CORE
Metadata, citation and similar papers at core.ac.uk
Provided by DigitalCommons@University of Nebraska

University of Nebraska - Lincoln
DigitalCommons@University of Nebraska - Lincoln

Agronomy & Horticulture -- Faculty Publications   Agronomy and Horticulture Department

2015

Tonoplast Sugar Transporters (SbTSTs) Putatively Control Sucrose Accumulation in Sweet Sorghum Stems

Saadia Bihmidine
University of Missouri

Benjamin T. Julius
University of Missouri

Ismail M. Dweikat
University of Nebraska-Lincoln, idweikat2@unl.edu

David M. Braun
University of Missouri

Follow this and additional works at: http://digitalcommons.unl.edu/agronomyfacpub

Part of the Agricultural Science Commons, Agriculture Commons, Agronomy and Crop Sciences Commons, Botany Commons, Horticulture Commons, Other Plant Sciences Commons, and the Plant Biology Commons

Bihmidine, Saadia; Julius, Benjamin T.; Dweikat, Ismail M.; and Braun, David M., "Tonoplast Sugar Transporters (SbTSTs) Putatively Control Sucrose Accumulation in Sweet Sorghum Stems" (2015). *Agronomy & Horticulture -- Faculty Publications*. 888.
http://digitalcommons.unl.edu/agronomyfacpub/888

This Article is brought to you for free and open access by the Agronomy and Horticulture Department at DigitalCommons@University of Nebraska - Lincoln. It has been accepted for inclusion in Agronomy & Horticulture -- Faculty Publications by an authorized administrator of DigitalCommons@University of Nebraska - Lincoln.

Published in *Plant Signaling and Behavior* 11:1 (2016), e1117721 (7 pages). doi 101080155923242
Copyright © 2016 Taylor & Francis Group, LLC. Used by permission.
Submitted October 23, 2015; accepted November 4, 2015.

UNIVERSITY OF Nebraska Lincoln
digitalcommons.unl.edu

Tonoplast Sugar Transporters (*SbTSTs*) Putatively Control Sucrose Accumulation in Sweet Sorghum Stems

Saadia Bihmidine,[1] Benjamin T. Julius,[1] Ismail Dweikat,[2] and David M. Braun[1]

1 Division of Biological Sciences, Interdisciplinary Plant Group, and Missouri Maize Center, University of Missouri, 116 Tucker Hall, Columbia, MO, USA

2 Department of Agronomy and Horticulture, University of Nebraska–Lincoln, 365 Keim Hall, Lincoln, NE, USA

Abstract

Carbohydrates are differentially partitioned in sweet versus grain sorghums. While the latter preferentially accumulate starch in the grain, the former primarily store large amounts of sucrose in the stem. Previous work determined that neither sucrose metabolizing enzymes nor changes in *Sucrose transporter* (*SUT*) gene expression accounted for the carbohydrate partitioning differences. Recently, 2 additional classes of sucrose transport proteins, Tonoplast Sugar Transporters (TSTs) and SWEETs, were identified; thus, we examined whether their expression tracked sucrose accumulation in sweet sorghum stems. We determined 2 *TST*s were differentially expressed in sweet vs. grain sorghum stems, likely underlying the massive difference in sucrose accumulation. A model illustrating potential roles for different classes of sugar transport proteins in sorghum sugar partitioning is discussed.

Keywords: Carbohydrate partitioning, sorghum bicolor, sucrose, SWEETs, TSTs

Abbreviations: TSTs, Tonoplast Sugar Transporters; SUTs, Sucrose Transporters; qRT-PCR, quantitative reverse-transcription polymerase chain reaction

Sucrose harvested from plants represents a multi-billion dollar (US) annual industry, with great interest in expanding production for food and biofuel uses.[1-5] Multiple crops have been independently bred to store high concentrations of sucrose in terminal storage organs, namely, the taproots of sugar beet (Beta *vulgaris* L.), and the stems of sweet sorghum (*Sorghum bicolor* L. Moench) and sugarcane (*Saccharum officinarum* L.).[5-9] However, the sucrose contents of these crops appear to be approaching maximal levels attainable from breeding efforts[10,11]; therefore, new approaches are needed to increase sucrose accumulation in storage organs. Hence, characterizing the genes that function in sucrose transport and storage will reveal potential new targets for future manipulations to enhance crop yields.[3,4,12-16]

