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REVIEW PAPER

Understanding and manipulating sucrose phloem loading, unloading, metabolism, and signalling to enhance crop yield and food security

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Abstract

Sucrose is produced in, and translocated from, photosynthetically active leaves (sources) to support non-photosynthetic tissues (sinks), such as developing seeds, fruits, and tubers. Different plants can utilize distinct mechanisms to transport sucrose into the phloem sieve tubes in source leaves. While phloem loading mechanisms have been extensively studied in dicot plants, there is less information about phloem loading in monocots. Maize and rice are major dietary staples, which have previously been proposed to use different cellular routes to transport sucrose from photosynthetic cells into the translocation stream. The anatomical, physiological, and genetic evidence supporting these conflicting hypotheses is examined. Upon entering sink cells, sucrose often is degraded into hexoses for a wide range of metabolic and storage processes, including biosynthesis of starch, protein, and cellulose, which are all major constituents for food, fibre, and fuel. Sucrose, glucose, fructose, and their derivate, trehalose-6-phosphate, also serve as signalling molecules to regulate gene expression either directly or through cross-talk with other signalling pathways. As such, sugar transport and metabolism play pivotal roles in plant development and realization of crop yield that needs to be increased substantially to meet the projected population demand in the foreseeable future. This review will discuss the current understanding of the control of carbon partitioning from the cellular to whole-plant levels, focusing on (i) the pathways employed for phloem loading in source leaves, particularly in grasses, and the routes used in sink organs for phloem unloading; (ii) the transporter proteins responsible for sugar efflux and influx across plasma membranes; and (iii) the key enzymes regulating sucrose metabolism, signalling, and utilization. Examples of how sugar transport and metabolism can be manipulated to improve crop productivity and stress tolerance are discussed.

Key words: Apoplasmic, invertase, maize, phloem, rice, sink, source, sugar, symplasmic.

Introduction

Most of the world's population obtains the vast majority of their daily calories from cereal grains. The carbohydrates stored in these grains are derived from carbon assimilated in leaves, which are net carbohydrate exporters (termed sources). Source leaves export sucrose long distances through the veins to non-photosynthetic tissues, such as roots, stems, flowers, fruits, and seeds (net carbohydrate importers, called sinks) (Turgeon, 1989; Ludewig and Flügge, 2013). These sink tissues are typically harvested and utilized for food, fibre, feed, and, more recently, as a source for renewable fuels (Bihmidine *et al.*, 2013). However, to produce enough food and renewable fuel for an expanding global population estimated to reach

9 billion people by 2050, agricultural productivity must be dramatically increased (Fedoroff *et al.*, 2010; Godfray *et al.*, 2010). This increase must be acieved without additional land for farming, with reduced inputs such as water, fertilizer, and pesticides, and within the context of uncertain climatic shifts (Beddington, 2010). Thus, a significant increase in crop output is needed to produce higher biomass and yields on a per plant basis. Despite the enormous agricultural and economic importance of carbohydrates, we are just beginning to uncover and understand the functions of genes responsible for controlling the whole-plant distribution of fixed carbon, particularly in crops (Baker and Braun, 2007; Braun and Slewinski, 2009; Slewinski and Braun, 2010*a*, *b*; Slewinski, 2011, 2012).

Understanding the sucrose delivery pathways from leaves to seeds is critical for designing novel approaches to improve crop yield and increase food security. Although cereal grains account for most human calories, most research to date on sucrose phloem loading has been performed in dicotyledonous species. Therefore, a brief overview of a typical dicot leaf structure and potential mechanisms of phloem transport are provided, prior to discussions specific for grasses. In particular, the review will focus on the mechanisms of sucrose phloem loading in grasses, comparing maize and rice, since more evidence is available concerning the loading mechanisms in these cereal crops. Intriguingly, it has been proposed that rice may utilize a different phloem loading pathway from that used in other closely related grasses based upon anatomical, physiological, and genetic studies (for a review, see Eom *et al.*, 2012). This will be followed by a discussion of recent findings on the delivery and utilization of sugars in sink tissues, as well as the roles of sugars as signalling molecules.

Leaf structure in relation to phloem transport

To understand the long-distance movement of carbohydrates in plants, it is important to be familiar with leaf anatomy (for a review of phloem loading in dicots and monocots, see Nelson and van Bel, 1998). In the following sections, the carbon assimilation to the sucrose phloem loading pathway is described for a dicot leaf. Differences in this process for a grass leaf will then be discussed.

Carbon dioxide is assimilated in the mesophyll (M) cells, where it is synthesized into sucrose, the primary transported sugar in most plants. Sucrose moves cell to cell through plasmodesmata (PD; pores in the cell wall connecting the cytoplasms of adjacent cells) into cells neighbouring the veins, called bundle sheath (BS) cells, and then into the vein for long-distance transport out of the leaf. In a dicot leaf, the veins exhibit reticulate branching comprised of multiple vein orders, with the smallest veins intergrading into successively larger veins until reaching the midvein (largest) (Turgeon, 1989). Sucrose exits the leaf through the midvein in the petiole and moves into the stem vasculature.

Contained within each vein are two prominent conducting tissues, xylem and phloem (Esau, 1977). Xylem transports water and minerals from the roots to the shoots. Phloem is composed of three cell types: sieve elements (SEs) that conduct assimilates, companion cells (CCs) that genetically and metabolically support the SEs, and phloem parenchyma (PP) cells. SEs are connected end-to-end longitudinally to a sieve tube (ST), which translocates assimilates from source to sink tissues (Evert, 1982).

The phloem system of a plant can be subdivided into three different, but overlapping functional regions (van Bel, 1996) (Fig. 1A). Collection phloem located in the small veins of source leaves is responsible for sucrose entry into the vein. Release phloem in the sink tissues is where sucrose exits from the phloem into the surrounding tissues for utilization or storage (unloading). Transport phloem connects the collection and release phloems, and represents the largest portion of the integrated phloem network in a plant (van Bel, 2003). Mass flow through the interconnected STs is driven by an osmotically generated difference in pressure between source and sink tissues. Sucrose entry into the collection phloem increases the solute concentration. In response, water from the xylem flows into the STs by osmosis, raising the pressure. In sink tissues, sucrose unloading from the release phloem decreases the solute concentration in the STs, resulting in water diffusing out of the STs into surrounding cells and lowering the pressure. The hydrostatic pressure gradient between the collection and release phloem propels the bulk flow of solutes (sucrose and other assimilates) from source to sink tissues (Lalonde *et al.*, 2003; Patrick, 2013). During long-distance movement from source to sink tissues, some sucrose leaks out of the transport phloem to provision axial sinks (e.g. stem parenchyma cells), and some of the effluxed sucrose is reloaded into the phloem (Minchin and Thorpe, 1987; Hafke *et al.*, 2005; Bihmidine *et al.*, 2013).

Mechanisms for phloem loading

The anatomical connections within a leaf impact the possible routes through which sucrose travels into the vein. Three principal pathways have been proposed in plants by which sucrose is transported into the collection phloem in source leaves (van Bel, 1993; Rennie and Turgeon, 2009; Slewinski and Braun, 2010*a*). In symplasmic phloem loading species, the entire route from M cells to phloem ST is connected via PD, and is so named because the cytoplasms of all of the intervening cells are joined into a single symplasm (Fig. 1B). The concentration of sucrose is highest within the M cell cytoplasm and moves down a concentration gradient to enter the phloem ST. This phloem loading mechanism is also known as passive loading, since there is no requirement for energy input into the system for sucrose to enter the ST, only diffusion down a concentration gradient (Rennie and Turgeon, 2009; Slewinski and Braun, 2010*a*). In symplasmic phloem loading, there is *a priori* no requirement for sucrose transporter proteins to move sucrose across a plasma membrane to enter into the collection phloem. However, there remains a need for sucrose transporters in the transport phloem, even in symplasmic loading species, as sucrose leaks out along the

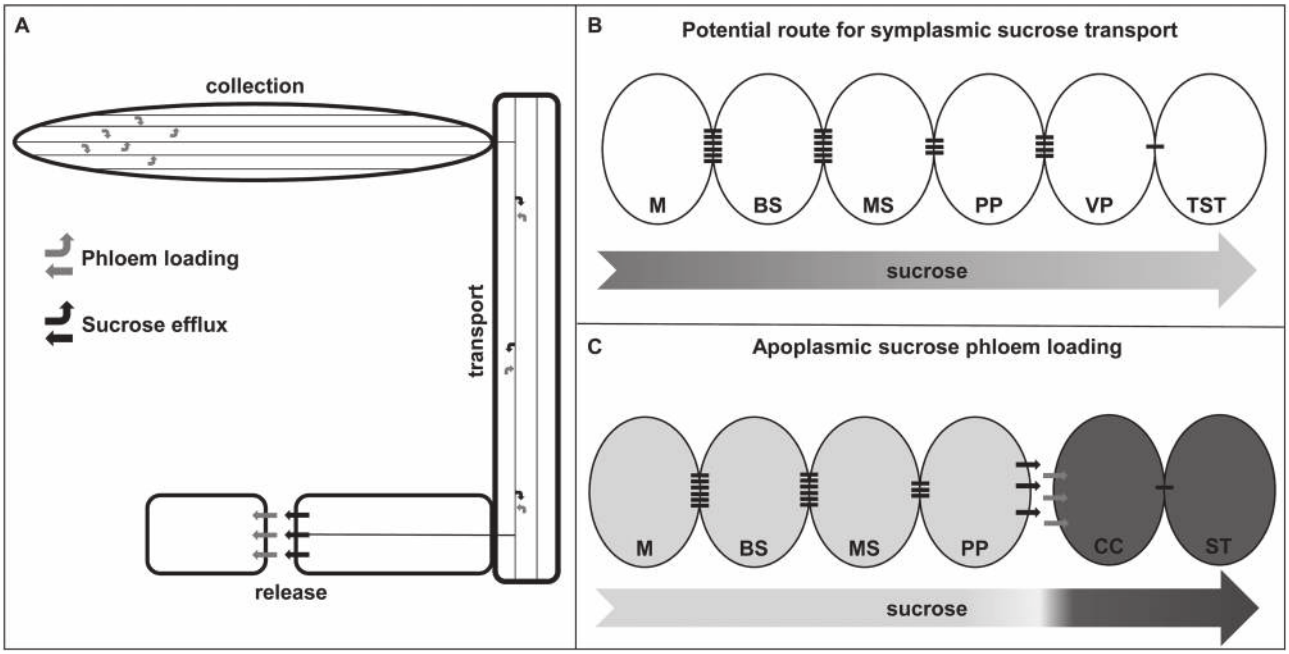

Fig. 1. Diagrams of the whole-plant phloem network, and potential symplasmic and apoplasmic sucrose transport routes in grass leaves. (A) Simplified representation of the integrated phloem network in a plant. Sucrose is loaded (grey arrows) into the minor veins within leaf blades in the collection phloem. Minor veins intergrade into the lateral veins (thin black lines), which exit the leaf and connect to the stem veins. In the transport phloem, sucrose is effluxed (black arrows) to support axial sink tissues, and is also reloaded into the transport phloem. In terminal sink tissues such as seeds, which are apoplasmically separated from the maternal tissue, sucrose is unloaded in the release phloem. (B) A putative route for symplasmic sucrose phloem loading in rice. Sucrose diffuses down a concentration gradient (shaded arrow) cell to cell through plasmodesmata (PD; black rectangles) from the highest concentration in the mesophyll (M) cells to the lowest concentration in the thick-walled sieve elements (TSTs). BS, bundle sheath; MS, mestome sheath; PP, phloem parenchyma; VP, vascular parenchyma. (C) Probable route for apoplasmic sucrose phloem loading in rice. The companion cells (CCs) and thin-walled sieve elements (STs) are virtually symplasmically isolated from neighbouring cells. Sucrose moves symplasmically via PD from the M into the BS, then into the MS, and into the PP cells. Sucrose is effluxed to the apoplasm from the PP cells probably by SWEETs (black arrows) and is actively imported against a concentration gradient (shaded arrow) into the CCs by sucrose transporters (grey arrows). Sucrose moves through PD into the STs for long-distance transport.

transport phloem and must be retrieved to maintain a high chemiosmotic gradient along the transport path.

