Genetic Control of Carbon Partitioning in Grasses: Roles of *Sucrose Transporters* and *Tie-dyed* Loci in Phloem Loading[1][C]

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Plants have specialized organs for distinct functions. Leaves perform photosynthesis and fix carbon, whereas roots absorb water and minerals. To distribute resources between these organs, plants have a vasculature composed of phloem and xylem. The xylem conducts water and minerals from the roots up to the shoots. The phloem transports carbon- and nitrogen-containing compounds from mature leaves to the roots and to other nonphotosynthetic organs such as flowers and fruits. Phloem tissue comprises two main cell types: sieve elements and companion cells. Sieve elements conduct nutrients, while companion cells metabolically support the sieve elements (van Bel and Knoblauch, 2000). The vascular system represents a highly integrated distribution network essential not only for the life of the plant but also for the life of the planet, as nearly all terrestrially produced chemical energy, including our food supply, is derived from plants.

Photosynthesis and carbon assimilation occur in leaf mesophyll cells and additionally in bundle sheath cells in C_4 plants. For distribution to distal tissues, fixed carbon must move out of the photosynthetic cells and into the phloem. If the photoassimilates (assimilated carbon) diffuse down a concentration gradient from the mesophyll cells into the phloem following an entirely cytoplasmic path through plasmodesmata (intercellular channels through which small molecules freely diffuse), it is referred to as symplastic phloem loading (Turgeon and Medville, 1998). However, if the assimilated carbon must cross a membrane prior to entering into the phloem, it is called apoplastic phloem loading (van Bel, 1993; Turgeon, 2006). In this case, the concentration of the transported assimilate is higher in the phloem than in the photosynthetic cells. Because apoplastic phloem loading involves movement against a concentration gradient, it requires energy in the form of a pH gradient generated by H^+-ATPases (Bush, 1993; Gaxiola et al., 2007).

Carbon partitioning is the process whereby assimilates are distributed throughout the plant body from photosynthetic tissues. For most plants, this occurs by loading Suc into the phloem and transporting it from source tissues (net exporters) to sink tissues (net importers), where Suc is unloaded (Turgeon, 1989; van Bel, 2003). This process is well characterized at the physiological, biochemical, and anatomical levels (Hofstra and Nelson, 1969; Fellows and Geiger, 1974; Evert et al., 1978; Nguyen-Quoc et al., 1990; Huber and Hanson, 1992; Evert et al., 1996a; Koch, 1996; Paul and Foyer, 2001). However, despite the obvious importance of this process for plant growth and development, few genes that function in carbon partitioning have been identified. Suc, K^+, and water transporters/channels have been characterized for their contribution to the transport of Suc in the phloem (Deeken et al., 2002; Lalonde et al., 2004; Maurel, 2007; Sauer, 2007). Of these, the best described genes that directly control Suc loading into the phloem encode Suc transporters (SUTs; Lalonde et al., 2004; Sauer, 2007).

In this review, we discuss phloem loading and the control of carbon partitioning in grasses, focusing on SUTs and highlighting similarities and differences with eudicots. Additionally, we cover related aspects of phloem loading, such as leaf anatomy, and discuss other genes regulating carbohydrate accumulation in grass leaves. For additional discussions of genes that function in phloem loading and carbon partitioning (e.g. H^+-ATPase, K^+ channel, Suc synthase, aquaporins), we refer interested readers to the following articles (Nolte and Koch, 1993; DeWitt and Sussman, 1995; Hannah et al., 2001; Deeken et al., 2002; Dinges et al., 2003; Ma et al., 2004; Hardin et al., 2006; Lu and Sharkey, 2006; Smith and Stitt, 2007).

LEAF ANATOMY

As the path of phloem loading is intimately related to leaf structure, we begin with a brief overview of grass leaf anatomy, describing a maize (*Zea mays*) leaf blade as a typical example (Fig. 1A). Three orders of veins are arranged longitudinally along the main axis of the leaf (Esau, 1977). The largest type contains large

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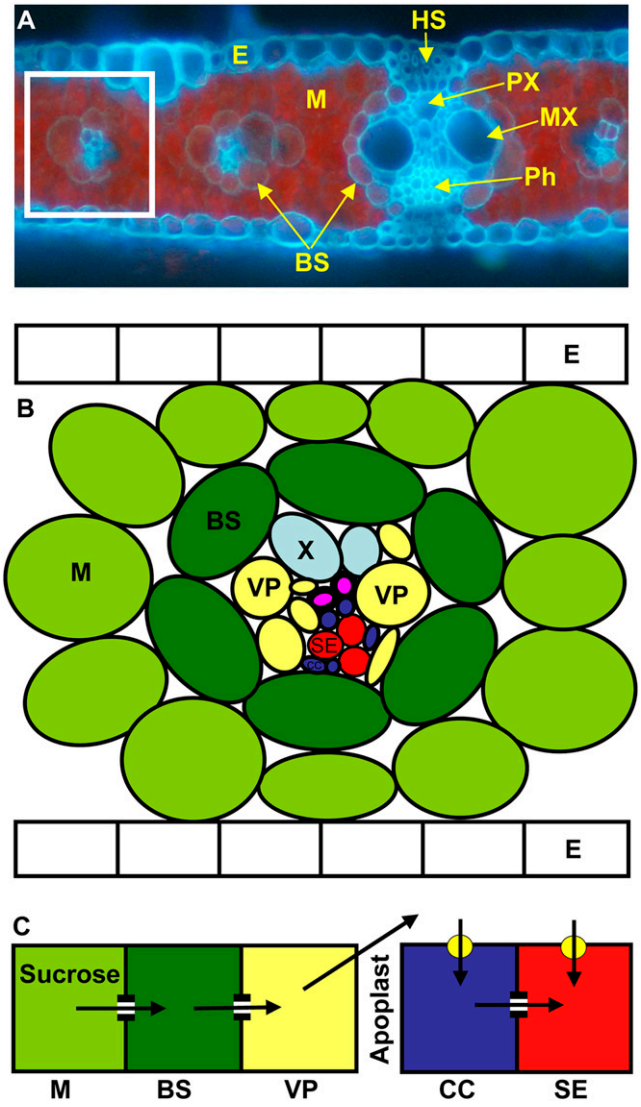

