences. The 3TM domain carries a highly conserved proline and glutamine motifs; therefore, SWEETs are referred to as PQ-loop family transporters (Faham et al., 2008). This domain was first identified in the legume *Medicago truncatula*, hence also named as MtN3 family transporters (Gamas et al., 1996). In-depth structural analysis was enabled once the crystal structure of *Oryza sativa* SWEET2 (OsSWEET2) was solved (Tao et al., 2015). The findings suggested that each triple-helix bundle (THB) has a characteristic 1-3-2 helix arrangement, similar to that of SemiSWEET THB (Cheung et al., 2015; Lee et al., 2015; Wang et al., 2014; Xu et al., 2014). The bacterial semiSWEET consisting only single THB, shows an intimate interaction between TM2-TM3 and TM1-TM3 with no less contact of TM1 and TM2, this interaction provides 1-3-2 arrangement to the semiSWEETs (Feng and Frommer, 2015). The major facilitator superfamilies (MFSs) depict a similar type of 1-3-2 structural arrangement of the helix (Wang et al., 2014; Yan, 2013). Further, the TM4 or the linker helix is inversely fused between the 3TM repeats, which leads to the parallel orientation of both THBs. The SWEET homologs in prokaryotes consist only of a single THB, while the eukaryotic SWEETs are consisting of 2 parallelly oriented THB, covalently fused by an inversion TM helix named as linker. This fusion of 2THB via linker give rise to an asymmetrical transport route to SWEETs for accommodation of various substrate (Feng and Frommer, 2015). The sequence of the linker helix is found to be non-conserved among the other members of the SWEET family. The N-terminal domain of the transporter consists of TM-4 and THB1, while the C-terminal domain consists mainly of THB2. The crystal structure study of OsSWEET2b has clearly shown that the linker helix lies closer to THB1 and barely contacts THB2 (Tao et al., 2015). Based on the phosphorylation of the membrane transporters that mainly occur at the C-terminal, it is predicted that the C-terminal domain faces the cytosolic side (Niittylä et al., 2007 7), while the N-terminal of the transporter faces the periplasmic side.

Through *in silico* modeling using SWISS-MODEL (Waterhouse et al., 2018), the orthogonal and surface structures of SWEET2 of *A. thaliana* (AtSWEET2), belonging to clade I, have been elucidated, clearly depicting the 3-1-3 topology of SWEETs in *A. thaliana* (Fig. 1A and Supplementary Table 1). Overlapping structures of AtSWEETs belonging to four different clades, including AtSWEET2, AtSWEET8, AtSWEET12, and AtSWEET17, were generated (Fig. 1B). Next, the structures of SWEETs in crop plants, including *Solanum tuberosum* and *Oryza punctata*, were generated using AtSWEET13 (PDB ID: 5XPD) as a template (Fig. 1C and D). Structural analysis of OsSWEET2b revealed an inward (cytosolic) open conformation of SWEETs (Tao et al., 2015). Structural comparison of AtSWEET2 and *S. tuberosum* SWEET (StSWEET) by superpositioning with the solved crystal structure of both AtSWEET13 and OsSWEET2b clearly showed the structures to be well aligned. Moreover, the analysis showed AtSWEET2 to be more similar to OsSWEET, with a root mean squared deviation (RMSD) of 0.151 Å. However, StSWEET and *O. punctata* SWEET showed structural similarity with AtSWEET13, with RMSDs of 0.074 Å and 0.331 Å, respectively. This suggests that model and crop plants share high similarity in the structure of SWEETs (Supplementary Fig. 1).

Besides, a few highly conserved and important amino acid residues were identified by creating mutations. A study by Han et al. (2017) revealed the presence of Tyr61 on TM2, Tyr183 on TM6, and Asp189 on loop 6–7 in AtSWEET13. A hydrogen bond is formed between the carboxyl side chain of Asp189 and tyrosine residues. Y61A and D189A mutations reduced the transportation activity, clarifying that the interaction among the residues plays an essential role in forming the functional transporter. The proline tetrad is another conserved and important residue. Prolines are thought to be associated with the structural rearrangement of the transporter. It acts as a hinge for gating the transport pathway. Moreover, compared to other heptahelical transporters like G-protein–coupled receptor (GPCR), Pnu transporter, etc., SWEETs show a completely different spatial transmembrane arrangement, which makes them an interesting class of transporters to investigate further (Jaehme et al., 2014, 2015; Yee et al., 2013).

