Sucrose homeostasis: Mechanisms and opportunity in crop yield improvement

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ABSTRACT

Sugar homeostasis is a critical feature of biological systems. In humans, raised and dysregulated blood sugar is a serious health issue. In plants, directed changes in sucrose homeostasis and allocation represent opportunities in crop improvement. Plant tissue sucrose varies more than blood glucose and is found at higher concentrations (cytosol and phloem ca. 100 mM v 3.9–6.9 mM for blood glucose). Tissue sucrose varies with developmental stage and environment, but cytosol and phloem exhibit tight sucrose control. Sucrose homeostasis is a consequence of the integration of photosynthesis, synthesis of storage end-products such as starch, transport of sucrose to sinks and sink metabolism. Trehalose 6-phosphate (T6P)-SnRK1 and TOR play central, still emerging roles in regulating and coordinating these processes. Overall, tissue sucrose levels are more strongly related to growth than to photosynthesis. As a key sucrose signal, T6P regulates sucrose levels, transport and metabolic pathways to coordinate source and sink at a whole plant level. Emerging evidence shows that T6P interacts with meristems. With careful targeting, T6P manipulation through exploiting natural variation, chemical intervention and genetic modification is delivering benefits for crop yields. Regulation of cereal grain set, filling and retention may be the most strategically important aspect of sucrose allocation and homeostasis for food security.

1. Introduction

Sucrose is the first major end-product of photosynthesis and starting point for growth and development. In this pivotal position between source and sink, sucrose plays a principal role in providing the carbon for the variety and diversity seen in the plant kingdom including for crop yields. The regulation of sugar homeostasis is a rule of life in biology that is more complex in plants than in other organisms as it involves more and varied components than for example the human insulin/glucagon control of blood glucose. Plant tissues have both symplasm (cytosol, phloem) where sucrose is most tightly controlled and apoplasm (vacuole, cell wall space) where sugar levels are more variable. Whereas sugar stability is important in human fitness, provision of increased levels of sucrose to harvested sinks looks a promising route for improving crop yields for both yield potential and resilience (Nuccio et al., 2015; Flavell, 2023; Shen et al., 2022). There is evidence of both historic and ongoing selection of components of sucrose and related carbohydrate metabolism and homeostasis for yield in wheat e.g. trehalose 6-phosphate (T6P) metabolism (Lyra et al., 2021), sucrose and starch metabolism e.g. sucrose synthase (Hou et al., 2014), cell wall invertase CWIN (Wang

et al., 2008), ADPG transport (Wang et al., 2019) and sugar transport e. g. Sugars Will Eventually be Exported Transporters (SWEET4) (Sosso et al., 2015). Sugar transporters, enzymes of sucrose synthesis and breakdown as well as the underlying regulatory systems e.g. trehalose 6-phosphate (T6P) signalling which interacts with SNF1 related protein kinase1 (SnRK1) and TOR (target of rapamycin) kinase master regulators are particularly important in determining tissue levels of sucrose (Zhang et al., 2009; Morales-Herrera et al., 2023). It is likely that selection pressures for crops compared to plants in the natural environment are different in the area of sucrose homeostasis and allocation especially for the setting and filling of reproductive structures. This may involve altered expression levels of genes, gene duplication and proliferation e.g. trehalose phosphate synthase (TPS) and trehalose phosphate phosphatase (TPP) genes (Paul et al., 2018; Lyra et al., 2021). For high yields, adequate provision of more sucrose to harvested plants of the plant e.g. grain, seed or tubers appears to have a high level of control downstream of photosynthesis. It is likely that photosynthetic improvement as a route to more sucrose will require effective integration of elevated photosynthesis within the source-sink and sucrose homeostatic system to improve crop yields of major crops such as cereals in

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typical agricultural conditions.

In this review, we summarise recent developments in understanding sucrose homeostasis in plants including Arabidopsis as a model system where mechanistic understanding is often best developed, but also focusing to a large extent on cereals, the main food security crops, highlighting strategic importance and opportunity in yield improvement.

2. The generation of sucrose in photosynthesis as starting point for sucrose homeostasis

Sucrose is generated as the first major end product of photosynthesis from triose phosphate exported from chloroplasts counter-exchanged with inorganic phosphate from the cytosol (Fig. 1). Flux of triose phosphate towards sucrose is catalysed by fructose 1,6-bisphosphatase (FBPase) as a major control point regulated by fructose 2, 6-bisphosphate (F26BP) (Stitt et al., 1984). F26BP regulates partitioning between sucrose and starch in fluctuating light and temperature in Arabidopsis (McCormick and Kruger, 2015), but not in wheat (Trevanion, 2002) and targeting F26BP has not been pursued as a target in crop improvement. Sucrose phosphate synthase (SPS) catalyses the next highly regulated step of carbon flux from photosynthesis to sucrose (Ruan, 2014) (Fig. 1). Overexpression of SPS can increase sucrose accumulation independently of photosynthetic rate at the expense of starch and amino acids in tomato (Laporte et al., 2001), but this transgenic strategy may not be readily reproducible and has not been deployed in crop improvement. Strong endogenous regulation of highly regulated steps by metabolites and phosphorylation control as for SPS (McMichael et al., 1995) may confound attempts to increase enzymatic activities in transgenic approaches. An enzyme not thought to be a strong control point in the flux to sucrose, sucrose phosphate

phosphatase (SPP) shows more promise for increasing sucrose content and yield. Jing et al. (2022) showed higher expression of SPP (Fig. 1) *TaSPP-5A* in *TaSPP-5A* haplotypes (a group of alleles that are inherited together from a single parent) in wheat seedling leaves compared to haplotypes of *TaSPP-5B* and *TaSPP-5D*. Higher expression of *TaSPP-5A* was associated with increased sucrose content and spike thousand-grain weight. Selection of favourable SPP haplotypes may provide opportunity in breeding programmes.