Different sorghum genotypes have been selectively bred to store carbohydrates in contrasting storage organs: sweet sorghums accumulate large quantities of soluble sugars, mostly sucrose, in stem tissues, whereas grain sorghums primarily store carbohydrates as starch in the seeds.[1,2,6,17,18] The molecular basis for the difference in carbohydrate partitioning between these sorghum types is unknown. Previous research found that sucrose accumulation within sweet sorghum stems was not correlated with the activities of enzymes involved in sucrose metabolism, invoking sucrose transport proteins as potentially controlling sucrose content.[19] Transport experiments using asymmetrically radio-labeled sucrose determined that sucrose movement into stems likely included an apoplasmic transport step.[20] Subsequent dye transport studies suggested the phloem tissues within sorghum stems are symplasmically isolated from surrounding tissues, supporting that sucrose phloem unloading occurs apoplasmically, and thus requires sucrose transport proteins.[21] However, other studies support a possible symplasmic transport route from phloem sieve elements to storage parenchyma cells in mature stems.[22] Quantitative reverse-transcription polymerase chain reaction (qRT-PCR) analyses indicated that *Sucrose Transporters* (*SUT*s), which function as H^+/sucrose symporters to transport sucrose across membranes, were not differentially expressed in the stem of a flowering sweet sorghum line, UNL71-2011, a sweet sorghum derived from cultivar Wray, in comparison to a similarly staged grain sorghum line, UNL3016, selected from cultivar Macia.[21] These data suggest other types of sucrose transport proteins may underlie sucrose accumulation within sorghum stem tissues.

Two additional distinct classes of sucrose transport proteins have recently been described. SWEETs are a family of sugar transport proteins, with different family members preferentially transporting hexoses or sucrose.[23-28] Clade III SWEET proteins, which localized to the plasma membrane, have been proposed to function as uniporters that facilitate the transport of sucrose down a concentration gradient. A different class of sugar transport proteins, Tonoplast Sugar Transporters (TSTs, also known as Tonoplast Monosaccharide Transporters), is located on the tonoplast and function a H^+/sucrose antiporters to transport sucrose into the vacuole.[29-32] Recently, a TST was shown to be responsible for sucrose accumulation within the sugar beet taproot.[12] Whether *TST* or *SWEET* genes have also been selected during the domestication of other major sucrose storage crops, such as sweet sorghum or sugarcane stem tissues, is not known. Since *SbSUT* genes were not differentially expressed between sweet and grain sorghum stem tissues,21 we decided to examine the expression of other predicted sucrose transport proteins, specifically, the clade III *SbSWEET* and the *SbTST* genes. For these studies, we compared gene expression between the sweet sorghum line UNL71-2011 at anthesis, when sugars are actively accumulating in the stem, with the equally staged grain sorghum line UNL3016, with low stem sugar content, to determine if any *SbSWEET* or *SbTST* genes are associated with sucrose accumulation in stem tissues. These lines are herein simply referred to as sweet and grain sorghums for clarity. As a point of reference, we found that the total solute levels, consisting primarily of sucrose, increased approximately 24-fold in sweet sorghum stems compared with grain sorghum stems during the ripening process from anthesis to physiological maturity.[21]

Bioinformatic analyses were used to identify *SbTST* and *SbSWEET* genes in the sorghum genome. Three *SbTST* genes and 20 *SbSWEET* genes were identified.[24] We next analyzed a sorghum gene expression database to determine which of these genes were expressed in leaf and stem tissues (Table 1).[33] *SbSWEET13A* was the most strongly expressed clade III gene within these tissues. Its expression was more than 10-fold higher than other clade III sweet genes; *SbSWEET13B* and *SbSWEET13C* had lower, but appreciable, expression relative to *SbSWEET13A*. The other clade III *SbSWEET* genes likely to transport sucrose were all very lowly or not detectably expressed (Table 1). Therefore, we selected the 3 *SbSWEET13* genes and all *SbTST* genes for further expression analyses. Gene-specific qRT-PCR primer sets were validated for each gene (Table 2). The qRT-PCR experiments and statistical analyses were performed as previously described.[21] In examining mature leaf and ripening stem tissues of both grain and sweet sorghum, we determined that *SbTST1*, *SbTST2*, and *SbSWEET13A* were reliably expressed, whereas *SbTST3*, *SbSWEET13B*, and *SbSWEET13C* were expressed at a much lower level (Figs 1–2). *SbSWEET13B* expression was at least 33-fold less than *SbSWEET13A* in all tissues examined. Similarly,

Table 1. The expression level of the SbTST and SbSWEET genes by RNA-seq in the leaf and stem tissues. The numbers represent the average expression obtained from the FPKM (fragments per kilobase of transcript per million mapped reads) plots.

Gene name	Phytozome reference no.	Gene ID.	Leaf	Stem
SbTST1	Sobic.001G312900	Sb01G030430	105	149
SbTST2	Sobic.004G099300	Sb04G008150	110	130
SbTST3	Sobic.010G276100	Sb10g031000	Not detected	Not detected
SbSWEET11A[III]	Sobic.007G191200	Sb07g026040	Not detected	10
SbSWEET11B[III]	Sobic.002G259300	Sb02g029430	Not detected	Not detected
SbSWEET12[III]	Sobic.001G373600	Sb01g035490	Not detected	Not detected
SbSWEET13A[III]	Sobic.008G094000	Sb08g013620	2250	200
SbSWEET13B[III]	Sobic.008G094300	Sb08g013840	28	2
SbSWEET13C[III]	Sobic.008G094400	Sb08g014040	120	20
SbSWEET14[III]	Sobic.005G123500	Sb05g018110	1	3
SbSWEET15[III]	Sobic.004G157100	Sb04g021000	Not detected	1
SbSWEET16[IV]	Sobic.001G377600	Sb01g035840	Not detected	Not detected
SbSWEET1A[I]	Sobic.003G377700	Sb03g041740	100	135
SbSWEET1B[I]	Sobic.009G143500	Sb09g020860	82	2
SbSWEET2A[I]	Sobic.003G182800	Sb03g024250	15	8
SbSWEET2B[I]	Sobic.003G269300	Sb03g032190	75	4
SbSWEET3A[I]	Sobic.009G080900	Sb09g006950	135	300
SbSWEET3B[I]	Sobic.003G015200	Sb03g001520	Not detected	1
SbSWEET4A[II]	Sobic.004G136600	Sb04g015420	85	35
SbSWEET4B[II]	Sobic.004G133500	Sb04g012910	20	30
SbSWEET4C[II]	Sobic.004G133600	Sb04g012920	Not detected	Not detected
SbSWEET5[II]	Sobic.009G252000	Sb09g030270	Not detected	Not detected
SbSWEET6[II]	Sobic.003G213000	Sb03g027260	30	10