3. Oligomerization of SWEETs

Oligomerization allows the formation of the functional pore that permits the efficient movement of sugar substrate. The crystal structure of a few SemiSWEETs and SWEETs revealed that the dimerization of these proteins is required for forming a functional pore (Lee et al., 2015; Wang et al., 2014; Xu et al., 2014). The oligomerization is also reported for plant aquaporins or aquaglyceroporins in the form of homotetramer, but it is not necessary for forming a functional pore. The aquaporins or aquaglyceroporins consist of 6TM, which is sufficient to form a functional pore for the transport of water or sugar alcohol (Gomes et al., 2009). For bacterial SemiSWEETs 3TM is insufficient to form a functional pore for sucrose transport. Therefore, it was posited that for the formation of the functional pore, 3TM SemiSWEETs dimerize, similar to that of 6TM aquaporins in plant (Xuan et al., 2013). Moreover, based on the structural analysis of other sugar transporters carrying 12TM domains, it was hypothesized that the 7TM of SWEETs might not be sufficient for creating a functional pore (Xuan et al., 2013). The mating-based split ubiquitin assay for all the 17 AtSWEET members of *A. thaliana* revealed that eight members could homooligomerize, while 47 heteromer combinations were found (Lalonde et al., 2004 Xuan et al., 2013) *In planta* protein–protein interaction analyzed by a split green fluorescent protein assay revealed that AtSWEETs could homooligomerize (Xuan et al., 2013). It is noteworthy that the co-expression of a mutated and non-functional AtSWEET1 with a functional AtSWEET1

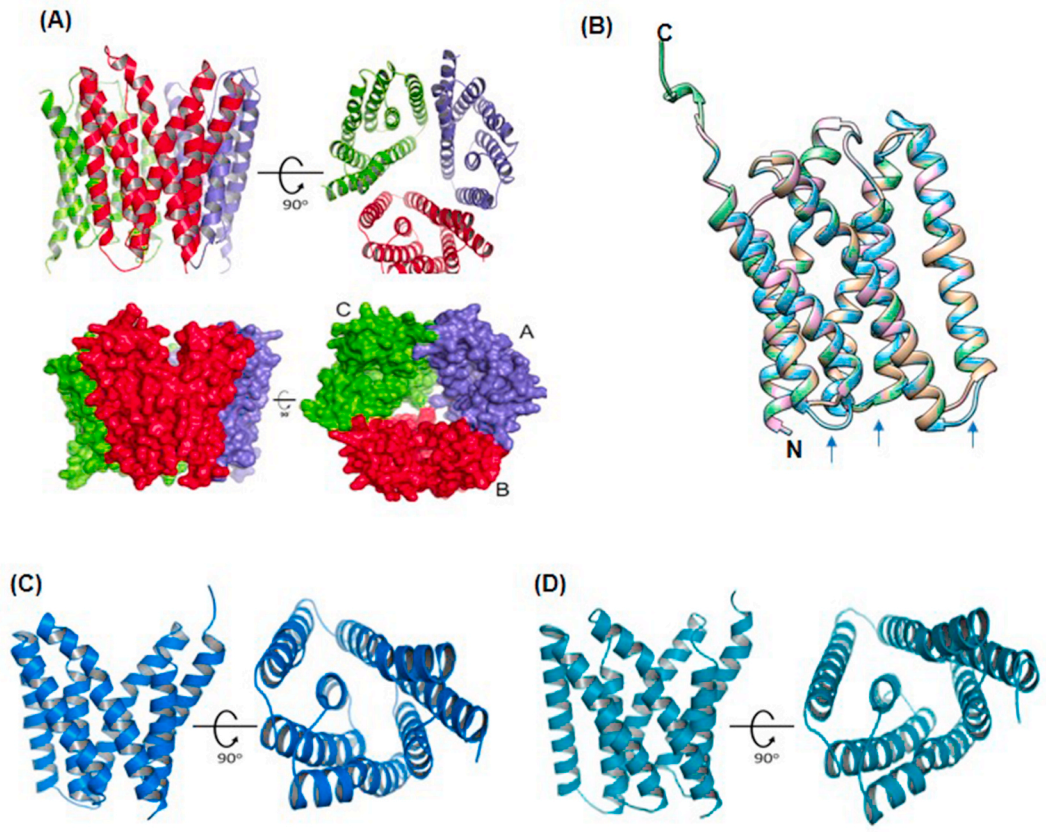

Figure 1. Structure of SWEETs in model and crop plants. (A) Orthogonal and surface view of AtSWEETs belonging to four different clades in Arabidopsis. Ribbon representation (above) and surface representation (below) of AtSWEET2 (clade I). **(B),** Overlap of modeled AtSWEET2 (clade I) in purple, AtSWEET8 (clade II) in green, AtSWEET12 (clade III) in blue, and AtSWEET17 (clade IV) in cyan. The structurally variable regions are indicated by arrows, and N- and C- terminals are marked. **(C–D),** Ribbon representation of SWEETs in crop plants **(C),** *Solanum tuberosum* and **(D),** *Oryza punctata*. The details regarding the topology of SWEETs are presented in the Supplementary Table 1.

inhibited the sugar transport activity. Similarly, another study showed that the oligomerization of the mutated form of OsSWEET11 (mSWEET11) with functional OsSWEET11 disrupted sugar transport activity (Gao et al., 2018). Thus, the homodimerization of SWEETs (SemiSWEETs tetramer) appears to be necessary for its transport function (Fig. 3).