The second type of phloem loading mechanism is known as apoplasmic loading. The apoplasm is the extracellular space outside of the symplasm, which is bounded by the plasma membrane continuum, and includes the cell wall, intercellular spaces, and xylem vessels. In apoplasmic phloem loading species, sucrose does not move symplasmically from the M all the way to the phloem due to the paucity of PD connecting the CC–SE complex to surrounding cells (Fig. 1C) (Evert *et al.*, 1978; Haritatos *et al.*, 2000; Braun and Slewinski, 2009). Instead, sucrose synthesized in the M cells moves through PD into the BS cell, and then via PD into the PP cell (Russin *et al.*, 1996; Haritatos *et al.*, 2000; Ma *et al.*, 2008). Sucrose could be exported into the BS–CC cell wall space, as in tobacco (*Nicotiana tabacum*) minor veins (Turgeon, 1984), or across the PP plasma membrane and delivered into the phloem apoplasm, by SWEET transporters prior to being imported into the CC–SE complex (Chen *et al.*, 2012) (see below). Sucrose is subsequently imported across the plasma membrane of the CC–SE complex by sucrose transporters (SUTs). Sucrose imported into the CC cytoplasm moves through PD into the ST for long-distance transport from source to sink tissues (Slewinski *et al.*, 2012; Baker *et al.*, 2013). Importation of sucrose into the CC–SE complex requires energy (i.e. phloem loading) since sucrose must move against its concentration gradient from a lower concentration in the apoplasm to a higher concentration in the ST symplasm (Giaquinta, 1983). Apoplasmic phloem loading species are able to attain very high concentrations of sucrose within the STs (>1 M) (Geiger *et al.*, 1973; Weiner *et al.*, 1991; Winter *et al.*, 1992; Berthier *et al.*, 2009) because they lack symplasmic connectivity to adjoining cells, otherwise sucrose would diffuse back through the PD towards the M cells to come to equilibrium (Rennie and Turgeon, 2009).

The third mechanism for phloem loading is called polymer trapping. In addition to sucrose, some plants transport additional sugars in their STs that are large polymers synthesized from sucrose, such as raffinose and stachyose (Rennie and Turgeon, 2009). These molecules are synthesized in specialized CCs, called intermediate cells (ICs). Sucrose moves from M cells through PD into the IC cytoplasm, where it is synthesized into raffinose or stachyose, which are thought to be too large to diffuse back through the PD into M cells.

However, along with sucrose, the raffinose or stachyose can move through the PD connecting the CC to the SE and be transported long-distance in the ST sap. Polymer trapping is not known to be present in monocots, so does not pertain to the discussions below of phloem loading in grasses.

It is important to note that plants may utilize more than one type of phloem loading mechanism simultaneously, even within a single vein (van Bel, 1993; Braun and Slewinski, 2009; Voitsekhovskaja *et al.*, 2009; Slewinski and Braun, 2010*a*; Slewinski *et al.*, 2013). Hence, when discussing the mechanism of phloem loading employed by a plant species, it should be kept in mind that it may only be the predominant phloem loading mechanism used, not the sole one. Plants probably possess plasticity in the mechanism of phloem loading utilized and may shift between different phloem loading mechanisms during development (see below) and/or in response to biotic stress (Gil *et al.*, 2011; Lemoine *et al.*, 2013), abiotic stress (Lemoine *et al.*, 2013), or genetic mutation (Srivastava *et al.*, 2008; Slewinski *et al.*, 2009).

Sugar transport proteins for phloem loading

In apoplasmic phloem loading species (e.g. *Arabidopsis thaliana* and Solanaceous species), sucrose must cross the plasma membranes of two different cell types to enter into the ST symplasm. First, proteins known as SWEETs are proposed to efflux sucrose to the phloem apoplasm (Baker *et al.*, 2012; Braun, 2012; Chen *et al.*, 2012), and, secondly, proteins called SUTs import sucrose from the apoplasm into the ST symplasm (Ayre, 2011). SWEETs are a recently identified class of sugar transport proteins that function as facilitated diffusers and transport sugar across a membrane down a concentration gradient (Chen *et al.*, 2010, 2012). SWEETs possess seven transmembrane domains and are encoded by a multigene family in plants, with 17 members in *Arabidopsis* and 21 members in rice (*Oryza sativa*) (Chen *et al.*, 2010; Liu *et al.*, 2011; Yuan and Wang, 2013). Subcellular localization studies of eight different SWEETs showed that they are plasma membrane proteins (Ge *et al.*, 2000; Chu *et al.*, 2006; Guan *et al.*, 2008; Chen *et al.*, 2010, 2012; Xuan *et al.*, 2013), although one SWEET protein has been shown to function on the tonoplast (Chardon *et al.*, 2013). Two closely related *Arabidopsis* genes, *AtSWEET11* and *AtSWEET12*, are expressed in a subset of PP cells in leaves, and both act as sucrose uniporters that reversibly transport sucrose across a membrane in response to a concentration gradient (Chen *et al.*, 2012). Single mutant plants for *atsweet11* or *atsweet12* had no visible phenotype, but *atsweet11; atsweet12* double mutant plants exhibited slower growth, mild leaf chlorosis, and excess carbohydrate accumulation in leaves (Chen *et al.*, 2012).

SUTs are proteins with 12 transmembrane domains that form a pore in the membrane to allow the passage of sucrose (Lalonde *et al.*, 2004; Sauer, 2007; Ayre, 2011; Geiger, 2011). SUTs function as sucrose/proton symporters that use the energy stored in the proton gradient across the membrane generated by H^+-ATPases to drive sucrose movement (Bush, 1990, 1993; Boorer *et al.*, 1996; Zhou *et al.*, 1997; Carpaneto *et al.*, 2005; Gaxiola *et al.*, 2007). In all plant genomes analysed, SUTs are encoded by a multigene family (Aoki *et al.*, 2003; Lalonde *et al.*, 2004; Sauer, 2007; Braun and Slewinski, 2009; Doidy *et al.*, 2012; Reinders *et al.*, 2012). Through comparing SUT protein sequences from different plants, phylogenetic analyses have distinguished multiple distinct clades of SUTs (Aoki *et al.*, 2003; Sauer, 2007; Braun and Slewinski, 2009; Reinders *et al.*, 2012). Biochemical studies of the transport properties and specificities for sucrose analogues of different SUT proteins support the phylogenetic groupings (Chandran *et al.*, 2003; Carpaneto *et al.*, 2005; Reinders *et al.*, 2006, 2008, 2012).

In apoplasmic phloem loading species, mutation of the phloem-expressed, plasma membrane-localized SUT responsible for sucrose import resulted in stunted plants that have chlorotic leaves with a hyperaccumulation of starch and soluble sugars, have altered biomass partitioning, and displayed root growth and reproductive defects (Riesmeier *et al.*, 1994; Bürkle *et al.*, 1998; Gottwald *et al.*, 2000; Hackel *et al.*, 2006; Srivastava *et al.*, 2008; Slewinski *et al.*, 2009). However, mutation of the vacuolar membrane-localized SUT in *Arabidopsis* produced no obvious phenotypes (Schneider *et al.*, 2012). In contrast, in plants characterized as symplasmic phloem loading species, mutation of the tonoplast-localized SUT resulted in phenotypes that included slow growth, altered biomass partitioning, elevated sugar content in source leaves, and reduced seed yield (Payyavula *et al.*, 2011). Furthermore, in a polymer trapping plant in which stachyose and sucrose enter the phloem ST symplasmically, silencing the expression of the presumably plasma membrane-localized, phloem-expressed SUT did not affect sucrose phloem loading, plant growth, photosynthetic rates, or leaf carbohydrate status (Zhang and Turgeon, 2009). Hence, in apoplasmic phloem loading species, the function of a plasma membrane-localized SUT is critical, but the tonoplast-localized SUT may not be; whereas, in plants with high symplasmic connectivity to the STs, the tonoplast-localized SUT is needed for normal growth, but the plasma membrane-localized SUT does not appear to be.

Phloem loading in grass leaves

Grass leaves can be divided into a distal blade portion that angles away from the stem to maximize capture of sunlight, and a proximal sheath region that wraps around and connects the leaf to the stem (Freeling and Lane, 1994). Within the blade, three classes of veins are arranged parallel to the long axis of the lamina (Russell and Evert, 1985). Major veins, also known as lateral veins, primarily function in long-distance transport of assimilates through the ST from source to sink tissues (Fritz *et al.*, 1989). Intermediate and small sized veins, collectively referred to as minor veins, are located in close association with the photosynthetic cells and are principally responsible for loading sucrose into the phloem (Fritz *et al.*, 1983). The minor veins intergrade into major veins as they progress down the leaf blade toward the sheath (Russell

and Evert, 1985). Photosynthate is transferred laterally from minor to major veins by transverse veins (Fritz *et al.*, 1989).