The numbers are estimated from the FPKM plots obtained from the MOROKOSHI sorghum transcriptome database (http://sorghum.riken.jp/morokoshi).[33] The different upper-case roman letter superscripts indicate the clade to which each SbSWEET gene belongs. Not detected means that the gene had no detectable RNAseq counts in the corresponding tissue. The underlined genes are those selected for this study.

Table 2. List of primers used.

Primer name	Primer sequence (50 – 30)	Product size (bp)
SbTST1-F	GATGGGCTGACCTGTTTG	175
SbTST1-R	GCAGAAGATGCGCTAAGG	175
SbTST2-F	TTGGAGGTTGGAGGAGAC	150
SbTST2-R	CTTGGAAGGTCGAGCAATC	150
SbTST3-F	CTGTTGCTTCGTCATGGG	146
SbTST3-R	TGACAGGAAGAGAGTAGGTG	146
SbSWEET13A-F	CGCTCACTACTGCTAAGTATTAT	96
SbSWEET13A-R	ACAGTAGTCTGGGATCGATTA	96
SbSWEET13B-F	CATGAGTCGAGTCCGAATG	116
SbSWEET13B-R	AGCTACGGTTGGATAAACG	116
SbSWEET13C-F	ACCCGTTTATCCAACCCTTAG	87
SbSWEET13C-R	TGAAATTCCTGCCTGGTTACA	87
Luciferase-F	CCAGGGATTTCAGTGGATGT	183
Luciferase-R	AATCTGACGCAGGCAGTTCT	183

SbSWEET13C was expressed at least 20-fold less than *SbSWEET13A* in leaves of both cultivars. *SbSWEET13C* was expressed approximately 6.7- and 29-fold lower than *SbSWEET13A* in grain and sweet sorghum stems, respectively; however, *SbSWEET13C* exhibited lower expression in sweet sorghum stems compared to grain sorghum stems, which is the opposite of what was hypothesized if the gene functioned to promote sucrose accumulation in sweet sorghum stems. Based on these results, we conclude that *SbTST3*, *SbSWEET13B*, and *SbSWEET13C* are minimally expressed in mature leaf and ripening stem tissues and therefore do not likely contribute substantially to sucrose accumulation within these tissues.

Based on the previous results, we examined *SbTST1*, *SbTST2*, and *SbSWEET13A* to determine if they are differentially expressed between sweet and grain sorghum leaf and stem tissues. In mature leaves, *SbTST1* and *SbTS2* showed ~3.5-fold and ~7.4-fold higher expression levels in sweet sorghum relative to grain sorghum ($p \leq 0.05$; Fig. 3A, B). Within stem tissues at anthesis, *SbTST1* and *SbTST2* showed significantly higher expression levels in