Once synthesised in the cytosol, sucrose can be transiently stored in the vacuole in leaves to high levels. Vacuoles occupy as much as 90 % of cell volume serving as short- and long-term sugar stores (Vu et al., 2020). Sugar exchange with vacuoles is enabled by tonoplast monosaccharide transporters (TMT also known as TST), plant sucrose/H+ cotransporters (SUT or SUC), vacuolar glucose transporters, early response to dehydration-like (ERDL) monosaccharide transporters and SWEETs (Wormit et al., 2007; Schulz et al., 2011; Hedrich et al., 2015; Julius et al., 2017; Jeena et al., 2019). Induction of fructan synthesis by sucrose serves as a polysaccharide reserve in vacuoles of vegetative tissues of cereals (e.g. forage grasses, wheat and barley) and in the perennating organs of chicory, artichoke, asparagus, dahlia and the onion family (Cairns, 2003). In a transgenic approach sugars were relocalised from the vacuole by modifying the tonoplast transporters TMT and tonoplast sucrose/H+ cotransporter and SUC4 expression in Arabidopsis (Anaokar et al., 2021) which moved sucrose to stimulate fatty acid and triacylglycerol synthesis in the cytosol. This may be a strategy to alter the accumulation of end products in plants. Interestingly, where sucrose is diverted to other end-products such as lipids as in *Lolium perenne*, in this case by introducing two genes of lipid synthesis and storage (Beechey-Gradwell et al., 2019), the transgenic plants exhibited increased photosynthesis and overall biomass. The accumulation of foreign end-products in the form of fatty acids may disengage
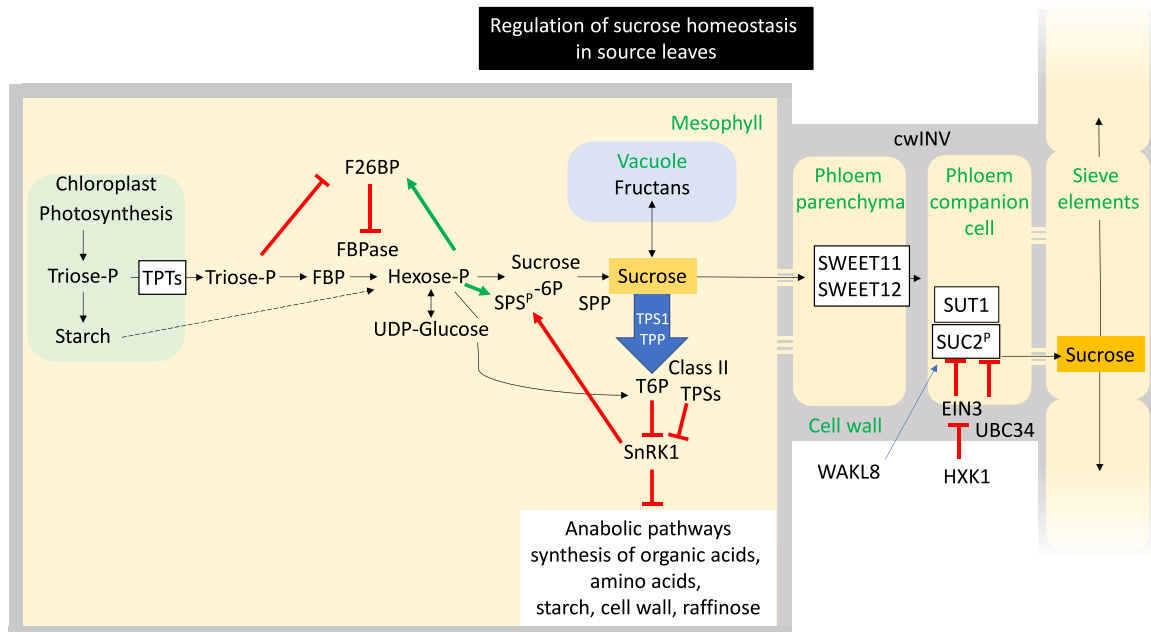