Some grass leaves, such as maize (*Zea mays*), have a single, prominent photosynthetic BS layer surrounding the vein (Esau, 1977; Huang *et al.*, 2009). Other grasses, such as rice, wheat (*Triticum aestivum*), sugarcane (*Saccharum officinarum*), and barley (*Hordeum vulgare*), have two rings of parenchyma cells, which comprise an inner, non-photosynthetic mestome sheath (MS), and an outer, photosynthetic BS (Esau, 1977; Kaneko *et al.*, 1980; Robinson-Beers and Evert, 1991; Dannenhoffer and Evert, 1994). Photosynthetic M cells flank the BS cells.

Unique to monocot leaves, it is noteworthy that the veins contain two types of STs in the phloem, termed thin-walled STs and thick-walled STs (TSTs), which have been proposed to have different functions relating to sucrose transport (Evert *et al.*, 1978; van Bel, 1993; Botha, 2005, 2013; Braun and Slewinski, 2009; Slewinski *et al.*, 2013). The STs are ontogenetically associated with CCs and are virtually symplasmically isolated from other cells in the vein; hence, sucrose entry into these cells probably requires an apoplasmic step (Evert *et al.*, 1978). These STs function in the long-distance transport of sucrose from source to sink tissues (Fritz *et al.*, 1983; Botha, 2013). In contrast, the TSTs do not have an associated CC. Instead, they are symplasmically connected to, but do not share a common mother cell with, the vascular parenchyma (VP) cells. Analogous to the CC supporting the STs, the VP cells support the TSTs. VP cells have PD connections to neighbouring cells, including other VP cells, PP cells, xylem parenchyma (XP) cells, and BS cells (Evert *et al.*, 1978). The VP cells/TSTs have been shown to function in solute retrieval from the xylem transpiration stream (Fritz *et al.*, 1983; Botha *et al.*, 2008) and may be involved in the symplasmic movement of compounds or macromolecules other than sucrose. It has been proposed that source to sink transport of assimilates occurs through the STs, not the TSTs (Botha, 2013). Evidence supporting this hypothesis includes that the number of TSTs decreases in larger vein classes compared with small veins, and the frequency of TSTs decreases from the tip of the blade to the sheath, as well as in the stem veins (Botha, 2013). Additionally, it was found that aphids preferentially feed from STs in comparison with TSTs, suggesting that the nutrient concentration or contents of the TSTs may be different from that of the STs (Botha and Matsiliza, 2004). Botha (2013) discusses additional evidence concerning the structure and functions of monocot STs and TSTs, and concludes that the TSTs are apparently not involved in long-distance transport of sucrose.

Below, a brief synopsis of the data concerning the path sucrose travels into the ST in maize, an apoplasmic phloem loading species, will be presented and compared with similar studies of rice leaves. Evidence consistent and inconsistent with symplasmic phloem loading in rice will be examined. After discussions of phloem loading in source tissues, the final part of the review will discuss recent progress in understanding the delivery and metabolism of carbohydrates in sink tissues as well as the roles of sugars as signalling molecules.

The apoplasmic sucrose phloem loading pathway in maize leaves

Maize leaves have been proposed to use an apoplasmic phloem loading pathway. This conclusion is supported by ultrastructural, physiological, and genetic data. Like all grass leaves, maize veins possess both STs and TSTs (Walsh, 1974; Evert *et al.*, 1978). From transmission electron microscope investigations, it was determined that the CC–SE complex has very few PD connecting it to other cells in the phloem; thus, sucrose import into the STs is proposed to require an apoplasmic step (Evert *et al.*, 1978).

Physiological experiments comparing the sucrose concentration in the maize leaf STs, apoplasm, and cytoplasm of the PP cell support the hypothesis of apoplasmic phloem loading. The sucrose concentration has been estimated to be in the hundred millimolar range in the M–BS–PP symplasm (Evert *et al.*, 1978), and measured at between 1 mM and 3 mM in the apoplasmic space (Heyser *et al.*, 1978; Lohaus *et al.*, 2000, 2001). Within maize leaf minor veins, the PP cells positioned close to the CC–SE complex have been proposed as the site for sucrose efflux to the apoplasm (Evert *et al.*, 1978; Fritz *et al.*, 1983). Similar to rice, the maize genome contains >20 SWEET genes, which probably mediate this step (Chen *et al.*, 2010; DMB, unpublished data). Measurements of solute concentrations in the CC–SE complex showed that it was greater than that in the M–BS–PP symplasm (Evert *et al.*, 1978). Moreover, the maize ST sap contains ~900–1400 mM sucrose (Ohshima *et al.*, 1990; Weiner *et al.*, 1991), significantly higher than the concentration in the photosynthetic cells, and consistent with an active loading (concentrating) step (Evert *et al.*, 1978). Sucrose was the only sugar identified in the ST sap (Ohshima *et al.*, 1990).

Additional physiological evidence supporting maize using apoplasmic phloem loading is that phloem translocation of sucrose is susceptible to anoxia, and, hence, is energy dependent (Thorpe and Minchin, 1988). Furthermore, treatment of maize leaf blades with a membrane-impermeable, sulphydryl-modifying chemical, para-chloromercuribenzenesulphonic acid (PCMBS), an inhibitor of SUT function, showed a decrease in phloem loading (Thompson and Dale, 1981; Thorpe and Minchin, 1988). Together, these data support the involvement of an energy-requiring step, probably mediated by a SUT, in the loading of sucrose into the collection phloem against a concentration gradient.

Reverse genetic studies of loss-of-function mutant alleles of *zmsut1* showed that maize SUT1 is required for sucrose phloem loading. *zmsut1* mutant plants are severely stunted in their growth, develop chlorotic leaves that hyperaccumulate carbohydrates, contain high concentrations of sucrose in their apoplasmic fluid, and prematurely senesce (Slewinski *et al.*, 2009, 2010). *zmsut1* mutant plants frequently fail to produce an inflorescence, presumably due to carbon starvation. However, rarely, a *zmsut1* mutant develops a tassel with a few functional spikelets that shed viable pollen capable of fertilization, indicating that ZmSUT1 is not strictly required for pollen germination or pollen tube growth. In support of this, self-fertilized heterozygous plants carrying a *zmsut1* mutant

allele produce 25% homozygous mutant progeny, indicating that *zmsut1* mutant alleles display normal Mendelian segregation through both male and female gametes. Finally, exogenous application of ^{14}C-labelled sucrose to *zmsut1* mutant leaves showed that the plants were severely inhibited in their ability to load and transport sucrose away from the site of application in comparison with the wild type (Slewinski *et al.*, 2009). Therefore, the totality of the anatomical, physiological, and genetic data support the conclusion that maize utilizes an apoplasmic loading mechanism involving ZmSUT1 to transport sucrose into the ST.

Does rice use a symplasmic phloem loading mechanism?

In contrast to maize, wheat, barley, sugarcane, and *Lolium perenne*, which are proposed to utilize an apoplasmic phloem loading mechanism (Evert *et al.*, 1978, 1996; Robinson-Beers and Evert, 1991; Aoki *et al.*, 2004; Berthier *et al.*, 2009), rice has been suggested to use symplasmic sucrose phloem loading (Kaneko *et al.*, 1980; Scofield *et al.*, 2007; Eom *et al.*, 2012). Anatomical studies on another grass leaf have also suggested that sucrose may be loaded into the veins via the symplasm because of the presence of PD between the VP cell and CC (Botha and Evert, 1988). However, the frequency of PD connecting the cytoplasms of the VP cell and CC reported in that study was only 1.4% of the total PD measured among cell types. Therefore, these data suggest that symplasmic transport into the ST is possible, but it probably does not account for the majority of sucrose entering the translocation stream. A more in-depth look into all of the evidence concerning the path of sucrose transport into the ST in rice leaves is warranted.

Rice leaf vein anatomical studies

From transmission electron microscopy investigations of small veins from rice leaf blades, Kaneko *et al.* (1980) suggested that a symplasmic transport route existed for sucrose movement from the M cells to the SE within the collection phloem. In contrast, the same research group examined the veins of rice leaf sheaths and suggested that sucrose was loaded into the transport phloem by an apoplasmic path, since the CC–SE complex was symplasmically isolated (Chonan *et al.*, 1984). Hence, from ultrastructural studies, the mechanism for sucrose phloem loading in rice leaves may involve a symplasmic path, an apoplasmic path, or both. However, it has long been recognized that the path travelled by sucrose to the vein cannot be determined entirely based upon cytoplasmic connectivity through PD (Giaquinta, 1983; Botha and Evert, 1988; Turgeon, 1989; Botha and van Bel, 1992; Oparka and Prior, 1992; van Bel, 1993; van Bel and Oparka, 1995; Liesche and Schulz, 2012), but needs to be validated by physiological and genetic experiments.

Rice leaf sucrose physiology

Using the aphid stylectomy procedure, ST sap has been recovered from rice leaves. Sucrose was the only sugar identified in the sap, consistent with it being the translocated form of carbohydrate (Fukumorita and Chino, 1982; Hayashi and Chino, 1990). The sucrose concentration in rice ST sap collected from leaf sheaths was measured at between 205 mM and 754 mM (Fukumorita and Chino, 1982; Hayashi and Chino, 1990). The sucrose content of a rice leaf M cell has apparently not been reported. In comparison, the sucrose concentration in the barley leaf M cell cytoplasm has been measured at between 150 mM and 230 mM (Lohaus *et al.*, 1995; Koroleva *et al.*, 1997, 1998). However, for a meaningful comparison, the sucrose concentration both in the rice leaf M cell cytoplasm and in the leaf ST sap needs to be collected from the same plant at the same time to determine whether the sucrose concentration in the M cell is higher or lower than that in the ST. If the sucrose content is similar between a barley and rice M cell, the highest M cell sucrose concentration could be just slightly greater than the lowest reported ST concentration, supporting the possibility of a symplasmic phloem loading path (Fig. 1B). In contrast, if the M cell sucrose concentration is less than one-third the higher value reported for the rice ST sucrose content, an active concentrating mechanism and apoplasmic loading would be required (Fig. 1C).

To examine whether sucrose transport in rice included an apoplasmic step, Furbank *et al.* (2001) treated rice leaf sheath and stem tissue sections with PCMBS, which partially inhibited [^{14}C]sucrose uptake and suggested the involvement of a SUT in the phloem transport of sucrose. However, these tissues are not the primary site of sucrose loading in the collection phloem, but instead are part of the transport phloem. Therefore, the inhibited activity could correspond to the impairment of the sucrose retrieval function of OsSUT1, rather than sucrose phloem loading activity. Hence, sucrose uptake into the collection phloem of minor veins in the leaf blade tissues needs to be tested for PCMBS sensitivity to determine if phloem loading is inhibited in rice. If so, it would support rice using the apoplasmic phloem loading pathway.