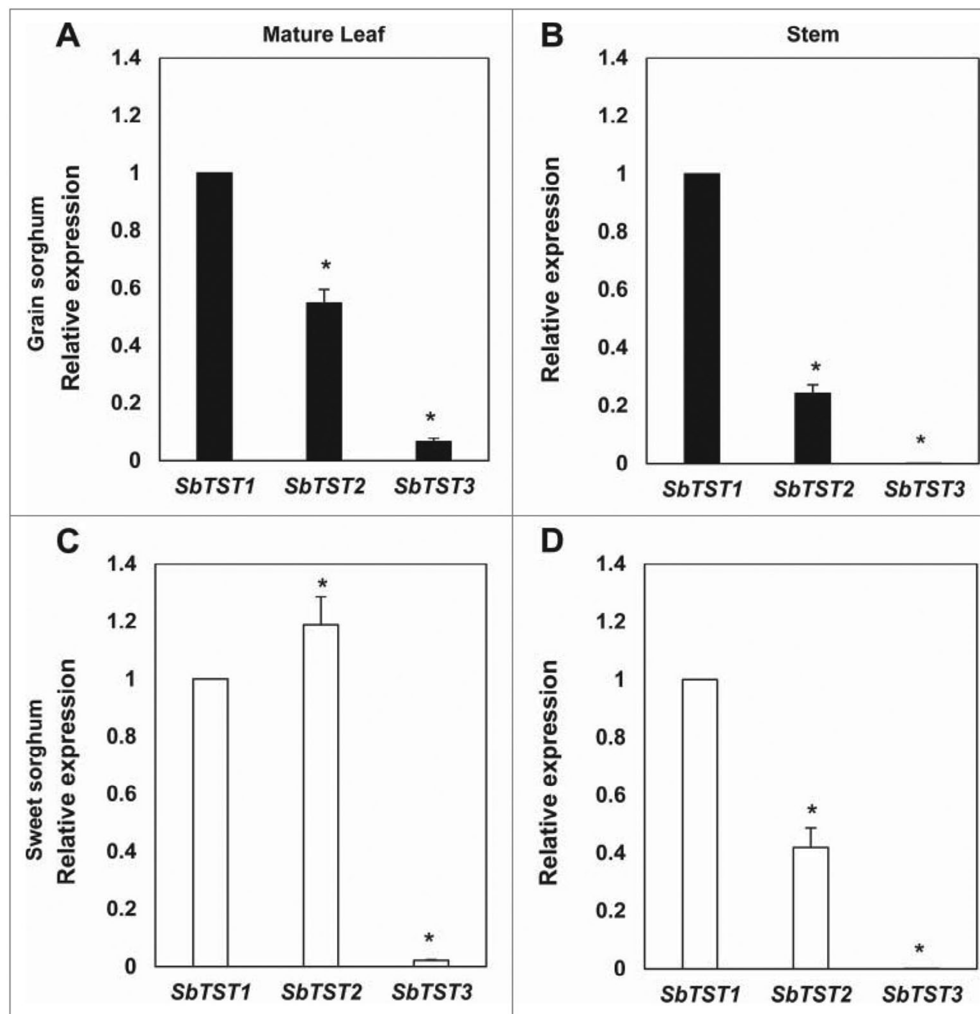

Figure 1. Expression levels of *SbTST2* and *SbTST3* relative to *SbTST1* in grain and sweet sorghum mature leaves and stems. A, B show grain sorghum (black bars), and C, D show sweet sorghum (white bars); A, C are mature leaf tissues, and B, D are flowering stems. Values are means ± standard error of N = 5 plants, and an asterisk indicates significantly different means between the 2 genes at $p \leq 0.05$. Relative gene expression is shown compared to exogenously added Luciferase RNA as a normalization control.[21]