Figure 1. Maize leaf anatomy and path of Suc movement. A, Cross section of a maize leaf blade viewed under UV light to visualize cellular anatomy. Chlorophyll autofluoresces red and cell walls autofluoresce blue. One lateral vein and three minor veins are shown. B, Schematic diagram of cell types associated with the minor vein boxed in A. Maize veins display Kranz anatomy, with mesophyll cells surrounding bundle sheath cells, which surround the vein. Individual cell types are color coded for identification. Thick-walled sieve elements are shown in pink. C, Suc moves symplastically from mesophyll to bundle sheath to vascular parenchyma cells through plasmodesmata. Suc is exported to the apoplast by vascular parenchyma cells and imported into the companion cell and/or thin-walled sieve element by SUTs (yellow circles). BS, Bundle sheath cells; CC, companion cell; E, epidermal cells; HS, hypodermal sclerenchyma cells; M, mesophyll cells; MX, metaxylem vessels; Ph, phloem cells; PX, protoxylem lacuna; SE, thin-walled sieve element; VP, vascular parenchyma cell; X, xylem tracheary element.

metaxylem vessels and a protoxylem lacuna (space; Fig. 1A). Additionally, as structural support, hypodermal sclerenchyma girders are located between the vein and the epidermal cell layers. Intermediate veins lack metaxylem vessels but have hypodermal sclerenchyma caps on one or both sides of the vein. The smallest veins have neither metaxylem vessels nor hypodermal sclerenchyma caps. Collectively, the intermediate and small veins are referred to as minor veins, while the largest veins are known as lateral or major veins. Minor veins intergrade into the lateral veins (Russell and Evert, 1985). Transverse veins with anatomy similar to the small veins connect adjacent longitudinal veins. All veins contain phloem cells that transport photoassimilates, but the various orders of veins play different roles in carbon partitioning. Minor veins are the site where photoassimilates are loaded into the vein (Fritz et al., 1983). Lateral veins principally function in the long-distance transport of assimilated carbon out of the leaf into the stem (Fritz et al., 1989). Transverse veins shunt assimilates from minor veins to lateral veins (Fritz et al., 1989).

SUC ENTRY INTO A VEIN

The majority of grasses are thought to utilize apoplastic phloem loading, based on transmission electron microscopy studies showing an almost complete symplastic isolation of the companion cell-sieve element complex from surrounding cells (Gamalei, 1989). Examples include maize (Evert et al., 1978), barley (*Hordeum vulgare*; Evert et al., 1996b), and sugarcane (*Saccharum* hybrid; Robinson-Beers and Evert, 1991). The proposed path of assimilate entry into the phloem in maize is illustrated in Figure 1, B and C. After Suc is synthesized in the cytoplasm of mesophyll cells (Lunn and Furbank, 1999), it diffuses through plasmodesmata into bundle sheath cells and then into vascular parenchyma cells. By an unknown mechanism, the vascular parenchyma cells export Suc to the apoplast. Suc is subsequently imported across the plasma membrane of phloem companion cells and/or sieve elements and then transported by bulk flow to sink tissues. Utilization of an apoplastic phloem-loading mechanism is proposed for C_3 grasses, such as barley and wheat (*Triticum aestivum*; Thompson and Dale, 1981), as well as for the C_4 grasses sugarcane and maize. However, the apoplastic loading path may not be universal in grasses, as it has been suggested that rice (*Oryza sativa*; a C_3 species; Kaneko et al., 1980) and *Themeda triandra* (a C_4 species; Botha and Evert, 1988) may instead use symplastic phloem loading, based on the high frequency of plasmodesmata connecting vascular parenchyma cells to companion cells.

Evidence supporting the vascular parenchyma cell as the site of Suc export to the apoplast comes from analysis of the *sucrose export defective1* (*sxd1*) mutant of maize (Russin et al., 1996; Provencher et al., 2001). *sxd1* mutants show reduced Suc export capacity and accumulate large quantities of carbohydrates in the distal regions of leaf blades. An examination of the cellular interfaces in these distal regions revealed that callose is ectopically deposited over the cell wall between the bundle sheath and vascular parenchyma cells in leaf

minor veins, thereby occluding the plasmodesmata (Botha et al., 2000). This defect is proposed to prevent Suc movement into the vascular parenchyma cells and result in the accumulation of carbohydrates in photosynthetic cells. *Sxd1* encodes tocopherol cyclase, the penultimate enzyme in tocopherol (vitamin E) synthesis (Sattler et al., 2003). It is not understood how a lack of tocopherol leads to ectopic callose deposition. However, this function is conserved in potato (*Solanum tuberosum*) and Arabidopsis (*Arabidopsis thaliana*), as plants deficient for the *Sxd1* orthologous gene show a similar callose-deposition and carbohydrate-accumulation phenotype (Hofius et al., 2004; Maeda et al., 2006).