The roles of rice SUTs in phloem loading

Toward understanding the mechanism of phloem loading and carbohydrate partitioning in rice, multiple SUT genes have been identified in the rice genome (Aoki *et al.*, 2003). *OsSUT1* was the first SUT to be cloned from a monocot and is expressed in source leaf blades and sheaths (Hirose *et al.*, 1997). RNA and protein localization studies determined that OsSUT1 is expressed in the phloem, in both the CC and SE of leaf blades and sheaths (Matsukura *et al.*, 2000; Scofield *et al.*, 2007), and that it is the predominantly expressed SUT in rice tissues (Sun *et al.*, 2012). From the light microscope images presented (Scofield *et al.*, 2007), it is not possible to discern whether OsSUT1 is expressed in both STs and TSTs.

To investigate the function of *OsSUT1* in sucrose phloem loading in rice leaves, two research groups independently generated antisense RNA lines. Antisense repression of *OsSUT1* expression had no observable effects on plant growth, photosynthesis, or carbohydrate assimilation, but did result in

decreased seed yield and germination (Ishimaru *et al.*, 2001; Scofield *et al.*, 2002). Possible explanations for why knockdown of *OsSUT1* did not lead to a measurable change in plant growth or leaf carbohydrate accumulation were that (i) the level of gene repression was insufficient to eliminate gene function; (ii) genetic redundancy with another rice *SUT* compensated for the loss of *OsSUT1*; and (iii) rice may utilize symplasmic phloem loading.

Subsequent research identified *Tos17* retrotransposon insertional mutations of *OsSUT1* that are null alleles. Unfortunately, self-fertilizing heterozygous plants harbouring the *Tos17* insertions in *OsSUT1* did not yield any homozygous mutant plants (Hirose *et al.*, 2010). The authors determined that *OsSUT1* function is required for pollen germination and/or pollen tube growth, but not for pollen development or female reproduction. Because *ossut1* homozygous mutants could not be produced, the effect of the *ossut1* mutant on phloem loading could not be assessed. To circumvent this, Eom *et al.* (2012) used anther tissue culture to regenerate homozygous *ossut1* mutant lines from one of the *Tos17* exon insertions in *OsSUT1*. Similar to the antisense *OsSUT1* lines, *ossut1* homozygotes were indistinguishable from wild-type plants during vegetative growth. These data support the conclusion that OsSUT1 is not required for apoplasmic phloem loading of sucrose in leaves. The rice genome contains five *OsSUT* genes (Aoki *et al.*, 2003), so it is possible that one of the other four *OsSUT* genes could compensate for the loss of *OsSUT1* function. According to semi-quantitative reverse transcription–PCR (RT–PCR) expression analyses, all five *OsSUT* genes are expressed in source leaves, with the two genes phylogenetically more closely related to *OsSUT1*, *OsSUT3* and *OsSUT5*, showing higher expression than *OsSUT1* (Aoki *et al.*, 2003). Through biochemical activity studies in *Xenopus laevis* oocytes, OsSUT5 was found to have higher substrate affinity for sucrose and less substrate specificity than OsSUT1, and thus it could potentially replace OsSUT1 function in sucrose phloem loading (Sun *et al.*, 2010). Cell type-specific expression studies are needed to determine whether any *OsSUT* genes overlap with *OsSUT1* expression in the CC–SE complex and could mediate sucrose import into the ST. Alternatively, the *ossut1* mutant characterization data may suggest that OsSUT1 may have a primary role in reloading sucrose leaked into the apoplasm from the ST (Scofield *et al.*, 2007; Eom *et al.*, 2012), or that rice uses a symplasmic phloem loading mechanism (Eom *et al.*, 2012).

OsSUT2 belongs to a phylogenetic clade different from OsSUT1 (Aoki *et al.*, 2003; Sauer, 2007; Braun and Slewinski, 2009; Reinders *et al.*, 2012), and closely related SUT proteins from other plants have been shown to localize to the tonoplast (Endler *et al.*, 2006; Reinders *et al.*, 2008; Payyavula *et al.*, 2011; Schneider *et al.*, 2012). Eom *et al.* (2011) determined that a OsSUT2::green fluorescent protein (GFP) translational fusion also localized to the vacuole membrane. The authors demonstrated that OsSUT2 has proton–sucrose symporter activity when expressed in yeast, and transgenic rice plants carrying a promoter *OsSUT2::β-glucuronidase* (*GUS*) reporter showed that *OsSUT2* was not expressed in leaf phloem cells, but was strongly expressed in the M cells. Hence, if another *OsSUT* genetically compensates for the loss of *ossut1* function, it is unlikely to be *OsSUT2*. To characterize the function of *OsSUT2*, a null mutant T-DNA insertion allele, *ossut2*, was identified. Compared with the wild type, *ossut2* mutant plants had decreased plant growth, no changes in photosynthesis, an ~2-fold increase in soluble sugar content (sucrose, glucose, and fructose) in the leaves, a 54% reduction in sugar export from the leaves, and diminished seed yield. Transgenic complementation with the *OsSUT2* cDNA expressed by the strong maize *Ubiquitin* promoter complemented the mutant phenotypes. These data support the conclusion that the tonoplast-localized OsSUT2 mediates sucrose flux from the vacuole into the M cell cytosol and is required to support normal growth in rice. A similar function is proposed for the phylogenetically related *PtaSUT4* from poplar (*Populus tremula*×*alba*) (Payyavula *et al.*, 2011). Based on the *ossut1* and *ossut2* mutant phenotypes, *OsSUT2* expression data, and the vein ultrastructural data of Kaneko *et al.* (1980), Eom *et al.* (2012) proposed that rice may use a symplasmic pathway to load sucrose into the phloem.

The roles of rice SWEETs in phloem loading and pathogenesis

If rice uses symplasmic phloem loading, there would be no need for sucrose to transit into the apoplasm prior to entering the ST. Therefore, it would imply that SWEET effluxers are not involved in sucrose movement from the photosynthetic cells to the ST. What are the functions of rice SWEETs then? Rice OsSWEET11 and OsSWEET14 are co-orthologous to AtSWEET11 and AtSWEET12 in *Arabidopsis* (Chen *et al.*, 2010; Yuan and Wang, 2013). OsSWEET11 and OsSWEET14 both preferentially transport sucrose and localize to the plasma membrane (Chu *et al.*, 2006; Chen *et al.*, 2012). Similarly to the *Arabidopsis AtSWEET11* and *AtSWEET12* genes, promoter::GUS reporter analysis of *OsSWEET11* indicated that the gene was preferentially expressed in VP cells (Chu *et al.*, 2006). Interestingly, *OsSWEET11*, also known as *Os8N3*, was first identified as the rice disease susceptibility gene *xa13* (Chu *et al.*, 2006; Yang *et al.*, 2006), and was also discovered to regulate copper distribution in plants (Yuan *et al.*, 2010; Zhang and Wang, 2013). Cultivars containing the recessive *xa13* allele are resistant to the rice pathogen *Xanthomonas oryzae* pv. *oryzae* strain PXO99^{A}, whereas other rice strains containing the dominant *Xa13* allele are susceptible. PXO99^{A} depends upon a type III effector gene *pthXo1* to introduce TAL (transcriptional activator-like) effector proteins into the host cell. Infection of *Xa13*-containing rice plants by PXO99^{A} induces expression of *OsSWEET11* >100-fold; however, *xa13*-containing rice lines have mutations in the promoter of *OsSWEET11* that prevent PXO99^{A} from inducing gene expression (Chu *et al.*, 2006; Yang *et al.*, 2006). Chen *et al.* (2010) proposed a model suggesting that PXO99^{A} induces *OsSWEET11* expression to transport sugars into the apoplasm where the bacteria proliferate, leading to bacterial blight.

During the evolutionary arms race between PXO99^A and *xa13* rice plants, the bacteria acquired other TAL effectors, for example AvrXa7, PthXo3, TalC, and Tal5, which induce expression of a different target gene, *Os11N3*, to defeat *xa13* resistance (Antony *et al.*, 2010; Yu *et al.*, 2011; Streubel *et al.*, 2013). Remarkably, Os11N3, also called OsSWEET14, has a similar function to OsSWEET11 and presumably effluxes sugar to the apoplasm, which facilitates bacterial growth. By up-regulating proteins that preferentially efflux sucrose, rather than hexoses, into the apoplasm, the bacteria probably minimize the induction of host defence responses (Herbers *et al.*, 1996; Essmann *et al.*, 2008; Kocal *et al.*, 2008). Insertional mutants or RNA interference (RNAi)-silenced lines of *OsSWEET14* are no longer susceptible to infection by AvrXa7-containing pathogens (Antony *et al.*, 2010). Recently, another *Xanthomonas oryzae* recessive disease resistance locus, *xa25*, was cloned in rice and found to encode a close paralogue of *OsSWEET14* (Liu *et al.*, 2011), suggesting that the bacteria co-opted the expression of multiple SWEETs to enable their pathogenicity.

OsSWEET11 and OsSWEET14 function as sucrose facilitators (Chen *et al.*, 2012). *OsSWEET11* is expressed at low levels in leaves and roots, and at higher levels in developing inflorescences and pollen (Yang *et al.*, 2006; Yuan and Wang, 2013). Rice lines containing the recessive alleles of *xa13* or *xa25* grow normally, as do transgenic plants silenced for *OsSWEET11* expression via RNAi (Yang *et al.*, 2006; Liu *et al.*, 2011; Li *et al.*, 2012). However, *OsSWEET11*-defective plants show reproductive defects and have low pollen viability and poor fertility (Yang *et al.*, 2006; Yuan and Wang, 2013). In contrast, *OsSWEET14* T-DNA insertional mutants display a stunted plant growth phenotype. Homozygous mutants require approximately twice the number of days to attain a plant size similar to the wild type, and show a reduction in seed size (Antony *et al.*, 2010). *OsSWEET14* shows higher expression in vegetative tissues and lower expression during panicle development (Yuan and Wang, 2013). The cellular expression pattern for *OsSWEET14* remains to be characterized; however, the *ossweet14* mutant phenotypes partially resemble those of the *Arabidopsis atsweet11; atsweet12* double mutant. It will be interesting to determine whether the *ossweet14* mutants have decreased sucrose in the phloem and hyperaccumulate carbohydrates in their leaves. If confirmed, these data may indicate that *OsSWEET14* has a similar function in rice leaves to *AtSWEET11* and *AtSWEET12* in *Arabidopsis*, and imply that OsSWEET14 may play a larger role than OsSWEET11 in sucrose export out of source tissues.