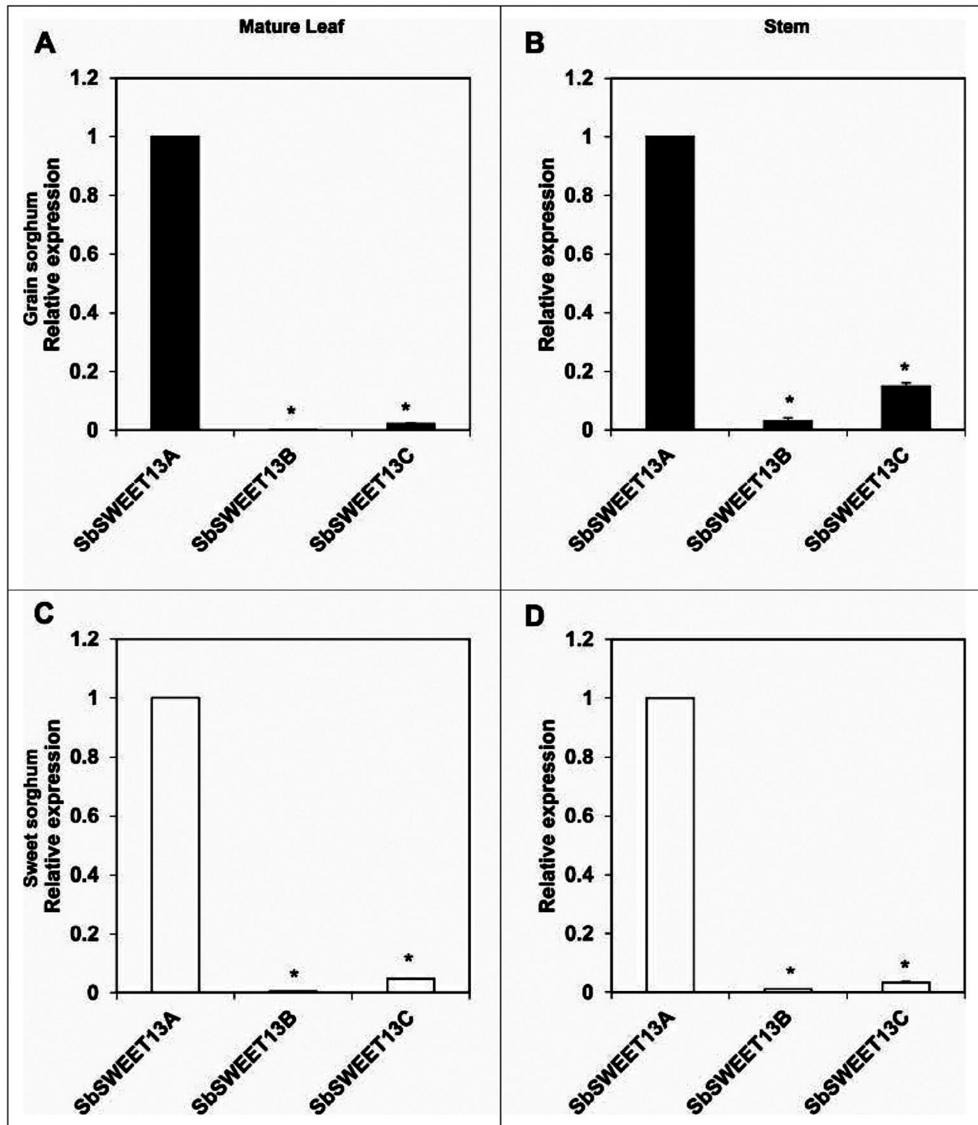

Figure 2. Expression levels of *SbSWEET13B* and *SbSWEET13C* relative to *SbSWEET13A* in grain and sweet sorghum mature leaves and stems. A, B show grain sorghum (black bars), and C, D show sweet sorghum (white bars); A, C are mature leaf tissues, and B, D are flowering stems. Values are means § standard error of N = 5 plants, and an asterisk indicates significantly different means between the 2 genes at $p \leq 0.05$. Relative gene expression is shown compared to exogenously added Luciferase RNA as a normalization control.[21]

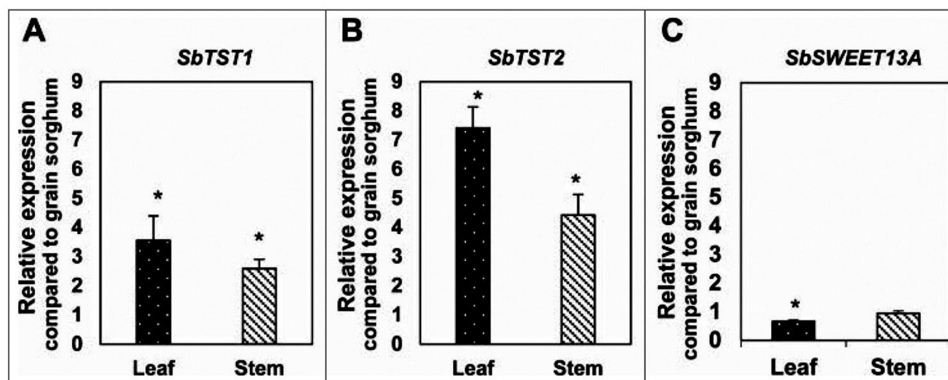

Figure 3. Expression levels of *SbTST1*, *SbTST2*, and *SbSWEET13A* in leaves and stems of sweet sorghum relative to grain sorghum. Expression levels are shown for *SbTST1* (A), *SbTST2* (B), and *SbSWEET13A* (C). An asterisk indicates significantly different means between the 2 lines at $p \leq 0.05$ of N = 5 plants.