Fig. 1. Schematic of regulation of sucrose homeostasis in source leaves. Triose phosphate from chloroplasts is converted to fructose 6-phosphate (hexose-P) which together with UDP glucose provide substrate for sucrose synthesis through sucrose phosphate synthase (SPS), inhibited by phosphorylation and sucrose phosphate phosphatase (SPP). Glucose 6-phosphate (hexose-P) and UDP glucose provide substrate for the synthesis of T6P (trehalose 6-phosphate) through trehalose phosphatase synthase 1 (TPS1) and trehalose phosphate phosphatases (TPP). Apoplastic sucrose loading to the phloem is mediated by the successive action of SWEETs and SUC2. SUC2 is phosphorylated by WALL-ASSOCIATED KINASE LIKE 8 (WAKL8) and its turnover rate is regulated by UBIQUITIN-CONJUGATING ENZYME 34 (UBC34), both in a light-dependent manner (Xu et al., 2020). ETHYLENE-INSENSITIVE3 (EIN3) directly inhibits SUC2 activity by binding to the SUC2 promoter. HEXOKINASE1 (HXK1) increases sucrose phloem loading in source tissues by destabilising EIN3 thereby elevating sucrose levels in sink roots (Tong et al., 2022). cwINV, cell wall invertase; FBP, fructose-1, 6-bisphosphate; FBPase, fructose 1,6 bisphosphatase; SnRK1, sucrose non-fermenting1 (snf1)-related kinase 1; SWEETs, sugars will eventually be exported transporters; Suc-6-P, sucrose 6-phosphate; SUT/SUC, sucrose transporter or carrier; TPTs, triose phosphate/inorganic phosphate translocators.

metabolism from normal endogenous sucrose homeostasis and feedback regulation of photosynthesis, thereby promoting overall productivity (Paul and Eastmond, 2020). This may represent a strategy of circumventing sucrose homeostatic mechanisms to increase productivity.

In addition to sucrose synthesis in cytosol and accumulation in vacuole, both chloroplasts and mitochondria contain invertases and it is generally accepted that sucrose metabolism occurs in these compartments too (Naegele et al., 2013; Martín et al., 2013; Vargas et al., 2008). For chloroplasts, invertases and a recently described chloroplastic H+ sugar antiporter may facilitate sucrose and hexose homeostasis between plastids and cytosol (Patzke et al., 2019). Overall, however, very little is known about the regulation of sucrose partitioning between sub-cellular compartments.

The synthesis of transitory starch in chloroplasts provides carbon storage and buffering of sucrose and a further means of sucrose homeostasis (Smith and Stitt, 2007). In leaves of grasses and cereals, however, diel starch turnover is small but there is substantial diel turnover of sucrose and accumulation of sucrose in leaves during the day (Smith and Zeeman, 2020). In wheat this was related to less inactivation of SPS by accumulating sucrose compared to starch-storing species (Trevanion et al., 2004). Other species do accumulate transitory starch during the day as main storage carbohydrate with models for the regulation of accumulation as either an overflow product from high photosynthesis or being more strongly related to the "anticipated" length of the night (in Arabidopsis) and hence regulated by growth rather than photosynthesis (Smith and Stitt, 2007; Mengin et al., 2017; Sharkey, 2017).

3. The key roles of T6P-SnRK1 and TOR

Trehalose 6-phosphate (T6P) is the sugar signal of a sucrose signalling mechanism in plants discovered over the past 27 years (Goddijn et al., 1997; Paul et al., 2001; Wingler, 2002). A powerful regulatory role for T6P was first shown when T6P was found to be indispensable for sucrose utilization (Schluepmann et al., 2003). T6P has been shown subsequently to be a universal mechanism of integrating sucrose supply into metabolism, development and physiology in plants and crops and hence sucrose homeostasis (Paul et al., 2020; Fichtner and Lunn, 2021). Both SnRK1 and TOR sense carbon and nutrient status and act as master regulators of metabolic homeostasis acting as antagonistic promoters and inhibitors of catabolism and anabolism, respectively. There have been excellent recent reviews on both SnRK1 and TOR (Peixoto and Baena-González, 2022; Artins and Caldana, 2022; Flavell, 2023) so detailed coverage of each is not provided here. Instead we cover mode of action of T6P, as a major regulator of sucrose homeostasis, which is thought to be explained at least in part from T6P inhibition of SnRK1 (Zhang et al., 2009) and recently through T6P activation of TOR (Morales-Herrera et al., 2023).

SnRK1 is activated by low energy (directly by AMP) which leads to metabolic reprogramming by transcriptional control of a large number of genes towards catabolism and energy conservation (Baena-González et al., 2007; Henninger et al., 2022). TOR is activated by favourable energy and nutrient conditions including amino acids, adjusting growth to available resources, particularly through regulation of cell cycle, promotion of synthesis of ribosomes, protein, nucleotide, lipids and amino acids, and suppression of the turnover of macromolecules through autophagy (Artins and Caldana, 2022). TOR can also induce SnRK1 under favourable conditions as a means of feedback control of itself (Jamsheer et al., 2021) and SnRK1 can inhibit TOR (Belda-Palazón et al., 2022).