Path of sucrose phloem loading in rice reconsidered

If rice uses a symplasmic phloem loading pathway, why does the *ossweet14* mutant show a reduction in plant growth? Could this indicate that sucrose is not being efficiently effluxed to the apoplasm in the mutant, restricting phloem loading and thereby growth? This question prompted a re-evaluation of the proposed sucrose transport pathway in rice based on anatomical considerations. Looking at Table 1 in the study by Kaneko *et al.* (1980), the authors clearly distinguish between early-formed, thin-walled metaphloem SEs (termed M_1SEs, denoted here as STs) and late-formed metaphloem SEs (M_2SEs, here called TSTs). The data of Kaneko *et al.* (1980) are reanalysed and presented in Table 1 and Fig. 2. In their study, the thin-walled SEs were found to be connected via PD to early-formed metaphloem CCs (M_1CCs, or simply CCs), but these CC–SE complexes were largely symplasmically isolated from other cell types in the veins, thereby strongly resembling the anatomical arrangement seen in apoplasmically phloem loading grass leaves (Evert *et al.*, 1978, 1996; Robinson-Beers and Evert, 1991; Berthier *et al.*, 2009). In contrast, the TSTs were only connected to late-formed metaphloem CCs (M_2CCs, here called VP cells). However, the VP cells had many PD connections to other parenchyma cells throughout the vein, including MS (referred to as thick-walled parenchyma) cells, as well as parenchyma cells adjacent to both xylem (XP cells) and phloem (PP cells) poles of the vein. In fact, VP cells had the fewest PD connections to TST cells in comparison with the other cell types (Table 1). Thus, Kaneko and colleagues were correct to conclude that a symplasmic path may be available for sucrose movement from the M cell to the SE (Fig. 1B). However, as noted above, the presence of PD connections is not sufficient to conclude whether symplasmic phloem loading is used.

In maize and rice leaf minor veins, the later forming metaphloem SEs are TSTs and associated with VP cells, whereas early-forming metaphloem SEs are STs and associated with CCs (Walsh, 1974; Evert *et al.*, 1978; Kaneko *et al.*, 1980). Botha and colleagues (2008, 2013) clarified that all longitudinally oriented veins in rice leaves contain both STs and TSTs. Hence, reinterpreting the PD frequency and ultrastructural data of Kaneko *et al.* (1980), the CC–SE complex in rice leaf blades is largely symplasmically isolated from other cells in the vein, whereas the VP cell–TST complex has ~5-fold higher potential symplasmic connectivity (11.1% PD between PP and VP cells compared with 2.2% PD between PP cells and CCs) to other parenchyma cells (Table 1). These data are in agreement with fluorescent dye retrieval data presented by Botha *et al.* (2008) who found that the TSTs are able to accumulate dye retrieved from the apoplasm by VP cells, but that the CC–SE complexes did not accumulate dye and were symplasmically isolated. Botha *et al.* (2008) discussed evidence suggesting that there may not be significant flux through the VP cell–TST pathway as dye transport studies reveal symplasmic connectivity, not dynamic transport rates. Additionally, it was found by feeding [^{14}C]sucrose through the xylem of cut maize leaves that the VP cells associated with TSTs were the first cells to retrieve the sucrose, which was subsequently transferred into the TSTs (Fritz *et al.*, 1983). Therefore, it is likely that the symplasmic sucrose movement pathway proposed by Kaneko *et al.* (1980) is used for sucrose retrieval from the xylem, rather than the mechanism of phloem loading for long-distance transport. Collectively, these data suggest that rice may in fact use an apoplasmic pathway to load sucrose into the STs in the collection phloem (Fig. 1C), which is the same anatomical and functional arrangement described

Table 1. *Relative plasmodesmata frequencies between cell types in small veins in an expanded rice leaf blade*

Data modified from Kaneko M, Chonan N, Matsuda T, Kawahara H. 1980. Ultrastructure of the small vascular bundles and transfer pathways for photosynthate in the leaves of the rice plant. *Japanese Journal of Crop Science* 49, 42–50. with permission from The Crop Science Society of Japan.

Cell types	BS	MS	PP	VP	CC
MS	29.8%	9.0	8.6	6.2	0
XP	12.0	5.6	2.8	4.3	–
PP	–	8.6	3.1	11.1	2.2
TST	–	–	0	2.2	0
ST	–	–	0	–	2.5

BS, bundle sheath cells, which correspond to parenchyma sheath cells in Kaneko *et al* (1980). MS, mestome sheath cells, which correspond to the thick-walled parenchyma cells. These cells are located at the periphery of the vein and are in contact with the BS cells, but not in as close proximity to the phloem CC–SE complex as PP cells. VP, vascular parenchyma cells, which correspond to M_2CC (late-formed CC) and are associated with the thick-walled SEs (TSTs). PP, phloem parenchyma cells, which are located internal to the MS cells and are in proximity to the CC–SE complexes. XP, xylem parenchyma cells, which are located adjacent to the xylem vessels. CC, companion cells, which correspond to M_1CC (early-formed CC). TST, thick-walled SE, which corresponds to M_2SE (late-formed SE). ST, thin-walled SE, which correspond to M_1SE (early-formed SE). – indicates that these two cell types do not share a common interface. Data for protophloem CC and SE are omitted for clarity.

for the rice transport phloem (Chonan *et al.*, 1984), as well as for the maize, sugarcane, *Lolium*, and barley collection phloem (Evert *et al.*, 1978, 1996; Robinson-Beers and Evert, 1991; Berthier *et al.*, 2009). However, the corollary of this hypothesis is the question of why *ossut1* mutants do not display a phenotype comparable with the inhibition of phloem loading in other apoplasmic species (Riesmeier *et al.*, 1994; Bürkle *et al.*, 1998; Gottwald *et al.*, 2000; Srivastava *et al.*, 2008; Slewinski *et al.*, 2009).

There are several possibilities to explain this lack of transport inhibition. It is possible that when *OsSUT1* function is disrupted, sucrose is not apoplasmically loaded into the CC–SE complex, and therefore the sucrose concentration in the STs is not expected to be greater than that of the photosynthetic cells. Instead, sucrose may passively enter the phloem translocation stream, moving down a concentration gradient from M cells to STs, through the few PD that connect the VP cells to the CCs. Though nearly symplasmically isolated, a small number of PD connect the CC–SE complex to adjacent cells (Evert *et al.*, 1978, 1996; Kaneko *et al.*, 1980; Botha and Evert, 1988; Robinson-Beers and Evert, 1991; Botha, 2013). These PD may possibly be used to traffic macromolecules, such as RNA or proteins, into the translocation stream (Ishiwatari *et al.*, 1995; Doering-Saad *et al.*, 2002; Tamaki *et al.*, 2007). Hence, while sucrose entry into the STs via the PD between the VP cells and CCs may normally account for only a small component of the sucrose in the translocation stream, this may become the primary route in the *ossut1* mutant. This shift from predominantly apoplasmic phloem loading could reflect the ability of rice to use symplasmic phloem loading conditionally, as has been previously suggested to be the case in maize and *Arabidopsis* when the principal SUT responsible for sucrose phloem loading was mutated (Srivastava *et al.*, 2008; Slewinski *et al.*, 2009). Heterogeneity in phloem loading mechanisms is common among angiosperms, even in so-called apoplasmic species such as *Arabidopsis* (Slewinski *et al.*, 2013). A second possibility for *ossut1* mutants not displaying a visible defective phloem loading phenotype would be that another rice SUT, such as OsSUT5, may compensate for the loss of OsSUT1 function. Genetic characterization of *ossut5* single and double mutants with *ossut1* would be informative for testing this hypothesis. It is also possible that another type of sugar transport protein could be responsible for apoplasmic phloem loading into the CC–SE complex in rice. Within the last couple of years, two other classes of membrane proteins were shown to be able to transport sucrose across a membrane (Wingenter *et al.*, 2010; Chen *et al.*, 2012). It is possible that one of these types or another unknown membrane protein could be largely responsible for apoplasmic sucrose phloem loading in rice. Hence, the evidence for rice normally using symplasmic sucrose phloem loading is weak, and further work is necessary to clarify the route by which sucrose is transported into the phloem in rice leaves.

Phloem unloading and post-phloem transport in sinks

Following phloem loading in source leaves, sucrose and other assimilates, such as amino acids and small peptides, are transported through STs to non-photosynthetic tissues (sinks) where they are unloaded for diverse uses. Sucrose is unloaded from the SE–CC complex either symplasmically through interconnecting PD, or apoplasmically into the surrounding cell wall matrix, or through a combination of both. The unloaded sucrose may be (i) taken up by adjacent recipient sink cells across the plasma membrane through SUTs (Weber *et al.*, 1997) or H^+/hexose symporters (HXTs) following hydrolysis by cell wall invertase, CWIN (Ruan *et al.*, 2010); or (ii) further transported to distant cells apoplasmically or symplasmically, through a process named post-phloem transport, depending on sink type, developmental stage, and species (Werner *et al.*, 2011). The control of phloem unloading and post-phloem transport is largely determined by the cellular pathways through which it occurs. Below, the paths of assimilate unloading and transport are evaluated in two major sinks, developing seeds and fruits, which constitute ~75% of total world food crop production.

Phloem unloading and post-phloem transport pathway in seeds

Developing seeds from all angiosperm species are characterized by two distinctive features. First, vascular bundles terminate in the maternal seed tissue, hence phloem unloading occurs there. Secondly, the filial tissues, namely the embryo and endosperm, are isolated from the maternal tissue by an apoplasmic compartment. The phloem unloading pathway has been most studied during seed development of

