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OPINION PAPER

Increasing crop yield and resilience with trehalose 6-phosphate: targeting a feast–famine mechanism in cereals for better source–sink optimization

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Abstract

Food security is a pressing global issue. New approaches are required to break through a yield ceiling that has developed in recent years for the major crops. As important as increasing yield potential is the protection of yield from abiotic stresses in an increasingly variable and unpredictable climate. Current strategies to improve yield include conventional breeding, marker-assisted breeding, quantitative trait loci (QTLs), mutagenesis, creation of hybrids, genetic modification (GM), emerging genome-editing technologies, and chemical approaches. A regulatory mechanism amenable to three of these approaches has great promise for large yield improvements. Trehalose 6-phosphate (T6P) synthesized in the low-flux trehalose biosynthetic pathway signals the availability of sucrose in plant cells as part of a whole-plant sucrose homeostatic mechanism. Modifying T6P content by GM, marker-assisted selection, and novel chemistry has improved yield in three major cereals under a range of water availabilities from severe drought through to flooding. Yield improvements have been achieved by altering carbon allocation and how carbon is used. Targeting T6P both temporally and spatially offers great promise for large yield improvements in productive (up to 20%) and marginal environments (up to 120%). This opinion paper highlights this important breakthrough in fundamental science for crop improvement.

Key words: Crops, photosynthesis, SnRK1, source–sink, sugar signalling, trehalose 6-phosphate.

Introduction

Global crop yields must double in the next 35 years to meet projected demand for food (Grassini *et al.*, 2013; Ray *et al.*, 2013). This requirement is coming at a time of unprecedented climatic variability (Lobell *et al.*, 2011). Crops that are simultaneously high yielding and resilient to fluctuating climatic conditions such as rainfall are a sought-after goal. However, a combination of high yield and resilience are traits that do not couple easily together in plants originating from most environments. In terms of metabolism, C_4 and Crassulacean acd metabolism (CAM) pathways are adaptations found in arid environments resulting in high water use efficiency, although C_4 maize is still sensitive to drought (see below). The CAM plants such as *Agave* and *Opuntia* have the potential to produce above-ground biomass rivalling that of C_3 and C_4 crops under optimal growing conditions (Cushman *et al.*, 2015). It is possible that some C_4 and CAM metabolic traits could be transferred to the agricultural environment. CAM in particular is an adaption to extreme drought not typical of most of agriculture, but some aspects of CAM and C_4, such as more water use-efficient gas exchange if this does

not result in leaves overheating, could be beneficial to crops. Interestingly, in maize, even though it already has C_4 photosynthesis, drought is still a major limitation and target for biotechnological improvement (Boyer and Westgate, 2004). Improvement strategies for the major cereal crops need to find a way to combine selection of high yields without stress with adaptations that enable good performance and yield under fluctuating water availability at all developmental stages.

What to target for increased yield and resilience?

The green revolution of the 1960s increased yields through two main approaches: stem shortening and disease resistance. Stem shortening meant that more photosynthate was available to move to the grain, improving harvest index and preventing lodging (Hedden, 2003). Disease resistance, particularly to rusts, further increased yield (Khush *et al*., 2001). These were traits that could be readily introduced into crops, in fact in over just a few years. Genetic variation was available; had stem height been highly conserved, improving this trait would have been far harder. Success of the stem height strategy depended on the trait not being already optimized for yield in the agricultural environment. Increasing stem height is a shade avoidance mechanism that enables plants to outgrow their neighbours, maximizing light interception. The trait ensures fitness in the natural environment, but is not best suited to high seed yield because resources are diverted away from seed production. This is selected in the natural environment and hence is more important for fitness and survival. In agriculture, increased drought, salinity, and heat tolerance are important targets for yield resilience improvements worldwide.

Can photosynthesis for crops such as wheat be targeted to improve crop yields?