We hypothesize that the oligomerization state of SWEETs might be involved in regulating sugar transport. In *A. thaliana*, Xuan et al. (2013)

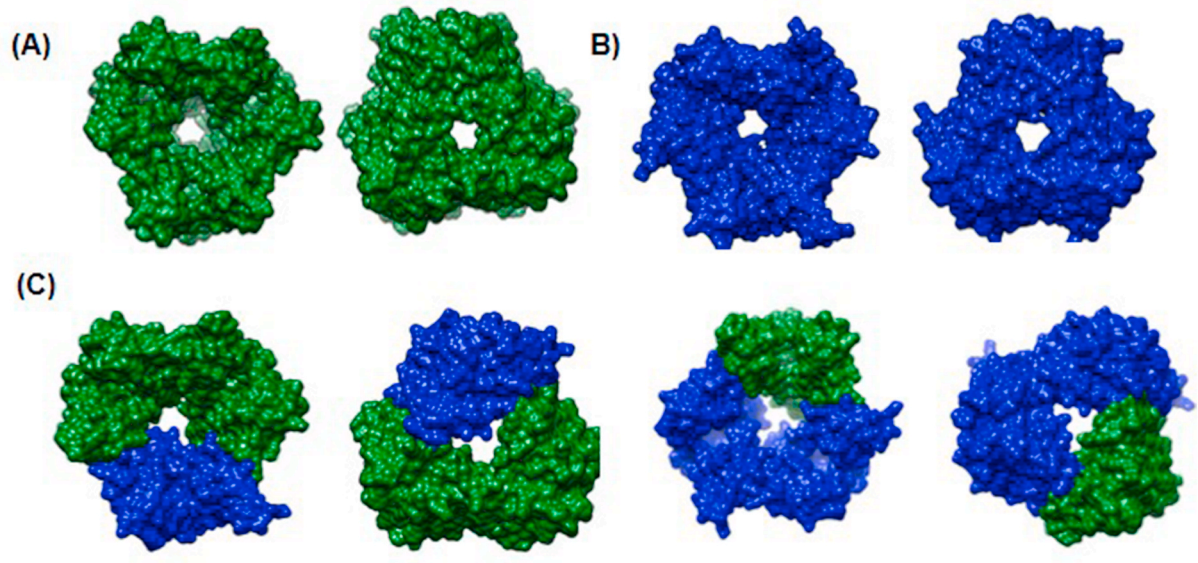

Fig. 2. Predicted structure of AtSWEET11 and AtSWEET12 homodimers and heterooligomers. (A–C), Surface view structure of **(A),** AtSWEET11 homooligomer **(B),** AtSWEET12 homooligomer **(C),** and AtSWEET11 and AtSWEET12 heterooligomer.

showed that two different AtSWEET monomers can also undergo heterooligomerization, including AtSWEET11 and AtSWEET12. Our *in silico* study revealed that homo- and heterooligomer structures of AtSWEET11 and AtSWEET12 transporters (Fig. 2) might be possible. These transporters are located in the plasma membrane of phloem parenchyma cells and are involved in sucrose transportation during phloem loading (Chen et al., 2012). Considering the location and role of AtSWEET11 and AtSWEET12 transporters, the oligomerization of both transporters might regulate their functioning. Although there are no studies available that can answer why SWEETs form heterodimers and how the heterodimerization affects the sugar transport activity in plants, a biochemical study showed that the heterooligomerization of SUT1 and SUT2 inhibited sucrose transport (Reinders et al., 2002). This leads us to predict that heterooligomerization of SWEETs might cease the transport activity and that it might be involved in regulating the sucrose transport dynamics to cope with abiotic or biotic stress.

4. Substrate specificity and binding pocket of SWEETs

Thus far, crystal structure and bioinformatics analyses of bacterial SemiSWEETs have revealed a lot regarding the architecture of the transport route and the mechanism of the transport (Guan et al., 2008; Lee et al., 2015; Xu et al., 2014). The transmembrane structure shields the transport route in such a way that it is only accessible from either the extracellular side in an outward open state or from the intracellular side in the inward open state (Feng and Frommer, 2015). The right substrate-binding pocket is present above the center of the transporter protein. Moreover, the size of the substrate-binding pocket varies for different bacteria. A large substrate-binding pocket may facilitate the transport of both disaccharides like, sucrose as well as monosaccharides like, glucose and fructose, while smaller sized pockets can only hold monosaccharides. Hence, the size of the pocket is very crucial for determining substrate specificity (Wang et al., 2014). It is interesting to note that SWEETs are capable of differentiating between mono- and disaccharides, and are involved in bidirectional transport of these sugars (Chen et al., 2010; Guo et al., 2014) (Supplementary Table 2).