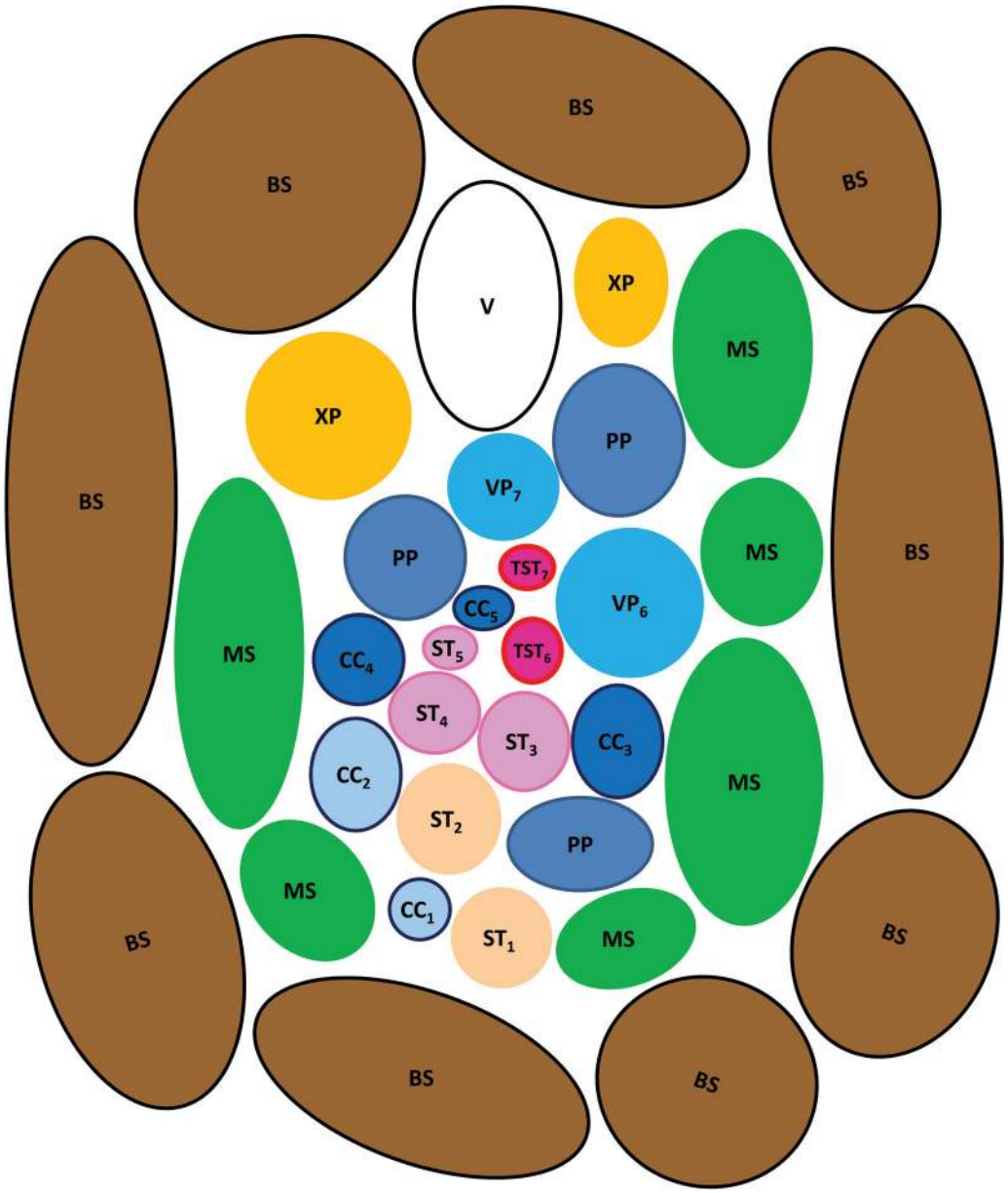

Fig. 2. Schematic of a rice leaf blade minor vein. The bundle sheath (BS) cells encircle the vein. The xylem vessel (V) is flanked by xylem parenchyma (XP) cells. Mestome sheath (MS) cells are located internal to the BS cells, adjacent to the xylem and phloem. Protophloem companion cells (CC_1 and CC_2) and protophloem sieve elements (ST_1 and ST_2) are located on the abaxial side of the vein. Phloem parenchyma (PP) cells are dispersed within the phloem. Early-formed metaphloem companion cells (CC_3–CC_5) and thin-walled sieve elements (ST_3–ST_5) are centrally located within the phloem. Late-formed metaphloem companion cells (VP_6 and VP_7) and thick-walled sieve elements (TST_6 and TST_7) are positioned in proximity to the xylem. Mesophyll cells outside of the BS cells are not shown. The figure is redrawn from Figs 1 and 2 in Kaneko M, Chonan N, Matsuda T, Kawahara H. 1980. Ultrastructure of the small vascular bundles and transfer pathways for photosynthate in the leaves of the rice plant. *Japanese Journal of Crop Science* 49, 42–50. with permission from The Crop Science Society of Japan.

legumes and cereals (Patrick and Offler, 2001; Zhang *et al.*, 2007; Bihmidine *et al.*, 2013). A symplasmic pathway for post-phloem sugar transport operates on both sides of the apoplasmic compartment [i.e. within the seed coat or filial tissues (Patrick, 1997)]. Because of the symplasmic isolation between maternal and filial tissues and between endosperm and embryo (Stadler *et al.*, 2005), sugars (sucrose or hexoses) must pass across at least two plasma membranes during transit from the maternal tissue to the filial tissue (Weber *et al.*, 2005), mediated by plasma membrane sucrose or hexose effluxers and influxers, respectively.

Recently, Werner *et al.* (2011) reported a dual switch in phloem unloading during ovule development in *Arabidopsis*. First, sucrose was unloaded through a symplasmic phloem pathway into ovule primordia, which follows a shift to an apoplasmic pathway when integuments are formed prior to

flowering. Subsequently, symplasmic unloading was switched on again in the integuments following fertilization. Although the cause of the underlying dual switch is unknown, this developmental shift in cellular route of assimilate transport resembles that in developing cotton fibres, which are single-celled seed trichomes. The fibre cells connect the underlying seed coat symplasmically in the initial and late phases of cell elongation, but are symplasmically isolated in the mid phase of elongation (Ruan *et al.*, 2001), probably due to temporary deposition of callose in the neck regions of PD (Ruan *et al.*, 2004). It is postulated that closure of the PD may provide a cellular basis for the fibres to maintain their cell turgor for further elongation. This view is supported by the high level of expression of plasma membrane transporters for sucrose and K^+ during PD closure (Ruan *et al.*, 2001), and the positive correlation between the duration of PD closure and fibre length attained across cotton species or genotypes (Ruan *et al.*, 2004, 2005). Overall, these observations suggest that symplasmic connectivity is highly regulated and varies not only between different sink types, but also between different developmental stages.

Phloem unloading and post-phloem pathway in fruit

Developing fruit typically features an apoplasmic unloading pathway operating at least for a certain period during pericarp development. In apple (*Malus domestica*), for example, phloem unloading follows an extensive apoplasmic pathway throughout fruit development. This conclusion is based on the absence of PD at the interface between the SE–CC complexes and their surrounding cells, and the inability of the symplasmic fluorescent dye carboxyfluorescein to move out of the phloem strands (Zhang *et al.*, 2004). Similarly, phloem unloading also follows an extensive apoplasmic pathway in developing cucumber (*Cucumis sativus*) fruit from anthesis to marketable mature stage (Hu *et al.*, 2011) and in walnut (*Juglans regia*) fruit pericarp (Wu *et al.*, 2004).

In some fruits, both symplasmic and apoplasmic unloading may operate sequentially, and the two unloading pathways may shift in response to developmental signals. For instance, in tomato (*Solanum lycopersicon*) fruit pericarp, a symplasmic pathway operates at the early stages of starch accumulation but shifts to an apoplasmic pathway during hexose accumulation (Ruan and Patrick, 1995). In grape (*Vitis vinifera*) berry, a symplasmic unloading pathway predominates during early and middle stages of berry development when sugar levels are low, but switches to apoplasmic unloading at the onset of ripening when the grape berry starts accumulating high concentrations of hexoses (Zhang *et al.*, 2006). The reason for this symplasmic to apoplasmic pathway shift may be that a symplasmic step would result in a back-flow of assimilates to the SEs due to the build up of cell turgor generated from the osmotic effect of high soluble sugar accumulation, while the apoplasmic unloading pathway bypasses this problem (Patrick, 1997). In jujube (*Zizyphus jujuba*) fruit, the phloem unloading pathway switches from an apoplasmic pathway early in development to a symplasmic route during the middle stage and then back to an apoplasmic path again in the late stage (Nie *et al.*, 2010). The cellular basis and the significance of this dual switch are unknown. However, the pathway switch may be regulated by developmental signals as indicated by decreased symplasmic conductivity in the shoot apical meristem during the transition from vegetative to reproductive stages (Gisel *et al.*, 2002).

Spatially, different unloading pathways may operate simultaneously in the same organ. For example, phloem unloading occurs symplasmically in pericarp but apoplasmically in the placenta connecting young seeds early in tomato fruit development (Jin *et al.*, 2009). The latter mode may provide a mechanism to generate glucose via CWIN to stimulate cell division for seed set (Ruan *et al.*, 2012). The observation highlights the complexity of cellular paths that different cell types may adopt to coordinate development at the tissue or organ level.

Phloem unloading and post-phloem transport pathways and sugar transporters

Symplasmic unloading and post-phloem transport require functional PD connecting the surrounding cells, whereas an apoplasmic pathway often couples with the expression of transporters to facilitate solute transfer across membranes. Similar to the situation in phloem loading, there are two classes of sugar transporters involved in phloem unloading and post-phloem transport: sucrose effluxers functioning to export sucrose, and sucrose or hexose influxers for uptake of sugars by the recipient sink cells, coupled with the H^+ gradient established through the activity of the plasma membrane H^+-ATPase.

A common phenomenon of apoplasmic unloading is the co-expression of CWIN and the plasma membrane hexose transporters either in the unloading region (Ruan *et al.*, 1997; Jin *et al.*, 2009) or at the post-phloem path, such as the interface between seed maternal and filial tissues (Weber *et al.*, 2005). In developing barley seed, HvCWINV2 and a H^+–hexose symporter, HvSTP2, are co-expressed in the pericarp unloading site at the very early stage of seed development, indicating their role in the development of the maternal seed tissue (Weschke *et al.*, 2003). In developing *Vicia faba* seed, the level of CWIN gene expression at the inner layer of the seed coat correlates with seed size, probably through co-expression of the hexose transporter in the adaxial epidermis of the cotyledons that take up CWIN-released glucose to promote mitotic cell division of the embryonic tissues (Weber *et al.*, 1996).

The importance of sugar transporters in sink development, which is coupled with phloem unloading and post-phloem transport, has been demonstrated by transgenic and mutational analyses. For example, suppression of a rice sucrose transporter, *OsSUT1*, and of tomato hexose transporters, *LeHXT 1*, *2*, or *3*, results in unfilled grains (Scofield *et al.*, 2002) and reduced fruit hexose levels (McCurdy *et al.*, 2010), respectively. On the other hand, overexpression of the potato (*Solanum tuberosum*) *StSUT1* gene in pea (*Pisum sativum*) increased sucrose uptake and the growth rate of the

cotyledons (Rosche *et al.*, 2002). Mutation of *AtSWEET8* and *OsSWEET11*, which encode sugar uniporters, blocked sugar efflux from tapetum cells in anthers, leading to unviable pollen grains in *Arabidopsis* and rice (Yang *et al.*, 2006; Guan *et al.*, 2008). As described previously, mutation of the *OsSWEET11* promoter prevented pathogenic bacteria from up-regulating transcription of the gene, and thereby sugar efflux from host cells, which consequently limited pathogen growth (Chen *et al.*, 2010). These studies provide valuable leads for future work to improve crop yield and disease resistance through manipulating sugar transporters.