SUTs

Due to the nearly complete symplastic isolation of the sieve element-companion cell complex in the leaf veins of many grasses, Suc entry into the phloem is assumed to require an apoplastic step. SUTs are proposed to mediate Suc transport across the phloem cell plasma membrane; however, until recently, a role for SUTs in phloem loading had not been functionally demonstrated in grasses (see below). SUTs function as Suc-proton symporters with a 1:1 stoichiometry (Bush, 1990; Boorer et al., 1996; Zhou et al., 1997; Carpaneto et al., 2005). The energy to transport Suc against its concentration gradient into the phloem is derived from the proton motive force generated by H^+-ATPases in the plasma membrane of phloem cells (Bush, 1993; DeWitt and Sussman, 1995). SUTs contain 12 transmembrane domains that form a pore permitting Suc transport through the membrane. Biochemical studies have shown Suc transport activity for numerous dicot as well as monocot SUTs (for recent reviews, see Aoki et al., 2003; Kühn, 2003; Lalonde et al., 2004; Sauer, 2007). In multiple plants, it has been shown that SUT RNA and/or protein abundance is regulated by Suc (Chiou and Bush, 1998; Aoki et al., 1999; Matsukura et al., 2000; Vaughn et al., 2002; Ransom-Hodgkins et al., 2003), but the genes that control SUT expression have not been identified.

Historically, *SUT* genes were named in the order in which they were identified; hence, *SUT1* in one species was orthologous to *SUT5* in another. Furthermore, in Arabidopsis and several other plants, some *SUT* genes are named *SUC* for Suc carriers. To clarify their evolutionary relatedness, a phylogeny of different SUT proteins is presented (Fig. 2). For simplicity and consistency, we have renamed three grass *SUT*s according to their orthology with rice *SUT* genes (Aoki et al., 2003; Fig. 2; Table I). We propose that all grass *SUT* genes that are identified in the future be named according to their phylogenetic relationships to avoid confusion.

Based on sequence homology and biochemical activity, SUTs were previously divided into three types: type I was composed exclusively of dicot sequences (e.g. AtSUC2), but type II (OsSUT1 and AtSUC3, which is identical to AtSUT2) and type III (HvSUT2 and AtSUT4, which is identical to AtSUC4) contained both monocot and dicot proteins (Aoki et al., 2003; Lalonde et al., 2004). With increasing numbers of SUT sequences available, phylogenetic analysis divided the SUTs into four clades, splitting the type II subfamily into two groups (Sauer, 2007). The recent completion of the draft genome sequences for sorghum (*Sorghum*