T6P inhibits SnRK1 to regulate metabolic pathways to promote anabolism at the transcriptional level (Zhang et al., 2009; Nunes et al., 2013a). In Arabidopsis rosettes T6P also regulates the enzymatic activity of key enzymes like phosphoenol pyruvate carboxylase (PEPC) and nitrate reductase through phosphorylation (Figueroa et al., 2016), starch metabolism through posttranslational redox activation of AGPase (Kolbe

et al., 2005) and starch degradation (Martins et al., 2013) thereby regulating sucrose homeostasis. T6P inhibition of SnRK1 is unique in plants; activities of the closely related AMPK and SNF1 of other organisms are not affected by T6P (Zhang et al., 2009). Zhang et al. (2009) showed that T6P inhibits SnRK1 in all Arabidopsis tissues except mature leaves and particularly strongly in sinks such as young inflorescences. In photosynthetic tissue where inhibition of SnRK1 by T6P is weakest, T6P levels were still related to SnRK1 marker gene transcripts (Peixoto et al., 2021; Avidan et al., 2023). It is likely that other interacting factors (Zhang et al., 2009) for example upstream protein kinase (Zhai et al., 2018) and class II TPSs (Van Leene et al., 2022) may modulate SnRK1 response to T6P, but exactly how this operates in mature leaves or indeed growing tissues is still incomplete. The sugar-phosphates glucose 6-phosphate and glucose 1- phosphate also inhibit SnRK1 (Nunes et al., 2013b; Avidan et al., 2023) in an additive and synergistic manner with T6P, respectively (Nunes et al., 2013b).

In actively growing sink tissue, T6P extends its role in cellular sucrose homeostasis to play a dynamic role in promoting growth and development and regulating plant architecture and crop yield. Within individual cells of sink tissues the T6P: sucrose balance or nexus seen in Arabidopsis rosettes (Figueroa and Lunn, 2016) can be overridden by cell type and developmental stage e.g. in wheat grain (Martínez-Barajas et al., 2011) and in maize cobs (Oszvald et al., 2018) see later for fuller consideration of cereal grain. Variation in SnRK1 and TOR has been directly associated with crop yield (Flavell, 2023), but as SnRK1 and TOR themselves are highly conserved and so central, such direct associations may be rare. A more successful approach to modify carbon-/energy signalling for yield through SnRK1 and TOR may be through more specific targeting or expression control of SnRK1/TOR at certain developmental time points, through TPSs and TPPs which alter T6P levels and signalling or through chemical intervention of T6P (Griffiths et al., 2016; Paul et al., 2020; Flavell, 2023).