Sequence analysis of 68 diverse SemiSWEETs revealed two highly conserved and essential residues in the substrate-binding pocket: Trp48 and Asp64. The role of these two residues was confirmed in sugar transport by mutagenesis studies carried out in two bacteria, *Leptospira biflexa* (LbSemiSWEET) and *Escherichia coli* (EcSemiSWEET) (Lee et al., 2015; Xu et al., 2014). These residues interact with sugar molecules via H-bonding or aromatic ring stacking. The substrate identified for LbSemiSWEET and EcSemiSWEET are glucose and sucrose respectively (Lee et al., 2015; Xu et al., 2014). Further, in OsSWEET2b, a conserved asparagine pair (Asn77 and Asn197) surrounds the binding pocket at the equivalent positions. In addition, the presence of Cys58 on THB1 and Phe181 on THB2 was reported in OsSWEET2b, while AtSWEET1 contains Ser54 and Trp176 at equivalent positions (Tao et al., 2015). When the Trp176 of AtSWEET1 was replaced by alanine, the transport activity was found to be abolished, but when replaced with phenylalanine, the transport activity was not affected. The substitution of Ser54 by alanine did not affect the transport activity. This suggests that the presence of one aromatic residue in THB2 instead of THB1 is important for transport activity, indicating the divergence of THBs in eukaryotic SWEETs to form an asymmetrical substrate-binding pocket. The substrate-binding pocket in SemiSWEETs is considered symmetrical, while in eukaryotic SWEETs it is highly asymmetrical.

Crystal structure study of AtSWEET1 has revealed an inward-facing conformation and the role of four major TM domains, TM1, 2, 5, and 6, in the formation of a transport route near the intrafacial side (Han et al., 2017). In addition, the presence of a proline tetrad in these domains plays a major role in structural rearrangement and conformational change of the transporters during transport activity. Replacing any of these prolines abolishes the sugar transport (Tao et al., 2015). Overall, the availability of the crystal structures of SemiSWEETs and eukaryotic SWEETs allowed us to better understand the structural features and the residues crucial for substrate-binding affinity and transport activity.

5. *In silico* prediction of SWEET regulation

PTMs, including phosphorylation, glycosylation, and ubiquitination, are involved in regulating the dynamics of protein structure and function in response to stimuli (Deribe et al., 2010). To date, the PTM via phosphorylation of the sugar transporter protein is the most common and well-studied regulatory mechanism. To identify the phosphorylation site, site-directed mutagenesis (Meier et al., 1997), mass spectrometry, and *in silico* prediction tools are being used. For *in silico* prediction of phosphorylation sites, several databases are available, such as UniProt, dbPTM (Lu et al., 2013), and PTMcode (Minguez et al., 2012).

Putative phosphorylation sites in a few members of AtSWEETs can be predicted using the NetPhos 3.1 server (http://www.cbs.dtu.dk/services/NetPhos/). On analyzing the topology of these transporters and the scores of NetPhos 3.1, we can find the potential S (serine)/T (threonine)/Y (tyrosine) residues present at the exposed C-terminal of AtSWEET transporters that may undergo phosphorylation (Supplementary Table 3). Further, through PyMOL (http://www.pymol.org/), the surface phosphorylation pattern of AtSWEET2 can be seen (Fig. 3). The number of residues that can undergo phosphorylation is predicted to be higher at the intrafacial C-terminal cytosolic side than at the intrafacial N-terminal cytosolic side. Therefore, these residues can be a putative site for phosphorylation and can be validated by analysing the available phosphoproteome profiles of plants and through other experimental studies. The large scale phosphoproteome profiling in *A. thaliana* revealed the phosphorylation of threonine residue located at 276 position of AtSWEET11 transporters (Reiland et al., 2009). This can be further validated by experimental analysis.