The argument is often presented that the green revolution increased yield through improving assimilate distribution which has now been optimized, and the next green revolution must improve the amount of carbon that is fixed (Zhu *et al.*, 2010). However, there is a whole breadth of opinion in the scientific literature about the degree to which photosynthesis actually limits yield in the field and whether photosynthesis is a good target for yield improvement (Gifford and Evans, 1981; Long *et al.*, 2006, 2015; Slafer 2007; Zhu *et al.*, 2010; Körner *et al*., 2015). This probably reflects the fact that photosynthetic regulation in the context of whole-plant growth, development, and yield formation is poorly understood, in contrast to the basic photosynthetic mechanism which is one of the best understood plant processes. Therefore, photosynthesis models can be produced which identify where the inefficiencies in photosynthesis are, but they cannot be integrated into growth and development models in agricultural environments. In fact, the balance of argument for what limits yield in crops at least for wheat is in favour of sinks being more limiting than sources (Slafer and Savin, 1994; Reynolds *et al.*, 2005, 2009; Slafer, 2007).

Selecting for better photosynthesis is difficult because the process of photosynthesis is highly conserved (Long *et al.*, 2015); there is not much variation that can be selected for. However, abundant variation exists in the processes that use sucrose rather than in the generation of sucrose in photosynthesis. For example, plants such as sugar beet accumulate high concentrations of sugar in their beet due to properties of the sink organ not because of variation in the efficiency of photosynthesis between sugar beet and closely related species (Jung *et al.*, 2015). The fact that photosynthesis is highly conserved is significant. It should also be remembered that evolution is acting continuously to improve the efficiency and optimize such fundamental processes. Any large obvious improvement in photosynthesis would presumably be seized upon in natural selection if this allows the plant to outgrow and outcompete its neighbour through faster growth or more efficient use of ATP. A ceiling may have been reached, and large obvious improvements cannot be made unless there is some way to shift boundaries of the photosynthetic process significantly and to ensure that this is compatible with plant growth and developmental processes that interface with photosynthesis. Large improvements in photosynthesis most probably require genetic modification or genome-editing methods.

Attempts to engineer the C_4 pathway in rice may confer benefit (von Caemmerer *et al.*, 2012), particularly as C_4 is found in areas of the world where rice is grown and has evolved independently >60 times (Sage *et al.*, 2012). An argument for C_4 wheat is less strong, except perhaps for Australian wheat, given that wheat is grown in more temperate areas, where C_4 is not naturally present. However, C_4 engineering does require transfer of a number of genes, and is one of plant biology's longer term grand challenges. Rubisco engineering is another way potentially for step change improvements in photosynthesis as evolution cannot work backwards to correct the problem of oxygenation given that Rubisco evolved initially in a high CO_2 low O_2 world. However, this again would be a major technological feat as Rubisco improvement requires engineering both nuclear and chloroplast genomes with several genes, in addition to ensuring that the new enzyme assembles correctly with chaperones and regulatory activases. Successfully completing this in crops such as wheat where chloroplast engineering has not yet been performed and where the genome is enormous makes genome engineering particularly trying. New Rubisco that has been engineered into tobacco has a high turnover rate, but only at substantially elevated CO_2 (Lin *et al.*, 2014); therefore, CO_2 pumps would simultaneously need to be incorporated for this strategy to be successful. Photorespiration is a further target in crop improvement (Hagemann and Bauwe, 2016). Photorespiration is a consequence of the oxygen reaction of Rubisco and converts 2-phosphoglycolate formed in oxygenation into 3-phosphoglycerate which then re-enters the Calvin cycle. Photorespiration requires extra energy and re-oxidizes one-quarter of the 2-phosphoglycolate carbon to CO_2, lowering potential maximum rates of photosynthesis. Attempts to engineer photorespiration show promise (e.g. in potato; Nölke *et al.*, 2014), although they still require validation in field environments. For the major food security crops, wheat

may be difficult to improve in this way (Sparks *et al.*, 2001); maize is already C_4, and C_4 rice is already underway. However, for crops such as potato, yield improvements could be substantial (Nolke *et al.*, 2014). Overexpression of the Calvin cycle enzymes sedoheptulose 1,7-bisphosphatase (SBPase) (Lefebvre *et al.*, 2005; Rosenthal *et al.*, 2011) and fructose-1,6-bisphosphatase (FBPase) (Tamoi *et al.*, 2006) has shown promise. In a higher CO_2 world, the balance of regulation within the Calvin cycle may have moved away from Rubisco more toward the generation of substrate RuBP, for which SBPase and FBPase are key regulatory enzymes. Recently, a novel way of photosynthesis engineering through accelerating recovery from photoprotection has increased biomass of tobacco in the field (Kromdijk *et al.*, 2016).