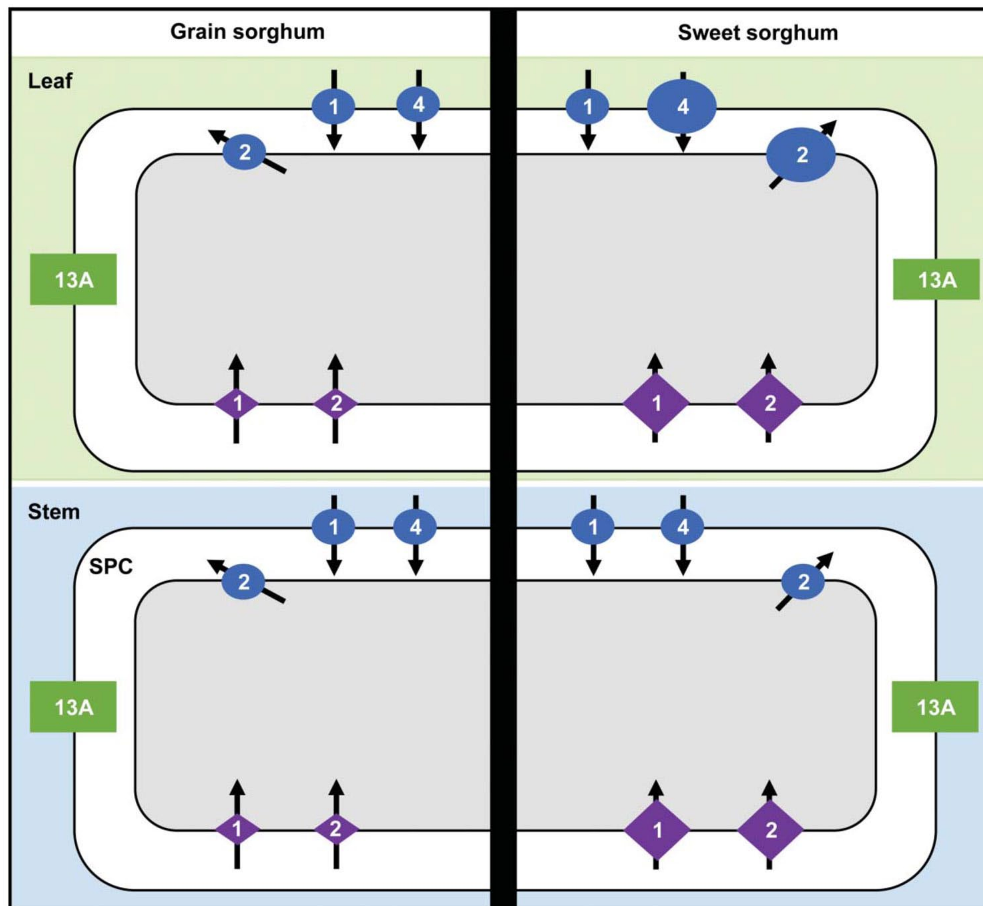

Figure 4. A model illustrates the roles for different sucrose transport proteins in sucrose movement across cellular membranes in sorghum leaf (green shaded background) and stem (blue shaded) tissues in grain sorghum (left) vs. sweet sorghum (right). The vacuole is shown in gray. SPC = stem parenchyma cell. SUT proteins are shown by a blue circle, with an arrow indicating the direction of sucrose movement, and the numbers correspond to SbSUT1, SbSUT2, or SbSUT4. Purple diamond with an arrow refers to a TST protein located on the tonoplast, and the numbers represent SbTST1 or SbTST2. The green boxes labeled 13A correspond to SbSWEET13A. The increased size of the shapes indicates increased expression of the corresponding gene in sweet (UNL 71-2011) vs. grain (UNL 3016) sorghum tissue.

sweet sorghum compared to grain sorghum (~2.6- and ~4.4-fold, respectively) (Fig. 3A, B). *SbSWEET13A* showed reduced expression in sweet compared to grain sorghum leaves and comparable expression in stem tissues of both genotypes (Fig. 3C). These data indicate *SbTST1* and *SbTST2* are significantly more highly expressed in leaves and stem tissues of sweet sorghum than in grain sorghum, and that *SbSWEET13A* expression was reduced in sweet sorghum leaves compared to grain sorghum but not differently expressed in stem tissues. Thus, these data suggest that differential expression of *SbTST1* and *SbTST2* genes, but not *SbSWEET13A* may play an important role in sugar accumulation in sweet sorghum stems. To our knowledge, no previous reports have shown the differential expression of *SbTSTs* associated with sugar accumulation in the stems of sweet vs. grain sorghum.

From our expression studies, we developed a model of the various sucrose transporter protein functions to explain the basis of sugar accumulation within sorghum leaf and stem tissues and to stimulate new directions in research (Fig. 4). Within leaves, *SbSUT2* and *SbSUT4*, but not *SbSUT1*, were more highly expressed in sweet sorghum than in grain sorghum, suggesting that *SbSUT4* may function to import sucrose into cells, and *SbSUT2* may function to export transitory stored sucrose from the vacuole.[21] *SbSUT1* function is likely conserved between grain and sweet sorghum, and based on orthology with the maize (*Zea mays*) *ZmSUT1* gene, it likely functions in sucrose phloem loading in leaves.[34-36] *SbSWEET13A* showed reduced expression in sweet compared with grain sorghum leaves, whereas *SbTST1* and *SbTST2* were both more highly expressed in sweet sorghum leaves, suggesting that they may function to import sugars into the vacuole for temporary storage during daylight. In stem tissues, none of the *SbSUT* or *SbSWEET13A* genes were differentially expressed, suggesting they do not account for the differences in sugar accumulation. However, both *SbTST1* and *SbTST2* were highly significantly expressed in sweet

sorghum stems, suggesting these genes function to import sucrose for storage in the vacuole within stem parenchyma cells. Based on these results, we hypothesize the ~24-fold increase in total stem solutes observed in sweet sorghum compared with grain sorghum is predominantly due to the significantly higher expression of *SbTST1* and *SbTST2* in sweet sorghum tissues.

In summary, based on both our previous and current results, we determined *SbTST1* and *SbTST2*, but probably not *SbSUTs* or *SbSWEETs*, are likely responsible for the substantial sugar accumulation in sweet sorghum stems. Testing of this hypothesis will potentially require characterizing loss-of-function mutations in both genes, since they were found to be partially functionally redundant in *Arabidopsis thaliana*.[29] These efforts are currently underway. Furthermore, these data suggest *TSTs* have been the target of selection for sugar accumulation in both the sweet sorghum stem and the sugar beet taproot. It will be interesting to determine whether *TSTs* have similarly been selected within sugarcane stem tissues. If so, it would indicate *TSTs* have been convergently selected during domestication of the world's 3 major sucrose storage crops. Our findings tantalizingly suggest *SbTST1* and *SbTST2* are candidate genes for the control of sucrose accumulation in sweet sorghum stems. Hence, modifying the expression or function of *TSTs* through genetic engineering or selective breeding, could potentially achieve greater sucrose accumulation and therefore enhancement of crop yields in sugar-storing organs, which would lead to direct benefits for food and fuel production.