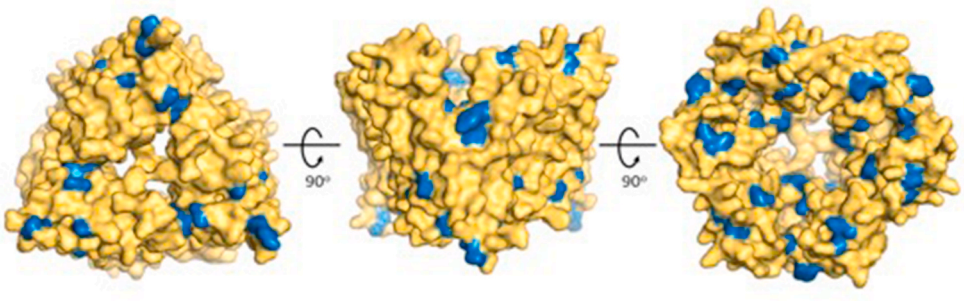

Fig. 3. Representative phosphorylation pattern of AtSWEETs in *Arabidopsis thaliana*. The surface phosphorylation pattern of AtSWEET2. The blue region represents the phosphorylated residues. The surface view of AtSWEETs is represented from intrafacial N- terminal cytosolic (left), extrafacial luminal (middle), and intrafacial C-terminal cytosolic (right) sides. The details of sequence analysis for phosphorylated residues are presented in Supplementary Table 3. The figures were generated using PyMOL 2.1.0. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

6. Regulatory mechanisms of other sugar transporters might reflect SWEET function

Sugar transporters are the major players in the distribution of photoassimilates to various heterotrophic sink organs. Therefore, they are required to be tightly regulated. Whenever plants experience any condition that enhances the rate of photosynthesis, there is a simultaneous demand for increased phloem loading to maintain the balance between the source and the sink (White et al., 2016). Plants are constantly exposed to fluctuating environments, which might affect transport activity. Besides abiotic modulations, plants are challenged by pathogen attacks, and it is already established that these sugar transporters are the prime target for many foliar pathogens once they colonize inside the host. Therefore, plants must regulate sugar transporters at various levels in a manner to minimize the leakage of metabolites towards the pathogens. Under all biotic stresses, plants tend to minimize the nutrient competition between its own tissues and the pathogens (Wang et al., 2012). Key sugar transporters such as SWEETs, SUCs, and STPs are connected to the sugar flux of plants but are not reported to be directly interconnected to each other. We speculate that the regulation mechanism of SUCs and STPs may influence the functioning of SWEETs directly or indirectly, or SWEETs might get modulated via some mechanisms similar to these two transporters.

Recently, the regulation of AtSTP13 in *A. thaliana* was investigated and showed the phosphorylation-dependent regulation of AtSTP13 to be critical for providing antibacterial defense (Yamada et al., 2016). AtSTP13 exists in a complex with FLS2 (leucine-rich repeat receptor of flg22 in *A. thaliana*) and a leucine-rich repeat-receptor kinase, BRASSINOSTEROID INSENSITIVE 1 associated receptor kinase (BAK-1) after flg22 treatment (Yamada et al., 2016). Sequence analysis of STP13 revealed the presence of many serine/threonine residues at its C-terminal. T485 is the site for BAK1-mediated phosphorylation in STP13. In a nutshell, the phosphorylation of STP13 at T485 position is very crucial for regulating the sugar content in the apoplast, which leads to the starvation of apoplast-colonized bacteria and hence confers resistance to the host plant. Investigations to ascertain the regulatory mechanisms of the sucrose transporter AtSUC2 in *A. thaliana* have shown that it contributes to phloem loading (Barberon et al., 2011; Gottwald et al., 2000; Jones et al., 2014; Rottmann et al., 2018; Stadler and Sauer, 1996; Truernit and Sauer, 1995). AtSUC2 is involved in the uptake of sucrose, which is effluxed by SWEETs in the apoplast (Chen et al., 2012). The phos-tag assays have shown that SUC2 can be phosphorylated. The yeast two hybrid (Y2H) assay of SUC2 predicted its interaction with a number of kinases (Jones et al., 2014), out of which WAKL8 has been verified to be a central player in the regulation of AtSUC2 via phosphorylation (Xu et al., 2020). Thus, the regulation of AtSTP13 and AtSUC2 occurs through phosphorylation that in turn controls the sugar levels in the apoplast during biotic and abiotic stresses.