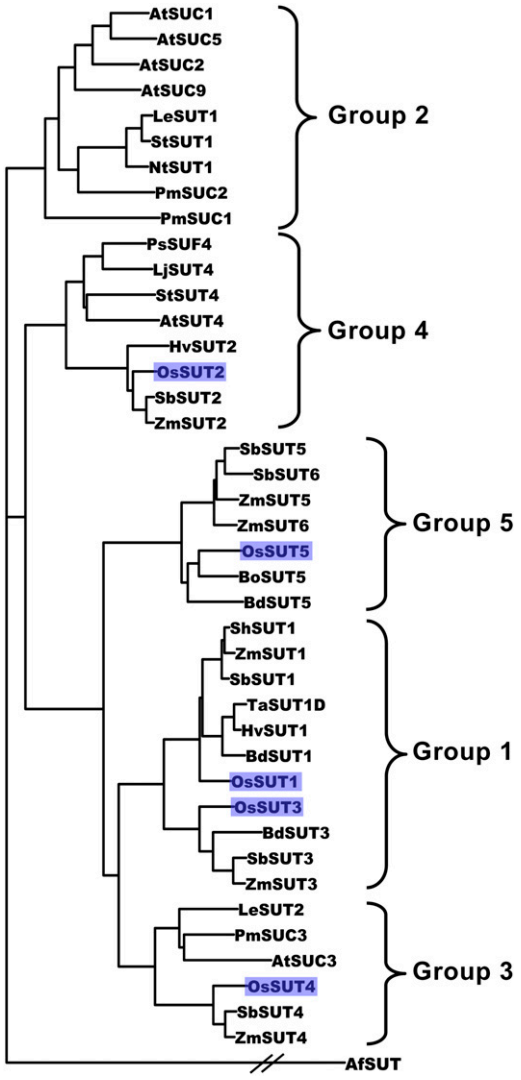

Figure 2. Phylogenetic tree of all grass and select dicot SUTs showing relationships among SUTs. The phylogenetic tree was created with PHYLIP based on the alignment of deduced amino acid sequences using the ClustalW program. The tree was rooted with the SUT-like sequence from *Aspergillus fumigatus* (AfSUT; accession no. EAL92728) as an outgroup. The five different groups of SUTs are indicated with brackets, and the locations of the rice SUTs are highlighted. GenBank accession numbers for grass SUTs are presented in Table I, and those from dicots are as follows: AtSUC1 (At1g71880), AtSUC2 (At1g22710), AtSUC3 (At2g02860), AtSUT4 (At1g09960), AtSUC5 (At1g71890), AtSUC9 (At5g06170), LeSUT1 (X82275), LeSUT2 (AAG12987), LjSUT4 (CAD61275), NtSUT1 (X82276), PmSUC1 (*Plantago major*; CAI59556), PmSUC2 (X75764), PmSUC3 (CAD58887), PsSUF4 (DQ221697), StSUT1 (CAA48915), and StSUT4 (AAG25923). [See online article for color version of this figure.]

Table I. *Grass SUTs*

Gene and Organism	SUT Name	GenBank Accession No. or Genome Locus	Reference
SUT1			
Rice	OsSUT1	AAF90181	Hirose et al. (1997)
Maize	ZmSUT1	BAA83501	Aoki et al. (1999)
Sorghum	SbSUT1	Sb01g045720[a]	
Brachypodium	BdSUT1	Super_1.4968[b]	
Barley	HvSUT1	CAB75882	Weschke et al. (2000)
Sugarcane	ShSUT1	AAV41028	Rae et al. (2005)
Wheat	TaSUT1D	AAM13410	Aoki et al. (2002)
SUT2			
Rice	OsSUT2	BAC67163	Aoki et al. (2003)
Maize	ZmSUT2	AAS91375[c]	
Sorghum	SbSUT2	Sb04g038030[a]	
Brachypodium	BdSUT2	Super_5.610[b,d]	
Barley	HvSUT2	CAB75881	Weschke et al. (2000)
SUT3			
Rice	OsSUT3	BAB68368	Aoki et al. (2003)
Maize	ZmSUT3	ACF86653[e]	
Sorghum	SbSUT3	Sb01g022430[a]	
Brachypodium	BdSUT3	Super_8.2211[b]	
SUT4			
Rice	OsSUT4	BAC67164	Aoki et al. (2003)
Maize	ZmSUT4	AAT51689[c]	
Sorghum	SbSUT4	Sb08g023310[a]	
Brachypodium	BdSUT4	Super_12.16[b,d]	
SUT5			
Rice	OsSUT5	BAC67165	Aoki et al. (2003)
Maize	ZmSUT5	ACF85284[e]	
Sorghum	SbSUT5	Sb04g023860[a]	
Brachypodium	BdSUT5	Super_5.1963[b]	
Bamboo (*Bambusa oldhami*)	BoSUT5	AAY43226[c]	
SUT6			
Maize	ZmSUT6	ACF85673[e]	
Sorghum	SbSUT6	Sb07g028120[a]	

[a]Sorghum Genome Project, Department of Energy Joint Genome Institute, www.phytozome.net. [b]*Brachypodium* Genome Project, Department of Energy Joint Genome Institute, www.brachypodium.org. [c]To use a uniform nomenclature across grass *SUT*s, the previously named ZmSUT2, ZmSUT4, and BoSUT1 sequences were renamed based on their orthology with the rice *SUT* genes. [d]BdSUT2 and BdSUT4 were not included in the phylogeny in Figure 2 due to incomplete sequences. [e]Maize Genome Project, www.maizesequence.org.

bicolor), *Brachypodium distachyon*, and maize facilitated the identification of their corresponding SUT sequences, and an additional fifth group emerged (Fig. 2). Groups 1 and 5 (formerly type II) consist entirely of monocot SUTs, group 2 (formerly type I) contains only dicot SUTs, and both group 3 (formerly type II) and group 4 (formerly type III) contain monocot and dicot SUTs (Fig. 2).

The Arabidopsis genome contains the largest *SUT* gene family characterized to date, with nine *SUT*-like genes, although two are categorized as pseudogenes (Sauer et al., 2004). Of the remaining seven, five are group 2 members, one is a group 3 member, and the last is a group 4 member. The rice genome contains five *SUT* genes: two group 1 members and a single gene each from groups 3, 4, and 5 (Aoki et al., 2003; Fig. 2; Table I). Analyses of the draft genomes of maize, sorghum, and *Brachypodium* indicate that they contain the same five *SUT* genes as rice (Fig. 2; Table I). In addition, *SUT5* appears to have been duplicated in maize and sorghum. As has been observed previously in dicots, in all the grass genomes available, there is only a single group 3 or 4 *SUT* gene (the two group 3 rice genes reported by Sauer [2007] are alleles from different cultivars). In dicots, the group 2 *SUT* genes underwent duplication and neofunctionalization (Baud et al., 2005; Sivitz et al., 2007, 2008), and in monocots, the group 1 SUTs similarly expanded (Fig. 2). Furthermore, it is possible that the grass SUT3 sequences, which are well resolved from the SUT1 sequences within group 1, are functionally distinct and may eventually be split into a group 6.

It is important to point out that all data currently available on monocot SUTs are derived solely from grasses. In comparison with dicots, few monocots have been characterized for leaf anatomy and

phloem-loading mechanism (Gamalei, 1989). Additionally, more monocot genomes need to be sequenced to better understand the evolution of the *SUT* gene family and whether SUT functions are conserved in nongrass monocots. Nevertheless, the functions of almost all grass *SUT* genes still remain to be determined.