Significant for crop yields is that emerging data reveal that T6P likely enables commitment of sucrose to reproductive development which forms the basis of cereal yields. It is likely that allocation of sucrose to reproductive tissues is not yet optimised for yield and in this regard targeting the T6P pathway presents great opportunity. Several TPS and TPP genes associated with reproductive traits were still undergoing positive selection in the HIBAP (high biomass association panel) wheat population (Lyra et al., 2021) indicating potential for further selection for yield. Whilst TPS and TPP genes clearly associate with grain yield traits (Lyra et al., 2021) detailed mode of action is not known. It is known that T6P regulates reproductive development through flowering time via *FLOWERING LOCUS T* (*FT*) in Arabidopsis (Wahl et al., 2013) shown to be dependent on SnRK1 in suppressor screens (Zacharaki et al., 2022). FT and TERMINAL FLOWER1 (TFL1) have been identified as closely related eukaryotic phosphatidylethanolamine-binding proteins (EuPEBPs) that integrate multiple environmental stimuli and act antagonistically to determine the optimal timing of the floral transition (Bennett and Dixon, 2021). FT and TFL1 act like hormonal signals, transported in the phloem from their primary site of expression in leaves to a primary site of action in the shoot meristem (Bennett and Dixon, 2021). In grasses compared to other species, copy number of EuPEBPs has expanded with unknown consequences for EuPEBP function. How T6P interacts with this expanded pathway in cereals is not known. Coincidently, the T6P pathway too has expanded in cereals (Paul et al., 2018; Lyra et al., 2021) and may fulfil a more general role in reproductive development beyond flowering time control. TPS and TPP genes are linked to several reproductive traits in wheat, particularly floret fertility and grain number (Lyra et al., 2021) (Fig. 2), and grain size and filling (Zhang et al., 2017; Liu et al., 2023). In maize, TPS genes are listed as domestication improvement genes (Hufford et al., 2012). However, the mechanistic details for the regulation of fertility, grain number and size by the T6P pathway are still sketchy. *TEOSINTE BRANCHED1* (*TB1*) in maize, a domestication gene responsible for loss of tillers and apical dominance regulates the T6P pathway by binding
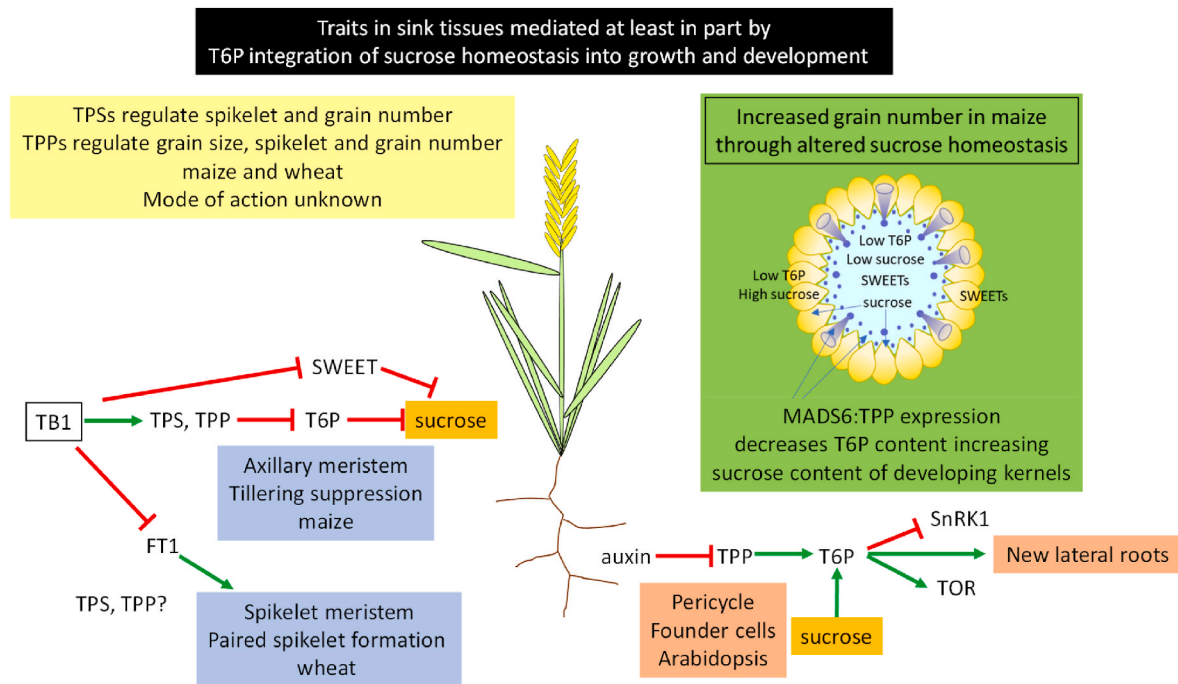
Fig. 2. Simplified schematic of integration of sucrose homeostasis through trehalose 6-phosphate (T6P) into growth and development in sinks underpinning traits that impact crop yield in maize and wheat, and in Arabidopsis. Regulation of spikelet and grain number by TPSs (trehalose phosphate synthases) and grain size, spikelet and grain number by TPPs (trehalose phosphate phosphatases) (Lyra et al., 2021) through unknown mechanisms. Wheat domestication-related gene *TB1* regulates axillary meristems and tillering in maize (Dong et al., 2019) and paired spikelet formation in spikelet meristems (Dixon et al., 2018a,b). Targeted expression of TPP in maize using MADS6 promoter breaks the link between T6P and sucrose enhancing the flow of sucrose into grain from pith tissue increasing grain number retention under drought (Oszvald et al., 2018). Regulation of lateral root growth in Arabidopsis involves T6P downstream of auxin signalling in pericycle lateral root founder cells and regulation of SnRK1 and TOR Morales-Herrera et al., 2023).

the promoter of class II TPS genes to promote their expression. Sucrose levels may be modulated through the *TB1* target, ZmSWEET15b (Dong et al., 2019) (Fig. 2). TB1 may therefore regulate architecture at least in part through control of sucrose homeostasis and T6P. Interestingly, TB1 also regulates meristems in spikes. Allelic variation of *TB1* in wheat affects the rate of spikelet meristem formation reducing FT1-dependent activation of spikelet meristem identity genes (Dixon et al., 2018a,b), affecting spike fertility and the number of spikelets and paired spikelets (Dixon et al., 2018a,b). Genes involved in the regulation of meristems such as TB1 and others such as in the *CLAVATA–WUSCHEL* pathway which regulate inflorescence meristem size show great promise in yield improvement (Liu et al., 2021). Regulation of meristem activity by sucrose/T6P is assuming greater importance than previously appreciated in axillary and spikelet meristems. Indeed, in axillary meristems regulation by T6P may supersede hormonal regulation (see Lindsay et al., 2024, for more general discussion). T6P also regulates new lateral roots from lateral root pericycle tissue (Morales-Herrera et al., 2023) (see later in this section).

Recently, several different interactors with T6P and SnRK1 have been found. A new regulatory module links SnRK1 and a TPP gene through a bHLH111 transcription factor *OsSGI1* in rice (Wang et al., 2022). OsSnRK1a-dependent phosphorylation of OsSGI1 enhanced the direct binding to the E-box *OsTPP7* promoter, inhibiting transcription of *OsTPP7*, which increased T6P content and decreased sucrose content. The knockout mutants, *sgi1-1/2/3*, had larger grain, enhanced seed germination and vegetative growth. In another regulatory node a NAC23 transcription factor represses a TPP driving a feedforward loop NAC23-TPP-T6P-SnRK1 to increase allocation of sucrose away from leaves towards grain and resulting up to 17 % higher yield due to more panicles and larger grains in paddy-field-grown rice and higher rates of photosynthesis (Li et al., 2022). Further, heterologous expression of a rice TPP in maize with a MADS6 promoter expressed in reproductive tissue decreased T6P in cobs during early reproductive development