Disclosure of potential conflicts of interest — No potential conflicts of interest were disclosed.

Acknowledgments — We thank R. Frank Baker for comments on the manuscript. We wish to clarify UNL71-2011 and UNL3016 are the same lines referred to as cultivars Wray and Macia, respectively, in our previous publication.[21] This research was supported by the US DOE Office of Science, Office of Biological and Environmental Research (BER), grant no. DE-SC0006810, and by the US National Science Foundation Plant Genome Research Program, grant no. IOS–1025976, to DMB.

References

1. Dweikat I, Weil C, Moose S, Kochian L, Mosier N, Ileleji K, et al. Envisioning the transition to a next-generation biofuels industry in the US Midwest. Biofuels Bioprod Bioref 2012; 6:376-86; doi 10.1002/bbb.1342
2. Slewinski TL. Non-structural carbohydrate partitioning in grass stems: A target to increase yield stability, stress tolerance, and biofuel production. J Exp Bot 2012; 63:4647-70; PMID:22732107; doi 10.1093/jxb/ers124
3. Braun DM, Wang L, Ruan YL. Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security. J Exp Bot 2014; 65:1713-35; PMID:24347463; doi 10.1093/jxb/ert416
4. Ruan YL. Sucrose metabolism: Gateway to diverse carbon use and sugar signaling. Annu Rev Plant Biol 2014; 65:33- 67; PMID:24579990; doi 10.1146/annurev-arplant-050213-040251
5. McGrath JM, Townsend B. Sugar Beet, Energy Beet, and Industrial Beet. In: Cruz V, Dierig D, eds. Industrial Crops: Springer New York, 2015:81-99.
6. Rooney WL, Blumenthal J, Bean B, Mullet JE. Designing orghum as a dedicated bioenergy feedstock. Biofuels Bioprod Bioref 2007; 1:147-57; doi 10.1002/bbb.15
7. Wang J, Nayak S, Koch K, Ming R. Carbon partitioning in sugarcane (*Saccharum* species). Front Plant Sci 2013; 4:201.
8. Bihmidine S, Hunter III CT, Johns CE, Koch KE, Braun DM. Regulation of assimilate import into sink organs: Update on molecular drivers of sink strength. Front Plant Sci 2013; 4:177; PMID:23761804; doi 10.3389/fpls.2013.00177
9. Calvino M, Messing J. Sweet sorghum as a model system for bioenergy crops. Curr Opin Biotech 2012; 23:323-9; PMID:22204822; doi 10.1016/j.copbio.2011.12.002
10. McCormick AJ, Watt DA, Cramer MD. Supply and demand: Sink regulation of sugar accumulation in sugarcane. J Exp Bot 2009; 60:357-64; PMID:19050062; doi 10.1093/jxb/ern310
11. Patrick JW, Botha FC, Birch RG. Metabolic engineering of sugars and simple sugar derivatives in plants. Plant Biotech J 2013; 11:142-56; PMID:23043616; doi 10.1111/pbi.12002
12. Jung B, Ludewig F, Schulz A, Meißner G, W€ostefeld N, Flugge UI, et al. Identification of the transporter responsible for sucrose accumulation in sugar beet taproots. Nat Plants 2015; 1:14001; doi 10.1038/nplants.2014.1
13. Braun DM, Slewinski TL. Genetic control of carbon partitioning in grasses: Roles of Sucrose Transporters and Tie-dyed loci in phloem loading. Plant Physiol 2009; 149:71 81; PMID:19126697; doi 10.1104/pp.108.129049
14. Ma Y, Slewinski TL, Baker RF, Braun DM. Tie-dyed1 encodes a novel, phloem-expressed transmembrane protein that functions in carbohydrate partitioning. Plant Physiol 2009; 149:181-94; PMID:18923021; doi 10.1104/pp.108.130971
15. Slewinski TL, Baker RF, Stubert A, Braun DM. Tie-dyed2 encodes a callose synthase that functions in vein development and affects symplastic trafficking within the phloem of maize leaves. Plant Physiol 2012; 160:1540-50; PMID:22932757; doi 10.1104/pp.112.202473
16. Slewinski TL, Braun DM. The psychedelic genes of maize redundantly promote carbohydrate export from leaves. Genetics 2010; 185:221-32; PMID:20142436; doi 10.1534/genetics.109.113357
17. Murray SC, Sharma A, Rooney WL, Klein PE, Mullet JE, Mitchell SE, Kresovich S. Genetic improvement of sorghum as a biofuel feedstock: I. QTL for stem sugar and grain nonstructural carbohydrates. Crop Sci 2008; 48:2165-79; doi 10.2135/cropsci2008.01.0016
18. Sukumaran S, Xiang W, Bean SR, Pedersen JF, Kresovich S, Tuinstra MR, Tesso TT, Hamblin MT, Yu J. Association mapping for grain quality in a diverse sorghum collection. Plant Genome 2012; 5:126-35; doi 10.3835/plantgenome2012.07.0016
19. Hoffmann-Thoma G, Hinkel K, Nicolay P, Willenbrink J. Sucrose accumulation in sweet sorghum stem internodes in relation to growth. Phys Plant 1996; 97:277-84; doi