FUNCTION OF GROUP 2 SUTs

Group 2 SUT family members are unique to dicots. In Arabidopsis, the best characterized group 2 SUT shown to function in phloem loading is AtSUC2. AtSUC2 RNA and protein are expressed in the companion cells of minor veins in a pattern reflective of the sink-to-source transition in leaves (Truernit and Sauer, 1995; Stadler and Sauer, 1996; Wright et al., 2003). AtSUC2 has a biochemical affinity for Suc consistent with a role in phloem loading from the apoplast (Chandran et al., 2003). Definitive proof of a function in phloem loading came from analyses of T-DNA insertion mutations in *AtSUC2* (Gottwald et al., 2000; Srivastava et al., 2008). Mutant plants have reduced Suc export from leaves, chlorotic leaves that accumulate carbohydrates, diminished shoot growth, and delayed flowering. Recently, using a tissue-specific promoter to complement an *Atsuc2* mutant, it was shown that the only essential function in photoassimilate distribution for AtSUC2 was to load Suc into the phloem in the leaf minor veins (Srivastava et al., 2008).

Phenotypes similar to those displayed by *Atsuc2* mutant plants have been reported in transgenic plants expressing antisense RNAs for *SUT1* in potato (Riesmeier et al., 1994), tobacco (*Nicotiana tabacum*; Bürkle et al., 1998), and tomato (*Solanum lycopersicum* [formerly *Lycopersicon esculentum*; Le]; Hackel et al., 2006), demonstrating that SUT1 functions in phloem loading in these plants. Solanaceous SUT1 protein was first reported as localizing to the plasma membrane of sieve elements (Kühn et al., 1997; Barker et al., 2000). However, a recent analysis found that SUT1 in tobacco, potato, and tomato localized to the plasma membrane of companion cells, suggesting that both solanaceous and Arabidopsis plants load Suc into the phloem companion cells (Schmitt et al., 2008). Contrary to this, another recent publication reports SUT1 in potato localizing to the sieve elements in stems and leaf minor veins (Krügel et al., 2008). In all of these papers, SUT1 localization was determined by immunofluorescence, which is dependent on the specificities of the different antibodies used and may explain the disparate results (for discussion, see Schmitt et al., 2008). It may be necessary to utilize a different approach to resolve which cells express SUT1 and are responsible for phloem loading in the Solanaceae.

GROUP 3, 4, AND 5 SUT FAMILY MEMBERS

The functions of group 3 SUTs are not clear. Based on sequence characteristics and an initial report of a lack of transport activity, it was postulated that AtSUT2/AtSUC3 might function as a Suc sensor (Barker et al., 2000). However, subsequent studies have questioned this hypothesis by finding that null mutations in *AtSUC3* have no obvious phenotype and that AtSUC3 protein has measurable biochemical activity (Meyer et al., 2000; Barth et al., 2003). Interestingly, the gene is expressed in many sink tissues and is strongly induced by wounding (Meyer et al., 2000, 2004). The only report characterizing a group 3 SUT in grasses showed that the rice *OsSUT4* gene is expressed in all tissues examined, although at higher levels in sink leaves (Aoki et al., 2003). More research is needed to understand the function of these SUTs in both grasses and dicot plants.

The first member of the group 4 SUT subfamily identified was *HvSUT2* (Weschke et al., 2000). HvSUT2 has Suc transport activity when expressed in yeast. The gene is expressed at the highest level in developing leaves and at approximately equal levels in roots, mature leaves, developing grains, anthers, and gynoecium tissues. Based on RNA expression, it was proposed that *HvSUT2* may play a general housekeeping role.

Endler et al. (2006) discovered that HvSUT2 was a vacuolar membrane-resident protein through a proteomic analysis of tonoplasts isolated from barley leaf mesophyll cells. Similar analyses showed that AtSUT4, the Arabidopsis homolog, was also a tonoplast-localized protein. These data were confirmed by HvSUT2-GFP and AtSUT4-GFP transient subcellular localization studies in onion (*Allium cepa*) bulb and Arabidopsis leaf epidermal cells. Based on their localization, it was suggested that these proteins function in Suc exchange between the vacuole and cytoplasm. Hence, the Suc uptake activity measured in yeast for these SUTs was likely due to mistargeting to the plasma membrane (Weise et al., 2000; Weschke et al., 2000).

Recently, Reinders et al. (2008) found that the *Lotus japonicus* group 4 homolog, LjSUT4, also localized to the vacuolar membrane, indicating that LjSUT4 may likewise function to transport Suc from the vacuole into the cytoplasm. Using *Xenopus laevis* oocytes, it was determined that LjSUT4 was a proton-coupled low-affinity Suc transporter. As the authors point out, fortuitous mislocalization of the tonoplast-resident protein to the oocyte plasma membrane permitted electrophysiological analyses. As oocytes do not contain vacuoles, it remains a possibility that the biochemical properties determined for the transporter do not completely reflect its endogenous role. It will be interesting to determine the function of the protein within its native context.