which altered sucrose allocation within the cob away from pith towards the developing grain. This modification also resulted in higher photosynthesis likely because of the higher sink demand from the sucrose stimulation of developing grain (Fig. 2) (Oszvald et al., 2018). Closer examination of cob tissues showed that pith cells with reduced T6P had less sucrose, but endosperm tissue with less T6P had more sucrose (Fig. 2), breaking the T6P: sucrose relationship seen in Arabidopsis rosettes (Figueroa and Lunn, 2016). Enhanced expression of SWEETs was proposed to account for altered sucrose allocation from pith to seed. Grain number retention and yield was increased, particularly under drought. The examples in Li et al. (2022) and Oszvald et al. (2018) both result in changes in sucrose allocation towards grain enhancing yield and photosynthesis (see fuller discussion in Paul et al., 2022). In wheat grain (Martínez-Barajas et al., 2011), it was found that in pericarp T6P was only related to high sucrose levels before grain filling, however in endosperm T6P/sucrose ratio was maintained during development. These results show strong tissue and developmental dependency of the T6P/sucrose nexus.

Other work has shown integration of T6P and auxin signalling. In pea, T6P promotes the expression of the auxin biosynthesis gene TRYPTOPHAN AMINOTRANSFERASE RELATED2 (TAR2) in developing seed (Meitzel et al., 2021). The resulting effect on auxin concentrations mediated the T6P-induced activation of starch storage processes. In this case auxin acts downstream of T6P to facilitate seed filling. In a different scenario in lateral roots in Arabidopsis, auxin acts upstream of T6P to promote lateral root growth (Morales-Herrera et al., 2023) (Fig. 2). Here, auxin inhibited expression of TPPB in lateral root founder cells from the pericycle leading to increased T6P levels. T6P then inhibits SnRK1 and activates TOR through phosphorylation to promote lateral root growth. Both examples show integration of hormone and metabolic signalling; Morales-Herrera et al. (2023) shows for the first time possible direct regulation of TOR by T6P and interaction with lateral root meristems.

In further examples that show the widespread and diverse impacts of T6P signalling, germination under anoxia in rice was promoted by a TPP gene expressed in seeds that underlay a quantitative trait locus for germination under flooding (Kretzschmar et al., 2015). The TPP gene is thought to reduce T6P levels to activate starch mobilisation through T6P/SnRK1 providing sugar to support germination. Beyond starch crops, oil metabolism can be enhanced by T6P in brassica (Zhai et al., 2018). This latter finding implies general upregulation of primary end-product accumulation (oil and starch) by T6P. A link between another end product, raffinose, and TPS1 was also found in Arabidopsis. Functionally distinct TPS1 isoforms differentially affected raffinose particularly under heat stress, which was associated with reduced endogenous sucrose levels and thermotolerance (Reichelt et al., 2023).

4. Export of sucrose from source leaves

Sucrose is loaded apoplastically into the phloem in leaves of most herbaceous plants, including crops, for transport from leaves to sinks (Fig. 1) (Braun, 2022). Sucrose diffuses through plasmodesmata from mesophyll into the phloem parenchyma and then is effluxed to the cell wall by SWEET transporters. SWEETs are a family of around 20 paralogues in angiosperms that mediate passive low-affinity and high-capacity transport of sugars across membranes following concentration gradients essential for phloem loading (Eom et al., 2015). In Arabidopsis, SWEET11 and 12 are expressed in phloem parenchyma cells adjacent to the companion cell sieve element complex (Chen et al., 2012) and are co-expressed with SPS for coordination of sucrose synthesis and transport. SWEET11 and 12 are regulated post-translationally by phosphorylation through drought- and abscisic acid-activated SnRK2 protein kinases (Chen et al., 2021). Activation through this mechanism enhances the oligomerisation and sucrose transport activity of SWEETs, elevating sucrose contents in roots, improving root growth and root: shoot ratio under drought stress, enhancing resilience to stress (Chen et al., 2021).

After the action of SWEETs in exporting sucrose into the apoplastic space, sucrose transporters (SUT/SUC) in minor vein phloem actively take up sucrose into phloem companion cells and sieve elements. Active sucrose uptake generates sucrose concentrations of up to an order of magnitude higher than surrounding cells (Braun, 2022). The proton gradient across the membrane of the companion cell-sieve element complex is created by an ATPase at the companion cell plasma membrane moving sucrose against its concentration gradient with a proton moving down its electrochemical potential (proton-sucrose symport). Once loaded in the sieve tubes sucrose moves by mass flow by the osmotically generated hydrostatic pressure difference between source and sink tissues according to the Munch pressure flow hypothesis. *AtSUC2* and its homologues in crops is expressed specially in companion cells (Julius et al., 2017). SUC2 plays a central role in the loading of sucrose into the phloem (Slewinski and Braun, 2010) by setting the rate of carbon export from source leaves. SUC2 overexpression in paddy rice increased grain size and yield (Wang et al., 2015) (Fig. 1). SUC2 is phosphorylated by WALL-ASSOCIATED KINASE LIKE 8 (WAKL8) and its turnover rate is regulated by UBIQUITIN-CONJUGATING ENZYME 34 (UBC34), both in a light-dependent manner (Xu et al., 2020). *ubc34* mutants of Arabidopsis increase phloem loading, which increases biomass and yield in Arabidopsis (Xu et al., 2020). Recently it was shown that transcription factor ETHYLENE-INSENSITIVE3 (EIN3) directly inhibits SUC2 activity by binding to the SUC2 promoter. HEXOKINASE1 increases sucrose phloem loading in source tissues by destabilising EIN3 thereby elevating sucrose levels in sink roots (Tong et al., 2022). A signalling role has been proposed for other members of the SUT gene family, most prominently for potato *SUT4* in shade avoidance and in influencing the hormonal regulation of flowering and tuberization, without making any known major contribution to sucrose transport itself (Chincinska et al., 2008, 2013). Interestingly, maize sucrose transporter1 (*sut1*) loss-of-function mutant had severely reduced