7. Conclusion and prospects

Recent research utilizing high-throughput techniques have rapidly advanced the current knowledge base regarding SWEETs. Various mutant analyses have uncovered the functions of different SWEET members, elucidating how these transporters are deeply involved in diverse physiological processes of plants. Their potential role in sugar translocation, seed filling, and nectar secretion make them pivotal plant transporters. The identification of SWEET homologs in various life forms and the structural clarification of their bacterial homologs, SemiSWEETs, have shed light on the evolutionary process of these transporters. Under biotic stress, pathogens residing inside the apoplast target SWEETs for facilitating sugar flow in the apoplast to maintain their population. Despite the advancement in the learnings about these transporters, many questions remain unanswered. For instance, it is still unclear how SWEETs regulate sugar flux under various stresses and adverse conditions. There must exist tight regulatory mechanisms to control substrate transportation according to the milieu. To this end, the regulatory mechanisms of other sugar transporters (AtSUC2 and AtSTP13) and our *in silico* analysis provide clues that SWEET transporters might also be regulated via phosphorylation. Second, *in silico* analysis of AtSWEET11 and AtSWEET12 yielded putative sites for phosphorylation, which are required to be tested experimentally, and crystal structure studies of AtSWEET13 and OsSWEET11 have enhanced current information on the details of SWEET structure. However, what makes these transporters recognize their specific substrate is yet to be clarified. Spectroscopic techniques such as nuclear magnetic resonance and Förster resonance energy transfer and detailed crystal structure study of all SWEET members will be key to decipher the dynamics of these proteins, substrate specificity, and the transport mechanism. For analyzing the entire regulatory network, the first step will be the identification of the interacting partners of SWEETs. Finding the answers to these with the help of advanced structural biology combined with molecular biology will definitely provide a promising new way to understand the regulatory mechanisms of SWEETs. Dissecting the regulatory mechanism of SWEETs will be a masterstroke in understanding the source–sink sugar balance, competition between pathogen and host for carbon source, and most importantly, will provide an opportunity to manipulate the sugar transport pathway for increasing crop productivity.

Credit author contribution statement

Anjali Anjali: Writing - original draft. **Urooj Fatima:** Writing - review & editing. **M.S. Manu:** Writing - contributed to figures & tables. **Sureshkumar Ramasamy:** Writing - contributed to figures & tables. **Muthappa Senthil-Kumar:** Writing - original draft, review & editing, Conceptualization.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Acknowledgment

The SWEET transporter project at MS-K's lab was funded by NIPGR core funding. UF acknowledges the DBT-SRF fellowship (DBT/2013/NIPGR/68) and NIPGR-SRF fellowship. Anjali acknowledges the CSIR fellowship (09/803(0168)/2019-EMR-I_367532).

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.plaphy.2020.08.043.

References

Asai, Y., Kobayashi, Y., Kobayashi, I., 2015. Increased expression of the tomato SISWEET15 gene during grey mold infection and the possible involvement of the sugar efflux to apoplasm in the disease susceptibility. J. Plant Pathol. Microbiol. 7, 329.

Barberon, M., Zelazny, E., Robert, S., Conéjéro, G., Curie, C., Friml, J., Vert, G., 2011. Monoubiquitin-dependent endocytosis of the iron-regulated transporter 1 (IRT1) transporter controls iron uptake in plants. Proc. Natl. Acad. Sci. U.S.A. 108 (32), E450–E458.

Chen, L.Q., Hou, B.H., Lalonde, S., Takanaga, H., Hartung, M.L., Qu, X.Q., Chermak, D., 2010. Sugar transporters for intercellular exchange and nutrition of pathogens. Nature 468 (7323), 527–532.

Chen, H.Y., Huh, J.H., Yu, Y.C., Ho, L.H., Chen, L.Q., Tholl, D., et al., 2015. The *Arabidopsis* vacuolar sugar transporter SWEET 2 limits carbon sequestration from roots and restricts Pythium infection. Plant J. 83 (6), 1046–1058.

Chen, L.Q., Qu, X.Q., Hou, B.H., Sosso, D., Osorio, S., Fernie, A.R., Frommer, W.B., 2012. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science 335 (6065), 207–211.