The SUT homolog PsSUF4 from pea (*Pisum sativum*), showing 73% amino acid identity to LjSUT4, was characterized by heterologous expression in yeast and shown to be a Suc facilitator, rather than a symporter, that functions independently of a H^+ gradient (Zhou et al., 2007). The subcellular localization of PsSUF4 in plants was not reported, but if similarly localized to the tonoplast, it may be responsible for Suc entry into the

vacuole. If so, this suggests the intriguing possibility that PsSUF4 (and maybe other group 4 SUTs) may facilitate Suc transport into and out of vacuoles. In agreement with this hypothesis, Suc uptake studies in barley leaf mesophyll cell vacuoles found that Suc uptake was not energy dependent and that transport was not influenced by protonophores, which destroy the H^+ gradient across the membrane (Kaiser and Heber, 1984). These data suggest that vacuolar import of Suc occurred by facilitated diffusion rather than active transport. Clearly, more work is needed to test the functions of these genes in plants to understand their role in transitory Suc storage in vacuoles.

The sole report on the in vivo function of a group 4 SUT concerns StSUT4 from potato (Chincinska et al., 2008). StSUT4 localized to both the plasma membrane and the endomembranes surrounding the nucleus in tobacco and potato leaves, but not to the tonoplast. This suggests that not all group 4 SUTs function in the tonoplast and that another SUT in potato may function to transport Suc between the vacuole and cytoplasm. Antisense reduction of *StSUT4* expression led to altered accumulation of soluble sugars in leaves and increased phloem export of Suc. These phenotypes are the opposite of those observed in antisense StSUT1 plants (Bürkle et al., 1998), suggesting that StSUT4 has a distinct biological function. Additionally, antisense StSUT4 plants flowered early, had higher tuber production, and had reduced shade avoidance. The authors proposed that StSUT4 may act in the interplay of carbon availability and flower induction pathways.

Very little is known about other grass members of the group 4 SUTs. The rice ortholog of *HvSUT2* is *OsSUT2*. From reverse transcription-PCR experiments, *OsSUT2* is constitutively expressed in vegetative and reproductive tissues, although expression decreases toward the end of seed development (Aoki et al., 2003). Determining the physiological functions of these genes awaits the characterization of plants containing the respective loss-of-function mutations.

Similarly, the functions of group 5 SUTs are unknown and remain to be determined. *OsSUT5* is expressed nearly ubiquitously and shows the highest expression level in sink leaves (Aoki et al., 2003).

FUNCTIONS OF GROUP 1 SUTs

Group 1 SUTs are present only in monocots and are subdivided into two clades represented by OsSUT1 and OsSUT3 (Fig. 2). Nothing is currently known of the functions of grass *SUT3* genes. Here, we briefly summarize what is known about the functions of grass *SUT1* genes in phloem loading in leaves as well as their roles in other tissues.

Rice

OsSUT1 was the first SUT cloned from monocots (Hirose et al., 1997). Reverse transcription-PCR shows that it is expressed at high levels in germinating seeds, source leaf sheaths, panicles, and developing grains and at lower levels in sink and source leaves (Aoki et al., 2003). *OsSUT1* transcript was expressed in companion cells in leaf sheaths and in the scutellar vasculature of germinating seeds (Matsukura et al., 2000; Furbank et al., 2001; Scofield et al., 2007a). OsSUT1 may also play a role in maternal tissues, as both transcript and protein have been localized to the nucellus, vascular parenchyma tissue, and nucellar projection (Furbank et al., 2001). Promoter:GUS analyses and immunolocalization experiments showed that OsSUT1 was expressed in the companion cells and sieve elements, where it may function in phloem loading of Suc for transport to seedling shoots and roots (Scofield et al., 2007a). OsSUT1 is also expressed in the phloem along the long-distance transport path from the flag leaf blade to the base of the filling grain (Scofield et al., 2007b). It was suggested that OsSUT1 may function in Suc retrieval from the apoplasm along the transport path.

OsSUT1 does not appear to have an essential function in phloem loading of Suc in mature leaf blades. This was shown by strongly reducing *OsSUT1* gene expression by antisense RNA suppression (Ishimaru et al., 2001; Scofield et al., 2002). Antisense lines with almost complete reduction in RNA or protein levels had normal vegetative growth and no alteration in photosynthesis or leaf carbohydrate contents, contrary to what would be expected if OsSUT1 principally functioned in phloem loading. Instead, transgenic plants had reduced grain filling and decreased germination, indicating that OsSUT1 plays an important role in transporting Suc to the developing grain and in remobilizing stored carbohydrates during early seedling growth. No visible phenotype was observed in leaves of OsSUT1 antisense lines, potentially due to genetic redundancy (Ishimaru et al., 2001; Aoki et al., 2003) or to utilization of a symplastic phloem-loading pathway (Kaneko et al., 1980). To our knowledge, these two reports are the only publications to date that directly assess the biological function of a grass SUT in vivo.