sucrose transport phloem pressure and mass flow due was maintained by increased K$^+$ loading which compensated for decreased sucrose loading (Babst et al., 2022). In potato at the onset of tuber development, the mode of sucrose unloading switches from apoplastic to symplastic mediated by SP6A, the FLOWERING LOCUS T (FT) homologue in potato. SP6A as a mobile signal in the phloem interacts with the sucrose efflux transporter SWEET11 blocking leakage of sucrose to the apoplast thereby promoting the alternative symplastic sucrose transport (Abelenda et al., 2019). This example shows the flexibility and complexity of sucrose transport and its regulation. Selection of beneficial SP6A alleles could alter plant architecture for improved tuber yield (Lehretz et al., 2021). Interestingly, when sucrose production by photosynthesis exceeds sucrose loading into the phloem, the surplus sucrose accumulated in the apoplast is carried toward the stomata by the transpiration stream promoting stomatal closure via hexokinase, to feedback regulate photosynthesis and also limit water loss (Kelly et al., 2013). Overall, in the export of sucrose from leaves, the coupled action of SWEETs and sucrose transporters SUC/SUT is a key component of sucrose homeostasis locally and at a whole plant level, representing possible targets to increase sucrose flow to sinks to increase crop yields.

5. Import of sucrose into cereal reproductive tissue

Sucrose import into yield-producing structures of crops is one of the most strategically important processes for food security. In cereals, sucrose import and homeostasis in the reproductive structures determine grain numbers (grain set and retention) and grain filling (grain size). In wheat spikelets between 6 and 12 floret primordia are initiated per spikelet yet fewer than 4–5 floret primordia survive to reach anthesis (González et al., 2003). Sucrose supply in the two weeks before anthesis is thought to be crucial in establishing grain number as part of the decision-making process in plants for allocating resources (Sinclair and Jamieson, 2006; Reynolds et al., 2021) although is also related developmental factors (Backhaus et al., 2023) in addition to assimilate supply.

Despite the discovery of genes that regulate grain number (Dixon et al., 2022) the molecular mechanisms through which sucrose regulates grain numbers are not known. Meristem fate in maize reproductive structures is regulated by putative sugar signalling genes TPPs *RAMOSA3* and *TPP4* but their mechanism of action remains unknown and may be independent of T6P (Claeys et al., 2019). Ovule initiation is thought to be controlled by sugar homeostasis and signalling through cell wall invertases (Liao et al., 2020). Sugar signals generated by invertases in the cell wall matrix via invertases (CWIN) may be sensed by and transmitted through plasma membrane hexose transporters which modulate cytosolic sugar homeostasis and signalling and/or receptor-like kinases to potentially interact with small GTPase (Rop) pathways. These CWIN-originated signals are then relayed to the nuclei to regulate the expression of genes encoding auxin signalling components and MADS-box transcription factors, thereby modulating ovule initiation and differentiation. Later, pollination is a key time when activation of sugar import and signalling during the transition of the ovary to grain establishes grain sink capacity to continue acquiring further carbon for grain survival and filling (Shen et al., 2020, 2023).

Sugar import into developing grains after pollination is conserved in cereals (Shen et al., 2023). Sucrose moves symplastically through phloem plasmodesmata connecting the terminal vascular bundle to the pedicel of maize grain or the equivalent ventral surface of wheat and rice grains in the innermost cell layer of maternal-facing endosperm. Sucrose then moves out of the maternal tissue into the apoplast before uptake into the endosperm through basal cells. Here in the extracellular space of the maternal-filial interface, CWIN hydrolyses apoplastic sucrose into hexoses, to play a crucial role in the modulation of sugar uptake into filial tissues. Grain basal cells are enriched in diverse SUC/SUT, hexose transporters (MST, STP) and SWEETs for sugar uptake into endosperm (Shen et al., 2023). Loss of function of these transporters and CWINs via

mutation or genome-editing in rice reduced grain weight and size (Wang et al., 2008; Ruan, 2022). Clade III SWEET uniporters localized on the innermost cell layers of maternal tissue are key players for export of sucrose into the apoplast, such as OsSWEET11/15 and ZmSWEET11 (Yang et al., 2018; Shen et al., 2022) and sugar transporters on outer cell layers of endosperm which take up sugars from the apoplast, including OsSUT1, HvSUT1/2, ZmSUT1, Clade III ZmSWEETs for sucrose and ZmSWEET4c for monosaccharides (Shen et al., 2022; Eom et al., 2015; Radchuk et al., 2017). In addition, a recent study identified a novel Sucrose and Glucose Carrier 1 (ZmSUGCAR1), a member of the nitrate transporter 1/peptide transporter family (NRT1/PTR), that acts as an active H^+-sucrose symporter at the basal endosperm transfer cell layer of maize (Yang et al., 2022).