Cheung, L.S., Li, S., Eom, J.S., Chen, L.Q., Xu, Y., Feng, L., 2015. Structure of a eukaryotic SWEET transporter in a homotrimeric complex. Nature *527* (7577), 259–263.
Chong, J., Piron, M.C., Meyer, S., Merdinoglu, D., Bertsch, C., Mestre, P., 2014. The SWEET family of sugar transporters in grapevine: VvSWEET4 is involved in the interaction with *Botrytis cinerea*. J. Exp. Bot. 22, 6589–6601.
Cohn, M., Bart, R.S., Shybut, M., Dahlbeck, D., Gomez, M., Morbitzer, R., 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), 1186–1198.
Deribe, Y.L., Pawson, T., Dikic, I., 2010. Post-translational modifications in signal integration. Nat. Struct. Mol. Biol. 17 (6), 666–672.
Eom, J.S., Chen, L.Q., Sosso, D., Julius, B.T., Lin, I.W., Qu, X.Q., Frommer, W.B., 2015. SWEETs, transporters for intracellular and intercellular sugar translocation. Curr. Opin. Plant Biol. 25, 53–62.
Faham, S., Watanabe, A., Besserer, G.M., Cascio, D., Specht, A., Hirayama, B.A., Abramson, J., 2008. The crystal structure of a sodium galactose transporter reveals mechanistic insights into Na+/sugar symport. Science 321 (5890), 810–814.
Feng, L., Frommer, W.B., 2015. Structure and function of SemiSWEET and SWEET sugar transporters. Trends Biochem. Sci. 40 (8), 480–486.
Gamas, P., Niebel, Fde C., Lescure, N., Cullimore, J., 1996. Use of a subtractive hybridization approach to identify new *Medicago truncatula* genes induced during root nodule development. Mol. Plant Microbe Interact. 9, 233–242.
Gao, Y., Zhang, C., Han, X., Wang, Z.Y., Ma, L., Yuan, D.P., Wu, J.N., Zhu, X.F., Liu, J.M., Li, D.P., Hu, Y.B., Xuan, Y.H., 2018. Inhibition of OsSWEET11 function in mesophyll cells improves resistance of rice to sheath blight disease. Mol. Plant Pathol. 19, 2149–2161.
Gomes, D., Agasse, A., Thiébaud, P., Delrot, S., Gerós, H., Chaumont, F., 2009. Aquaporins are multifunctional water and solute transporters highly divergent in living organisms. BBA-Biomembranes 1788 (6), 1213–1228.
Gottwald, J.R., Krysan, P.J., Young, J.C., Evert, R.F., Sussman, M.R., 2000. Genetic evidence for the in-planta role of phloem-specific plasma membrane sucrose transporters. Proc. Natl. Acad. Sci. U.S.A. 97 (25), 13979–13984.
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, 852–863.
Guo, W.J., Nagy, R., Chen, H.Y., Pfrunder, S., Yu, Y.C., Santelia, D., et al., 2014. SWEET17, a facilitative transporter, mediates fructose transport across the tonoplast of Arabidopsis roots and leaves. Plant Physiol. 164 (2), 777–789.
Han, L., Zhu, Y., Liu, M., Zhou, Y., Lu, G., Lan, L., Zhang, X.C., 2017. Molecular mechanism of substrate recognition and transport by the AtSWEET13 sugar transporter. Proc. Natl. Acad. Sci. U.S.A. 114 (38), 10089–10094.
Jaehme, M., Guskov, A., Slotboom, D.J., 2014. Crystal structure of the vitamin B3 transporter PnuC, a full-length SWEET homolog. Nat. Struct. Mol. Biol. 21, 1013–1015.
Jaehme, M., Guskov, A., Slotboom, D.J., 2015. The twisted relation between Pnu and SWEET transporters. Trends Biochem. Sci. 40 (4), 183–188. https://doi.org/10.1016/j.tibs.2015.02.002.
Jones, A.M., Xuan, Y., Xu, M., Wang, R.S., Ho, C.H., Lalonde, S., Su, T., 2014. Border control—a membrane-linked interactome of Arabidopsis. Science 344 (6185), 711–716.
Lalonde, S., Wipf, D., Frommer, W.B., 2004. Transport mechanisms for organic forms of carbon and nitrogen between source and sink. Annu. Rev. Plant Biol. 55, 341–372.
Lee, Y., Nishizawa, T., Yamashita, K., Ishitani, R., Nureki, O., 2015. Structural basis for the facilitative diffusion mechanism by SemiSWEET transporter. Nature comm 6, 6112.
Lin, I.W., Sosso, D., Chen, L.Q., Gase, K., Kim, S.G., Kessler, D., et al., 2014. Nectar secretion requires sucrose phosphate synthases and the sugar transporter SWEET9. Nature 508 (7497), 546–549.
Lu, C.T., Huang, K.Y., Su, M.G., Lee, T.Y., Bretana, N.A., Chang, W.C., Huang, H.D., 2013. DbPTM 3.0: an informative resource for investigating substrate site specificity and functional association of protein post-translational modifications. Nucleic Acids Res. 41, D295–D305.
Meier, R., Alessi, D.R., Cron, P., Andjelković, M., Hemmings, B.A., 1997. Mitogenic activation, phosphorylation, and nuclear translocation of protein kinase Bβ. J. Biol. Chem. 272 (48), 30491–30497.