In support of a symplastic loading pathway in rice, fluorescent dyes were recently used to show that a xylem sap retrieval pathway functions in rice leaf blades to transfer solutes from the xylem transpiration stream into adjacent vascular parenchyma cells and into the phloem sieve elements (Botha et al., 2008). Potentially related to a functional symplastic phloem-loading pathway, grass veins contain two types of sieve elements: thick walled and thin walled (Kuo and O'Brien, 1974; Walsh, 1974; Evert et al., 1978; Botha, 2005). Botha and coworkers (2008) showed that the thick-walled sieve elements were connected by plasmodesmata to vascular parenchyma cells and accumulated significant amounts of fluorescent dye. In contrast, the thin-walled sieve elements and their companion cells were virtually symplastically isolated from other cells and contained much less dye. An intriguing possibility that needs more attention is that

grass leaves may be able to use both symplastic (via thick-walled sieve elements?) and apoplastic (thin-walled sieve elements) phloem-loading mechanisms (Chonan et al., 1985; van Bel, 1993; Botha, 2005). Perhaps if the apoplastic pathway is not functional, the ability to symplastically load Suc into the phloem sustains plant growth. In fact, Srivastava et al. (2008) recently discussed the possibility that Arabidopsis may similarly be able to load Suc directly into the phloem using a completely symplastic pathway. Hence, it is possible that plants thought to use apoplastic phloem loading based on anatomical considerations are also conditional symplastic loaders (for discussion of mixed loading, see van Bel, 1993).

Wheat

In wheat, three homeologous genes known as *TaSUT1A*, *TaSUT1B*, and *TaSUT1D* (corresponding to the A, B, and D progenitor genomes that make up the genome of hexaploid wheat) have been characterized (Aoki et al., 2002). The three are almost equally expressed in leaves, internodes, and developing grains (Aoki et al., 2004). RNA in situ hybridization showed that the *TaSUT1* RNA accumulated specifically in companion cells in leaves. However, immunolocalization experiments with an antibody raised against the rice OsSUT1 protein determined that TaSUT1 is localized to the plasma membrane of sieve elements in both leaves and stem tissues. This suggests that phloem loading of Suc occurs in the sieve elements in wheat and that the protein trafficks through plasmodesmata (Aoki et al., 2004). Based on its expression pattern, TaSUT1 has been proposed to function in phloem loading in source leaves and in retrieval of leaked Suc along the transport phloem in sheath and stem tissues. In developing grains, TaSUT1 localized to the plasma membrane of modified aleurone and subaleurone cells adjacent to the endosperm cavity, where it has been proposed to play a role in Suc uptake into filial tissues (Bagnall et al., 2000; Aoki et al., 2006).

Barley

HvSUT1 in barley is expressed at very high levels in developing seeds during the time of starch deposition (Weschke et al., 2000). RNA in situ hybridization determined that *HvSUT1* transcript mainly accumulated in the maternal nucellar projection and in the endosperm transfer layer, which correspond to the site of Suc exchange between maternal and filial tissues. HvSUT1 is proposed to function in developing seeds in Suc uptake for delivery to starchy endosperm cells and in Suc retrieval in maternal tissue. *HvSUT1* is expressed at lower levels in sink and source leaves, but its function in these tissues is not known. *HvSUT1* RNA has been detected in phloem sap, indicating that the RNA trafficked through plasmodesmata from the companion cells to sieve elements (Doering-Saad et al., 2002). Expression of HvSUT1 in *Xenopus* oocytes determined that the transporter had moderate Suc affinity and was more substrate selective than AtSUC2 (Sivitz et al., 2005).

Sugarcane

Sugarcane internodes have the remarkable ability to store massive amounts of Suc, depositing it in both the vacuoles of stem storage parenchyma cells and the apoplast surrounding these cells (Welbaum and Meinzer, 1990). To prevent apoplastic backflow of Suc to the phloem, the stem veins are surrounded by sclerenchyma cells, which contain suberin and lignin in their cell walls (Walsh et al., 2005). This produces an apoplastic barrier to solute movement, as shown by the fact that xylem sap contains no Suc (Welbaum et al., 1992). Therefore, Suc must take an entirely symplastic route from the phloem into the storage parenchyma cells, where it is exported to the apoplast. Fluorescent dye tracer studies support this transport path (Rae et al., 2005; Walsh et al., 2005).

ShSUT1 RNA is expressed at the highest levels in tissues experiencing high sugar flux, specifically, mature exporting leaves and sugar-accumulating internodes (Rae et al., 2005). Immunolocalization experiments with an antibody raised against a ShSUT1 peptide indicated that the protein is not expressed in the phloem and therefore does not function in phloem loading or retrieval in these tissues (Rae et al., 2005). Instead, ShSUT1 localized to vascular parenchyma and mestome sheath cells (inner bundle sheath cell layer) in leaf and stem tissues. It was proposed that ShSUT1 may function as a biochemical barrier to apoplastic transport of Suc and retrieve any Suc leaked to the apoplast (Rae et al., 2005). Consistent with this idea, electrophysiological studies of ShSUT1 in oocytes determined that ShSUT1 had the highest selectivity for Suc of any SUT known, along with a relatively low Suc affinity (Reinders et al., 2006). It is likely that additional SUTs function in phloem loading of Suc in sugarcane, as an antibody raised against rice OsSUT1 immunolocalized to phloem tissue in leaves and stem (Rae et al., 2005). Further studies are needed to characterize additional sugarcane SUTs to determine which family member(s) function in the phloem.