Improving development, yield, and abiotic stress tolerance by altering sugar metabolism

Once sucrose has reached the sink cells following phloem unloading, it must be either hydrolysed by invertase (INV; EC 3.2.1.26) into glucose and fructose, or degraded by sucrose synthase (Sus; EC 2.4.1.13) into uridine-diphosphoglucose (UDP-glucose) and fructose for various metabolic, biosynthetic, or signalling processes (Fig. 3). In general, INVs appear to play regulatory roles in plant growth and development (Ruan *et al.*, 2009), whereas Sus is mainly involved in the biosynthesis of sugar polymers, including starch and cellulose, and generation of energy (ATP) (Chourey *et al.*, 1998; Coleman *et al.*, 2009). In parallel to their major roles in primary carbon metabolism, both INV and Sus play important signalling roles in plant development (Ruan, 2012). There is now compelling evidence that, in contrast to that in wild species, where INV or Sus activities may not exert evident control over the growth of small sinks such as *Arabidopsis* seeds, the development of bulky sinks in crop species is more sensitive to changes in INV or Sus expression levels or activities (Xu *et al.*, 2012). In the sections below, several case studies discuss the regulation of sink development, crop yield, quality, and stress tolerance by some major players in sugar metabolism.

Invertase and its inhibitor

INVs may be located in the cell wall, cytoplasm, and vacuole, and hence are named CWIN, CIN, and VIN, respectively, which are encoded by different genes with different molecular and biochemical characteristics (Ruan *et al.*, 2010). Apart from transcriptional and translational regulation, CWIN and VIN activities are highly regulated at the post-translational

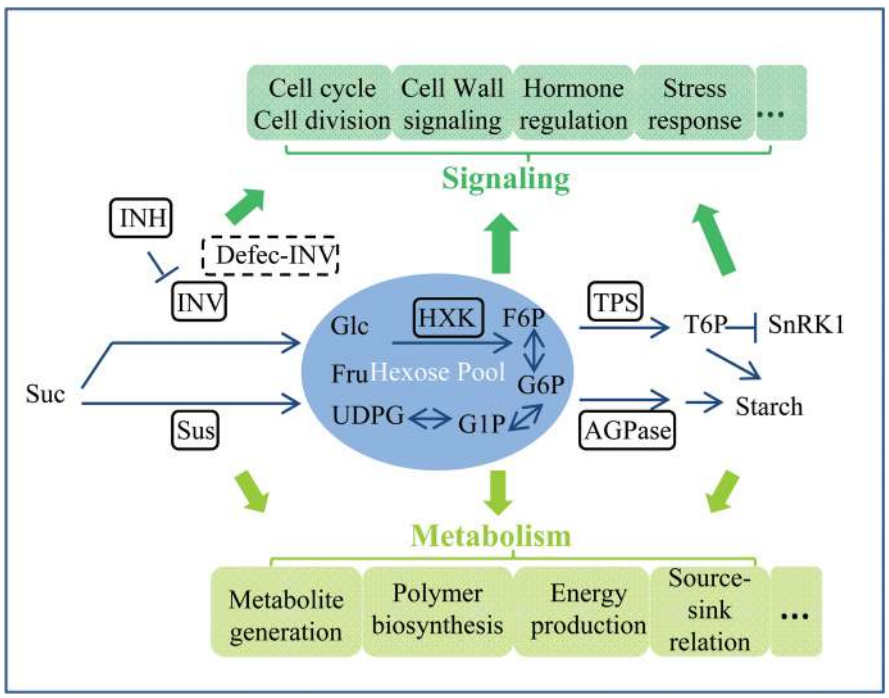

Fig. 3. Roles of sugar metabolism in sink tissue. A selection of crucial players is presented. Phloem-imported sucrose (Suc) must be either degraded by sucrose synthase (Sus) into uridine-diphosphoglucose (UDPG) and fructose (Fru), or hydrolysed by invertase (INV) into glucose (Glc) and Fru. INV activity is subject to post-translational regulation by their inhibitors (INH), and the putative defective invertase (Defec-INV). Generated Glc and Fru could be converted by hexokinase (HXK) into glucose-6-phosphate (G6P) and fructose-6-phosphate (F6P). Conversions among F6P and G6P, glucose-1-phosphate (G1P), and UDPG can be achieved by a series of enzymes. G6P and UDPG could be used by trehalose-6-phosphate synthase (TPS) to generate trehalose-6-phosphate (T6P), a signal metabolite promoting biosynthetic reactions by inhibiting sucrose non-fermenting related kinase-1 (SnRK1) activity. G1P could be catalysed by ADP-glucose pyrophosphorylase (AGPase) to form the initial substrate, ADP-glucose, for starch biosynthesis. These sugar metabolic pathways play important signalling roles in plant growth and development, in parallel to their functions in metabolism. Arrows (→) signify the direction or stimulation of the reaction, while an inhibitory interaction is represented by the ⊣ symbol.

level through interaction with their inhibitors, a group of small peptides with molecular masses ranging from 15 kDa to 23 kDa (Ruan *et al.*, 2010).

The critical role of CWIN in sinks has been demonstrated through mutational and transgenic analyses. Loss of function of an endosperm-specific *Incw2* gene resulted in an abnormal basal endosperm transfer cell and miniature seed (Cheng *et al.*, 1996). The phenotype is probably due to suppressed auxin biosynthesis (LeClere *et al.*, 2010) and reduced mitotic activity and cell size in the endosperm (Vilhar *et al.*, 2002) in response to a decreased glucose level in the mutant seed. A similar role for CWIN in seed development was found in rice (Wang *et al.*, 2008) and tomato (Zanor *et al.*, 2009). In tobacco, pollen development is inhibited by antisense suppression of Nin88, initially considered to be a CWIN (Goetz *et al.*, 2001), but more recently shown to be a putative defective CWIN that may enhance CWIN activity (Le Roy *et al.*, 2013). A strong positive correlation has been established between CWIN expression or its activity and abiotic stress tolerance in reproductive organs, particularly with regard to male and female fertility in both monocot and dicot species (Ruan *et al.*, 2012).

Several attempts have been made to exploit the functional coupling between CWIN and sink development for improving crop yield or accelerating breeding. Here, silencing a CWIN inhibitor, INVINH1, in tomato (i) restored CWIN activity in old leaves to the young leaf level, leading to delayed leaf senescence; and (ii) increased CWIN activity in fruit, resulting in increased fruit hexose level and mature seed weight (Jin *et al.*, 2009). Similarly, overexpressing CWIN in rice grains driven by it native promoter increased grain size (Wang *et al.*, 2008). These examples indicate huge potentials to improve plant productivity through modulating CWIN activity.

Recent studies have also significantly advanced our understanding of the roles of VIN and CIN in development. VIN has long been considered as a major player in cell expansion. This is achieved by hydrolysing sucrose into glucose and fructose, thereby doubling the osmotic potential of sucrose to attract an influx of water that generates a high turgor. The osmotic role of VIN, however, only applies to sinks that accumulate high level of hexoses, such as cotton fibres, and tomato and grape fruits. In tissues with low sugar levels, such as *Arabidopsis* roots, VIN promotes cell expansion in an osmotic-independent manner, probably through cross-talk between sugar signalling and wall-associated kinases or hormonal pathways (Wang *et al.*, 2010; Wang and Ruan, 2013). Application of VIN manipulation has been best demonstrated in preventing post-harvest sweetening of potato tubers during storage. Cold storage triggers an accumulation of reducing sugars in tubers, which induces sprouting and pathogen infection, and causes dark-coloured, bitter-tasting products during high temperature processing of potato chips. The latter products have high levels of acrylamide, a neurotoxin and potential carcinogen (Bhaskar *et al.*, 2010). This sweetening problem can be effectively alleviated or even eliminated by silencing the potato *VInv* gene (Bhaskar *et al.*, 2010) or by expression of a tobacco vacuolar invertase inhibitor (NtVIF) (Greiner *et al.*, 1999).

CIN has traditionally been thought to be a cytosolic protein. However, recent studies showed that CIN can be localized in multiple intracellular organelles, including the chloroplast, mitochondria, and nucleus, and it may have functions well beyond metabolism (Wang and Ruan, 2013). Indeed, evidence has been provided to show that mitochondrial CIN could play a major role in maintaining homeostasis of reactive oxygen species (ROS) through coupling with mitochondrial hexose kinase (Xiang *et al.*, 2011). Since ROS function as important signalling molecules in development and stress responses, the connection between CIN and ROS opens up exciting opportunities to dissect CIN-mediated signalling pathways.

Sucrose synthase

Sus has long been considered to be a biochemical marker for sink strength. This is generally the case in crop species. In maize, for example, mutation of one Sus protein, SH1, disrupts endosperm cellularization, probably due to reduced production of UDP-glucose for cellulose biosynthesis, leading to a shrunken seed phenotype; whereas, loss of another Sus, Sus1, decreases starch accumulation in the endosperm (Chourey *et al.*, 1998). More recently, Xu *et al.* (2012) found that overexpression of Sus in cotton reduces seed abortion, which results in more mature seeds and higher cotton fibre yield, probably due to increased sink strength. Consistently, increased Sus enzyme activity in the developing xylem of transgenic poplar promotes secondary cell wall cellulose biosynthesis without affecting other aspects of plant growth, resulting in thicker xylem secondary cell walls and consequently improved wood density (Coleman *et al.*, 2009). In *Arabidopsis*, however, Sus does not appear to exert evident control over plant growth and development (Barratt *et al.*, 2009), although the degree of reduction in Sus activity in the mutants analysed remains disputable (Baroja-Fernández *et al.*, 2012). The above analyses suggest that the roles of Sus in sink development may have been enhanced through domestication, and it remains a valid target for improving sink strength through gene technology in crops.

ADP-glucose pyrophosphorylase

ADP-glucose pyrophosphorylase (AGPase) is a key enzyme regulating starch biosynthesis (Keeling and Myers, 2010). It catalyses the production of ADP-glucose (ADPG) and pyrophosphate from glucose-1-phosphate and ATP. ADPG serves as the initial substrate for the starch biosynthetic pathway. The native AGPase is a heterotetramer in diverse plant species. In maize endosperm, it is comprised of two small subunits, encoded by the *Brittle2* (*Bt2*) gene, and two large subunits, encoded by the *Shrunken2* (*Sh2*) gene, and is localized both in amyloplasts and in the cytosol (Hannah, 2005; Ruan and Chourey, 2006). A number of studies have shown that increasing AGPase activity enhances seed starch biosynthesis and yield. For example, expression of the maize

AGPase gene, *Sh2r6hs*, in wheat increased grain weight by 38% due to enhanced sink strength (Smidansky *et al.*, 2002). *Sh2r6hs* is an AGPase mutant with an altered allosteric domain of SH2, which reduces its sensitivity to phosphate, an inhibitor of AGPase activity. Similarly, overexpressing AGPase in maize under the control of an endosperm-specific promoter enhanced AGPase activity, seed weight, and starch content compared with the wild-type plants (Li *et al.*, 2011*b*). AGPase is highly unstable under heat stress, which could account for reduction of starch biosynthesis and yield loss in hot weather. In this regard, a heat-stable variant of SH2 exhibited an improved interaction with the BT2 subunit, resulting in a more heat-stable and active heterotetramer AGPase (Greene and Hannah, 1998). This represents a valuable tool to enhance crop tolerance to heat and perhaps other forms of abiotic stress as well.