Minguez, P., Letunic, I., Parca, L., Bork, P., 2012. PTMcode: a database of known and predicted functional associations between post-translational modifications in proteins. Nucleic Acids Res. 41 (D1), D306–D311.
Niittylä, T., Fuglsang, A.T., Palmgren, M.G., Frommer, W.B., Schulze, W.X., 2007. Temporal analysis of sucrose-induced phosphorylation changes in plasma membrane proteins of Arabidopsis. Mol. Cell. Proteomics 6 (10), 1711–1726.
Reiland, S., Messerli, G., Baerenfaller, K., Gerrits, B., Endler, A., Grossmann, J., Gruissem, W., Baginsky, S., 2009. Large-scale Arabidopsis phosphoproteome profiling reveals novel chloroplast kinase substrates and phosphorylation networks. Plant Physiol 150 (2), 889–903.
Reinders, A., Schulze, W., Kühn, C., Barker, L., Schulz, A., Ward, J.M., Frommer, W.B., 2002. Protein–protein interactions between sucrose transporters of different affinities colocalized in the same enucleate sieve element. Plant Cell 14 (7), 1567–1577.
Rottmann, T.M., Fritz, C., Lauter, A., Schneider, S., Fischer, C., Danzberger, N., Stadler, R., 2018. Protoplast-esculin assay as a new method to assay plant sucrose transporters: Characterization of AtSUC6 and AtSUC7 sucrose uptake activity in Arabidopsis Col-0 ecotype. Front. Plant Sci. 9, 430.
Stadler, R., Sauer, N., 1996. The *Arabidopsis thaliana* AtSUC2 gene is specifically expressed in companion cells. Bot. Acta 109 (4), 299–306.
Sun, M., Huang, X., Yang, J., et al., 2013. Arabidopsis RPG1 is important for primexine deposition and functions redundantly with RPG2 for plant fertility at the late reproductive stage. Plant Reprod. 26, 83–91. https://doi.org/10.1007/s00497-012-0208- Tao, Y.
Tao, Y., Cheung, L.S., Li, S., Eom, J.S., Chen, L.Q., Xu, Y., et al., 2015. Structure of a eukaryotic SWEET transporter in a homotrimeric complex. Nature 527 (7577), 259–263.
Truernit, E., Sauer, N., 1995. The promoter of the *Arabidopsis thaliana* SUC2 sucrose-H+ symporter gene directs expression of β-glucuronidase to the phloem: evidence for phloem loading and unloading by SUC2. Planta 196 (3), 564–570.
Wang, K., Senthil-Kumar, M., Ryu, C.M., Kang, L., Mysore, K.S., 2012. Phytosterols play a key role in plant innate immunity against bacterial pathogens by regulating nutrient efflux into the apoplast. Plant Physiol. 158 (4), 1789–1802.
Wang, J., Yan, C., Li, Y., Hirata, K., Yamamoto, M., Yan, N., Hu, Q., 2014. Crystal structure of a bacterial homologue of SWEET transporters. Cell Res. 24 (12), 1486–1489.
Waterhouse, A., Bertoni, M., Bienert, S., Studer, G., Tauriello, G., Gumienny, R., Heer, F. T., de Beer, T.A.P., Rempfer, C., Bordoli, L., Lepore, R., Schwede, T., 2018. SWISS-MODEL: Homology modelling of protein structures and complexes. Nucleic Acids Res. 46 (W1), W296–W303.
White, A.C., Rogers, A., Rees, M., Osborne, C.P., 2016. How can we make plants grow faster? A source–sink perspective on growth rate. J. Exp. Bot. 67 (1), 31–45.
Xu, Y., Tao, Y., Cheung, L.S., Fan, C., Chen, L.Q., Xu, S., Perry, K., Frommer, W.B., Feng, L., 2014. Structures of bacterial homologues of SWEET transporters in two distinct conformations. Nature 515 (7527), 448–452. https://doi.org/10.1038/nature13670.
Xu, Q., Yin, S., Ma, Y., Song, M., Song, Y., Mu, S., Li, Y., Liu, X., Ren, Y., Gao, C., Chen, S., Liesche, J., 2020. Carbon export from leaves is controlled via ubiquitination and phosphorylation of sucrose transporter SUC2. Proc. Natl. Acad. Sci. U.S.A. 117 (11), 6223–6230.
Xuan, Y.H., Hu, Y.B., Chen, L.Q., Sosso, D., Ducat, D.C., Hou, B.H., Frommer, W.B., 2013. Functional role of oligomerization for bacterial and plant SWEET sugar transporter family. Proc. Natl. Acad. Sci. U.S.A. 110 (39) https://doi.org/10.1073/pnas.1311244110.
Yamada, K., Saijo, Y., Nakagami, H., Takano, Y., 2016. Regulation of sugar transporter activity for antibacterial defense in Arabidopsis. Science 354 (6318), 1427–1430.
Yan, N., 2013. Structural advances for the major facilitator superfamily (MFS) transporters. Trends Biochem. Sci. 38 (3), 151–159. https://doi.org/10.1016/j.tibs.2013.01.003.
Yee, D.C., Shlykov, M.A., Västermark, Å., Reddy, V.S., Arora, S., Sun, E.I., Saier Jr., M.H., 2013. The transporter–opsin–G protein-coupled receptor (TOG) superfamily. FEBS J. 280 (22), 5780–5800.