Maize

ZmSUT1 is highly expressed in photosynthetic tissues, with maximal expression in leaf blades at the end of the day and minimal expression during the night (Aoki et al., 1999). Additionally, in an expanding leaf, *ZmSUT1* is expressed in a gradient, with highest levels in mature tissues at the tip and lowest levels in the immature base, a pattern reflective of the sink-to-source transition. Based on its expression pattern and biochemical activity, it has been proposed that ZmSUT1 functions in phloem loading in source tissues (Aoki et al., 1999; Carpaneto et al., 2005). However, as the closely related genes *OsSUT1* and *ShSUT1* (Fig. 2)

apparently do not function in phloem loading, it is not certain what role ZmSUT1 plays in planta. Complicating the matter, ZmSUT1 has also been shown to be capable of transporting Suc across the plasma membrane in both directions in *Xenopus* oocytes if the membrane potential, Suc, and pH gradients are reversed (Carpaneto et al., 2005).

We recently determined that ZmSUT1 functions in phloem loading by characterizing a knockout mutation (Slewinski et al., 2009). *Zmsut1* mutant plants developed chlorotic leaves that hyperaccumulated carbohydrates and prematurely senesced. Application of [^{14}C]Suc to abraded leaves demonstrated that Suc export was greatly diminished in *Zmsut1* mutants compared to wild type. In addition, mutant plants had reduced plant height, fewer leaves, delayed flowering, and stunted tassel development. Presumably, these phenotypes result from a reduction in assimilates delivered to sink tissues due to the failure to export Suc from source leaves. These phenotypes are similar to those reported in dicot plants containing mutations in *SUT* genes that are responsible for phloem loading (Riesmeier et al., 1994; Bürkle et al., 1998; Gottwald et al., 2000; Hackel et al., 2006; Srivastava et al., 2008). Hence, it appears, at least in maize, that SUT1 function is essential for phloem loading of Suc. Determining the biological functions of the five additional SUTs in maize (Fig. 2) will require characterizing plants that harbor mutations in each gene.

Tie-dyed Loci

In addition to *Sxd1* and *ZmSUT1*, several other maize genes that affect carbohydrate accumulation in leaves have been characterized. The *tie-dyed1* (*tdy1*) mutant was identified by its variegated yellow and green leaf phenotype (Braun et al., 2006). *tdy1* sectors violate the clonal cell lineages in maize leaves, suggesting that a mobile signal is responsible for sector formation. Phenotypic characterization of the mutant revealed that the *tdy1* yellow sectors hyperaccumulated soluble sugars and starch, indicating that carbon partitioning was perturbed. Additionally, *tdy1* mutants showed reduced plant stature, delayed flowering, and decreased yield, phenotypes that resemble dicot and maize SUT mutants (see above). However, because *tdy1* mutants are variegated, it was proposed that the gene functions as an osmotic stress or sugar sensor to promote phloem loading, rather than acting directly to transport sugars (Braun et al., 2006). Through a clonal mosaic analysis, *Tdy1* function was mapped to the middle layer of leaves, consisting of the interveinal mesophyll, bundle sheath, and vascular cells (Baker and Braun, 2007). To further dissect the function of *Tdy1* in carbohydrate partitioning, epistasis analysis was used to determine that *Tdy1* does not play a role in the photosynthetic cells in starch metabolism (Slewinski et al., 2008). In addition, it was shown that *Tdy1* functions independently of *Sxd1* in controlling carbohydrate accumulation in leaves (Ma et al., 2008). However, based on its nearly identical mutant phenotype and a dosage-sensitive genetic interaction, *Tdy1* was found to function in the same pathway as *Tdy2* (Baker and Braun, 2008). It was hypothesized that TDY1 and TDY2 may form a protein complex that promotes phloem loading.

To understand the function of *Tdy1*, we recently cloned the gene (Ma et al., 2009). *Tdy1* encodes a novel transmembrane protein found only in grasses, although two conserved protein subdomains are present in monocots and dicots. RNA in situ hybridization studies determined that *Tdy1* RNA was expressed exclusively in phloem cells. In fact, *Tdy1* is expressed as soon as protophloem cells mature, indicating that *Tdy1* may be a useful marker for early phloem cell differentiation. Monitoring symplastic solute movement with a fluorescent dye in wild-type and mutant leaves revealed that phloem loading was impaired in *tdy1* mutants. Therefore, it was proposed that *Tdy1* may function to promote phloem loading, potentially through modulating the activity of a SUT. Future investigations to examine the genetic and biochemical interactions between TDY1 and maize SUTs will test this hypothesis.

CONCLUDING THOUGHTS

Our understanding of the genetic regulation of carbon partitioning is just beginning. In addition to the genes described above, we have identified many other maize loci that regulate leaf carbohydrate accumulation. Characterization of the function of these genes and all SUT family members will open up new avenues of investigation into the control of carbon partitioning in plants. Moreover, it will afford exciting prospects for biotechnological approaches to enhance crop yield and biofuels production. As a case in point, it was recently demonstrated that the sugar content of sugarcane stems could be doubled with no apparent defect to plant growth, illustrating the potential to greatly modify carbon partitioning patterns (Wu and Birch, 2007). In addition, hyperaccumulation of carbohydrates in maize leaves redirected significant amounts of carbon to cellulose in the cell wall (Baker and Braun, 2008). These examples indicate that tremendous opportunities exist to manipulate carbon partitioning pathways in grasses. Additional research is needed to uncover the genetic pathways and control points regulating carbohydrate partitioning in plants to realize these goals.

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