AGPase also plays important roles in transitory starch biosynthesis in tomato fruit, thereby contributing to fruit sugar levels and final yield. Here, introgression of a stably expressed large subunit of AGPase ($AgpL1^H$), sustained AGPase activity and increased fruit starch content, sugar levels, and fruit size (Petreikov *et al.*, 2006). More recently, Centeno *et al.* (2011) confirmed the relationship between AGPase activity and tomato sugar content by altering malate metabolism, in which they showed that AGPase is post-translationally regulated by cellular redox status. AGPase activity is also required for transitory starch accumulation in maize leaves (Slewinski *et al.*, 2008). The *agps* mutants had increased sugars in their leaves and exhibited decreased grain yields, indicating that the carbon remobilization from transitory starch in leaves makes a significant contribution to crop yield (Slewinski *et al.*, 2008; Schlosser *et al.*, 2012).

Trehalose-6-phosphate and trehalose: new tools for crop improvement

One notable discovery in sugar metabolism and signalling research in recent years is the importance of the disaccharide trehalose and its precursor trehalose-6-phosphate (T6P) in plant development. Trehalose is a non-reducing glucose disaccharide widely present in bacteria, fungi, invertebrates, and plants, where it serves as a carbon resource, structural component, and stress protectant in living cells (Elbein *et al.*, 2003). In higher plants, the trehalose biosynthesis pathway consists of a two-step process: a condensation reaction catalysed by trehalose-6-phosphate synthase (TPS) using UDP-glucose and glucose-6-phosphate (G6P) as substrates to generate T6P; and then a dephosphorylating step by trehalose-6-phosphate phosphatase (TPP) to form trehalose (Cabib and Leloir, 1958). UDP-glucose and G6P are two central molecules of plant metabolism. UDP-glucose is a known precursor for synthesis of cell wall components, cellulose and callose; and G6P is the starting point for energy metabolism, which is also used to generate NADPH for reductive biosynthesis (Ruan *et al.*, 2005; Masakapalli *et al.*, 2010). Therefore, T6P/trehalose synthesis is at the centre of carbon metabolism and plant development (Fig. 3). Moreover, T6P itself is an important signalling metabolite, which inhibits the catalytic activity of sucrose non-fermenting related protein kinase1 (SnRK1), and interacts with auxin and light signalling, in response to sucrose availability to support plant growth (O'Hara *et al.*, 2013). As revealed by studies in *Arabidopsis* and other crop species, T6P/trehalose biosynthesis plays indispensable roles in regulating plant development, ranging from vegetative growth (van Dijken *et al.*, 2004), the transition to flowering (van Dijken *et al.*, 2004), inflorescence morphogenesis (Satoh-Nagasawa *et al.*, 2006), flowering time control (Wahl *et al.*, 2013), and embryogenesis (Gómez *et al.*, 2010), to leaf senescence (Wingler *et al.*, 2012). Apparently, T6P/trehalose metabolism has to be tightly controlled in different plant organs, tissues, and cell types, and in response to environmental changes. Generally, the amount of trehalose as well as T6P is very low in the majority of higher plants (Vogel *et al.*, 2001). A balance between trehalose synthesis and breakdown is achieved by the participation of trehalase, which hydrolyses trehalose into two molecules of glucose.

In light of the protective ability, stability, and low reactivity of trehalose (Crowe *et al.*, 1984), and its natural accumulation in organisms upon stress (Karim *et al.*, 2007; Iordachescu and Imai, 2008), many attempts have been made to create stress-tolerant plants by introducing microbial trehalose biosynthetic genes. This has been done in tobacco (Holmstrom *et al.*, 1996; Romero *et al.*, 1997; Karim *et al.*, 2007), potato (Goddijn *et al.*, 1997), rice (Garg *et al.*, 2002; Jang *et al.*, 2003), tomato (Cortina and Culiáñez-Macià, 2005), and *Arabidopsis* (Karim *et al.*, 2007; Miranda *et al.*, 2007). Many transgenic plants with increased trehalose levels exhibit enhanced abiotic stress tolerance, but often are associated with pleiotropic growth aberrations, such as stunted roots, lancet-shaped leaves, and growth retardation (Romero *et al.*, 1997 Cortina and Culiáñez-Macià, 2005), which are probably due to the altered levels of T6P, and subsequent perturbations of developmental processes (Schluepmann *et al.*, 2003, 2004). Several approaches have been described to circumvent such growth defects without losing the improved stress tolerance. One is to introduce a bifunctional TPS–TPP construct into a plant under the control of a stress-inducible or tissue-specific promoter. Using this approach, researchers have created drought-, salt-, and cold-resistant rice (Garg *et al.*, 2002), drought-resistant tobacco (Karim *et al.*, 2007), and drought-, salt-, cold-, and heat-resistant *Arabidopsis* (Miranda *et al.*, 2007). Other methods include overexpressing the plant endogenous *TPS1*, such as in *Arabidopsis* (Avonce *et al.*, 2004) and rice (Li *et al.*, 2011*a*), and overexpressing the trehalase gene, for example *AtTRE1* in *Arabidopsis* (Van Houtte *et al.*, 2013), which results in various responses, including an increase in abiotic stress tolerance (Li *et al.*, 2011*a*). The above strategies and associated outcomes indicate a promising prospect of stress tolerance improvement by modifying trehalose metabolism in crop species.

In addition to manipulating T6P biosynthesis and degradation for better adaption to stress, studies in *Arabidopsis* have also proved that the T6P/trehalose status plays important roles in regulating carbon resource allocation (Schluepmann *et al.*, 2004; Lunn *et al.*, 2006). By inhibiting the catalytic

activity of SnRK1, T6P was linked with energy status in young and metabolically active heterotrophic tissues, where its accumulation was revealed to promote carbon utilization and anabolism, and hence growth (Schluepmann *et al.*, 2003; Zhang *et al.*, 2009). Moreover, T6P has been associated with the redox activation of AGPase required for starch deposition under high sucrose conditions in leaves (Wingler *et al.*, 2000; Kolbe *et al.*, 2005; Lunn *et al.*, 2006). An induction of starch accumulation by trehalose was also revealed in *Arabidopsis* shoots, which was attributed to the inhibition of starch breakdown mediated by the transcription factor ABI4 (Ramon *et al.*, 2007). Similarly, experimental results obtained from wheat grains suggest a similar T6P/trehalose regulatory mechanism existing in sink tissues, where it could activate starch synthesis and accumulation (Martínez-Barajas *et al.*, 2011). Overall, T6P is strongly associated with biosynthetic processes, including those for starch and possibly cell wall biosynthesis (Fig. 3).

Toward improving yield and quality for major harvestable sink tissues, such as seeds, fruits, tubers, and roots, the findings discussed above represent new ways to improve crop yield by modifying the T6P/trehalose pathways. However, it is worth noting that during wheat grain filling, high T6P is predominantly present in the endosperm, but little is found in the embryo and pericarp (Martínez-Barajas *et al.*, 2011). Associated with the expression pattern of SnRK1, the observation suggests that the inhibition of SnRK1 by T6P activates or promotes starch synthesis in the endosperm (Martínez-Barajas *et al.*, 2011). Interestingly, transgenic potatoes with elevated T6P levels specifically in their tubers displayed reduced starch content compared with those in the wild type; however, transgenic tubers with significantly diminished T6P showed no change in starch content when calculated on a fresh weight basis, and a strongly reduced tuber yield (Debast *et al.*, 2011). As learned from wheat grain and transgenic potato tubers, future studies will have to investigate the exact role of T6P/trehalose in anabolism and signalling. Clearly, by interfacing primary metabolism and signalling, the T6P pathway is a sensitive target for genetic engineering to improve crop yield. However, to achieve desirable outcomes, highly targeted approaches need to be pursued to manipulate T6P/trehalose metabolism more precisely in time, space, and magnitude within plant tissues and cells.

Other areas to improve plant development through adjusting T6P/trehalose metabolism include manipulation of plant architecture and flowering. A study on the *Arabidopsis* TPP gene family showed that disruption of the *TPPB* gene led to increased cell numbers and thus larger sized leaves, while the double mutant *tppa; tppg* exhibited changes in expression of genes involved in stimulating tip growth, and a hairy root phenotype (Van Houtte *et al.*, 2013). These observations shed light on the feasibility of targeting the T6P/trehalose pathway to regulate plant morphology. Moreover, by altering T6P levels, the floral transition and flowering time were affected (Avonce *et al.*, 2004; van Dijken *et al.*, 2004; Gómez *et al.*, 2010; Wahl *et al.*, 2013), indicating a potential feasibility to alter flowering time control, which is important in determining successful pollination, as well as seed and fruit set, and hence yield potential in most crop species (Ruan *et al.*, 2012).

Concluding comments

Within four decades, it is estimated that the human population will surpass nine billion people. In order to provide food, feed, fibre, and fuel sustainably for humanity, plant scientists are faced with significant challenges of increasing crop yield with fewer inputs. To engineer greater sucrose delivery to, and utilization within, harvestable sink organs, it is imperative to understand the regulation of sucrose phloem loading, transport, unloading, and storage processes in the major crops. Within cereals, the sucrose phloem loading pathway in maize, wheat, and barley is primarily through the apoplasm. However, prior to modifying phloem loading in rice, the path by which sucrose travels into the vein needs to be clarified. From available anatomical, physiological, and genetic evidence, it is highly likely that rice primarily utilizes the apoplasmic pathway to load sucrose into the collection phloem, similar to other characterized grasses. Additionally, because seed filial tissues are symplasmically isolated from maternal tissues, the functions and regulation of the sugar effluxers and influxers responsible for their provisioning must be elucidated. Finally, to enhance crop yield, further studies of the post-phloem usage and storage of imported carbohydrates within sinks are required. Recent results have suggested that new opportunities exist to improve crops for greater productivity. By understanding and manipulating the functions of these sugar transport, metabolism, and signalling pathways, it should be possible to enhance plant yield significantly.

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