After anthesis, grain can be lost due to abiotic stress such as drought and heat (Rajala et al., 2009) regulates at least in part by sugars. Lower soluble carbohydrate levels induce autophagy and floret death, especially in florets of the distal part of the spikelet (Ghiglione et al., 2008). Drought suppresses sugar transporters at the maternal filial interface, yet promotes uptake into filial tissues (Shen et al., 2022). Abortion can be alleviated by supplying sucrose (Zinselmeier et al., 1995). Sucrose utilization and transport e.g. through CWIN, T6P, SWEETs and the equilibrium between ethylene and spermine regulate the abortion process under drought (Shen et al., 2020).

Starch is a major consumer of sucrose during grain filling and constitutes the main component of cereal seed at harvest. A recent study in wheat showed the expression of 74 genes linked to starch accumulation (Gu et al., 2021) that are expressed in the endosperm during the grain-filling stage after 8 DAA. SUT1 and sucrose synthase were proposed as important for the entry of sucrose into starch biosynthesis in wheat (Gu et al., 2021), with several transcription factors coordinating the expression of starch biosynthesis genes (Huang et al., 2021). In rice, sucrose synthase involved in the entry of carbon into starch synthesis has been targeted successfully to increase grain weight (Fan et al., 2019).

6. Supra accumulation of sucrose in cereals

Different aspects of the sucrose homeostatic mechanism are causally involved in hyperaccumulation of sucrose. In sugar cane, many components of sucrose metabolism appear necessary for high levels of sucrose accumulation, including miRNA-target mRNA pairs which target transcription factors involved in sugar metabolism (Wang et al., 2022). Sugar cane contrasts with sugar beet, where tonoplast sugar transporters (BvTST2.1) are responsible for sucrose accumulation of up to 18 % of fresh weight in beet (Jung et al., 2015). Despite knowledge of the regulation of sugar accumulation, translating it into the improvement of sugar content in these sugar crops is still a challenge and there are no reports of this having been achieved. Sweetcorn accumulates very high concentrations of sugars in the kernels through naturally occurring mutations in genes encoding enzymes in starch biosynthesis, *sugary-1* (*Su1*) a starch-debranching enzyme (*isoamylase-1*) and *shrunken-2* (*Sh2*) and *Brittle-2* (*Bt2*) encoding subunits of AGPase (Halford et al., 2011). Mutated AGPase also was found as another target underpinning high sugar accumulation in tomato fruit in a screening of tomato mutants (Matsukura et al., 2007). In sorghum, manipulation of T6P metabolism by TPPs appears to be causally related to the large differences in assimilate partitioning between sweet and grain sorghum (Li et al., 2019) underlying active starch, sucrose and cell wall metabolism and sugar accumulation in tall stems of sweet sorghum compared to the down-regulation of these pathways in stems of grain sorghum. Beyond sorghum there are no examples of T6P metabolism being related to hyperaccumulation of sucrose in cereals or indeed other crops. Rather in cereals T6P regulates and integrates sugar supply with starch metabolism and reproductive development, spikelet formation, and final grain number and size.

7. Concluding remarks

Sucrose homeostasis in plants is regulated by a complex interaction of enzymes and transporters orchestrated at least in part by master regulators SnRK1 and TOR which respond to energy, carbon and nutrients. T6P-SnRK1 and TOR regulate metabolic reprogramming and growth and development in relation to resource supply to maintain sucrose homeostasis and link sucrose with growth and development. For the complex trait of yield, some domestication genes that affect architecture such as *TB1* will inevitably have affected sucrose homeostasis, in this case likely through T6P signalling. Changing sucrose homeostasis for yield could be enabled by simple interventions such as of sugar transporters and metabolic enzymes but given the complexity of the yield trait may require several changes or modification of master regulators for larger coordinated changes in source and sink. Modifying sucrose integration into yield traits through plant architecture, grain setting and filling and starch accumulation in sinks provides great opportunity to increase yield itself. Modulation of SnRK1 activity by T6P is providing successful examples of yield improvement which represents a major opportunity for selection of natural variation in the T6P pathway, gene editing, transgenic and chemical intervention strategies.

CRediT authorship contribution statement

Javier A. Miret: Writing – original draft. **Cara A. Griffiths:** Writing – review & editing. **Matthew J. Paul:** Conceptualization, Funding acquisition, Project administration, Supervision, Writing – original draft, Writing – review & editing.

Declaration of competing interest

MJP and CAG are founders and shareholders of spinout company SugaROx. Other than that, the authors have no competing financial interests or personal relationships that would influence the work reported in this paper.

Data availability

Data will be made available on request.

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