10.1034/j.1399-3054.1996.970210.x

20. Tarpley L, Vietor DM. Compartmentation of sucrose during radial transfer in mature sorghum culm. BMC Plant Biol 2007; 7:33; PMID:17584916; doi 10.1186/1471-2229-7-33
21. Bihmidine S, Baker RF, Hoffner C, Braun DM. Sucrose accumulation in sweet sorghum stems occurs by apoplasmic phloem unloading and does not involve differential Sucrose transporter expression. BMC Plant Biol 2015; 15:186; PMID:26223524; doi 10.1186/s12870-015-0572-8
22. Milne RJ, Offler CE, Patrick JW, Grof CPL. Cellular pathways of source leaf phloem loading and phloem unloading in developing stems of *Sorghum bicolor* in relation to stem sucrose storage. Funct Plant Biol 2015; 42:957-70; doi 10.1071/FP15133
23. Baker RF, Leach KA, Braun DM. SWEET as sugar: New sucrose effluxers in plants. Mol Plant 2012; 5:766-8; PMID:22815540; doi 10.1093/mp/SSS054
24. Eom J-S, Chen LQ, Sosso D, Julius BT, Lin IW, Qu X-Q, Braun DM, Frommer WB. SWEETs, transporters for intracellular and intercellular sugar translocation. Curr Opin Plant Biol 2015; 25:53-62; PMID:25988582; doi 10.1016/j.pbi.2015.04.005
25. Braun DM. SWEET! The pathway is complete. Science 2012; 335:173-4; PMID:22246760; doi 10.1126/science.1216828
26. Chen LQ. SWEET sugar transporters for phloem transport and pathogen nutrition. New Phytol 2014; 201:1150-5; PMID:24649486; doi 10.1111/nph.12445
27. Chen LQ, Qu XQ, Hou BH, Sosso D, Osorio S, Fernie AR, Frommer WB. Sucrose efflux mediated by SWEET proteins as a key step for phloem transport. Science 2012; 335:207-11; PMID:22157085; doi 10.1126/science.1213351
28. Feng L, Frommer WB. Structure and function of Semi SWEET and SWEET sugar transporters. Trends Biochem Sci 2015; 40:480-6; PMID:26071195; doi 10.1016/j.tibs.2015.05.005
29. Wormit A, Trentmann O, Feifer I, Lohr C, Tjaden J, Meyer S, Schmidt U, Martinoia E, Neuhaus HE. Molecular identification and physiological characterization of a novel monosaccharide transporter from *Arabidopsis* involved in vacuolar sugar transport. Plant Cell 2006; 18:3476-90; PMID:17158605; doi 10.1105/tpc.106.047290
30. Martinoia E, Meyer S, De Angeli A, Nagy R. Vacuolar transporters in their physiological context. Annu Rev Plant Biol 2012; 63:183-213; PMID:22404463; doi 10.1146/annurev-arplant-042811-105608
31. Schulz A, Beyhl D, Marten I, Wormit A, Neuhaus E, Poschet G, Buttner M, Schneider S, Sauer N, Hedrich R. Proton-driven sucrose symport and antiport are provided by the vacuolar transporters SUC4 and TMT1/2. Plant J 2011; 68:129-36; PMID:21668536; doi 10.1111/j.1365-313X.2011.04672.x
32. Wingenter K, Schulz A, Wormit A, Wic S, Trentmann O, Hoermiller II, Heyer AG, Marten I, Hedrich R, Neuhaus HE. Increased activity of the vacuolar monosaccharide transporter TMT1 alters cellular sugar partitioning, sugar signaling, and seed yield in *Arabidopsis*. Plant Physiol 2010; 154:665-77; PMID:20709831; doi 10.1104/pp.110.162040
33. Makita Y, Shimada S, Kawashima M, Kondou-Kuriyama T, Toyoda T, Matsui M. MOROKOSHI: Transcriptome database in *Sorghum bicolor*. Plant Cell Physiol 2015; 56:e6; PMID:25505007; doi 10.1093/pcp/pcu187
34. Slewinski TL, Garg A, Johal GS, Braun DM. MaizeSUT1 functions in phloem loading. Plant Sig Behav 2010; 5:687-90; PMID: 20404497; doi 10.4161/psb.5.6.11575
35. Slewinski TL, Meeley R, Braun DM. Sucrose transporter1 functions in phloem loading in maize leaves. J Exp Bot2009; 60:881-92; PMID:19181865; doi 10.1093/jxb/ern335
36. Rotsch D, Brossard T, Bihmidine S, Ying W, Gaddam V, Harmata M, Robertson JD, Swyers M, Jurisson SS, Braun DM. Radiosynthesis of 60 automated synthesis and its utility to study in vivo sucrose transport in maize (*Zea mays*) leaves. PLoS ONE 2015; 10:e0128989; PMID:2602452; doi 10.1371/journal.pone.0128989-deoxy-60