Source–sink optimization

The optimization of the source–sink ratio is a promising approach in improving yield and resilience in crops. Photosynthetic capacity is considered the source, and developing parts of the plant are considered the sinks. The partitioning of photo-assimilates from the source to the sink depends on many factors (photosynthetic capacity, environmental stress, nutrient availability, etc.). Crop improvement on the basis of increasing photosynthetic capacity was discussed above, and highlights the need to look at the optimization of how and where photo-assimilates are transported at a whole-plant scale. Recently, it was shown that on crossing two elite varieties of double haploid wheat, sink strength was stronger, resulting in higher yields in optimal environmental conditions (Bustos *et al.*, 2013). The strategy for this in these plants is presumably to pull more photosynthate from source leaves to the developing grain, leading to increased yield. In drought or salinity stress, crop yield losses are mostly accounted for by the reduced activity of sink leaves and reduced size or number of sink organs. The focus of the source–sink ratio in these conditions should be centred around delayed senescence and water use efficiency mechanisms in the leaves, and maintenance of activity in sink organs, for which the approach of Bustos *et al*., (2003) may be beneficial (reviewed in Albacete *et al.*, 2014). Several studies have indicated that an increase/decrease in source can be altered by changed environmental conditions and mechanical defoliation; for example, higher ambient CO_2 concentrations increase the source, and normal source–sink ratios are then restored through defoliation (Bryant *et al.*, 1998; Ainsworth *et al.*, 2003; Rogers *et al.*, 2009). In a similar manner, sink strength can be reduced through removal of developing grains, lowering temperature, and decreasing nitrogen (Arp, 1991; Nunes *et al.*, 2013). However, recent studies suggest that a successful approach in increasing sink strength involves genetic modification (Weichert *et al.*, 2010; Nuccio *et al.*, 2015) or a chemical approach (Griffiths *et al.*, 2016). There are likely to be benefits to optimizing leaf and root architecture further to maximize light interception and nutrient and water uptake, respectively, which will undoubtedly impact the source–sink ratio. However, it will be necessary to minimize overinvestment in shoots and roots to avoid maintenance costs and diversion of carbon away from harvested organs. It is quite clear that changes in either the source or sink have large effects on plant growth, and the key to a successful strategy in improving crop yield will be to take a multifaceted approach in source–sink optimization, potentially by integrating photosynthesis with sink processes.

Integration of photosynthesis with sink processes to increase crop yield

Photosynthesis is a conserved process, but variation in photosynthetic rate per unit leaf area can be found (Driever *et al.*, 2014). It is not clear, however, whether such variations in photosynthesis drive higher yield or are being pulled by higher yield. Photosynthesis per unit leaf area itself is only one measure of plant photosynthesis. Whole-plant photosynthesis rather than the rate of photosynthesis per unit leaf area is what ultimately is linked to yield. Total plant photosynthesis depends on leaf area, leaf area index, leaf area duration, and architecture of the canopy, which all affect the amount of light the plant intercepts. It is unclear what effect genetic modification of photosynthesis per unit leaf area will have on whole-plant photosynthesis. Overexpression of SBPase, for example, can result in an increase in leaf area of ~30% (Lefebvre *et al.*, 2005). Increasing leaf area can impose a yield penalty. For example, removing a proportion of leaves prior to full expansion increased yield of soybean by 8% (Srinivasan *et al.*, 2016) because of the carbon costs of maintaining larger leaf area. Hence, the considerations are complex.

It is by no means certain that genetic engineering of photosynthesis will be tolerated by crops because of the sugar homeostatic mechanisms that exist in plants unless increased photosynthesis can be linked to improved sucrose use in growth and development associated with yield formation. Sucrose levels are regulated by trehalose 6-phosphate (T6P) (Yadav *et al.*, 2014) and, unless some way is found to break the link between sucrose and T6P, deviation of sucrose levels in the long term for higher yield may be difficult to achieve. Many studies of photosynthesis conducted at elevated CO_2 show down-regulation of photosynthesis over the long term (Stitt, 1991). Additionally, the overall stimulation of photosynthesis by elevated CO_2 that does take place may have been overestimated to some extent because negative results from such experiments often go unreported (Haworth *et al.*, 2016). Simultaneous engineering of photosynthesis and other regulatory processes to enable toleration of higher sugar levels may be necessary for large increases in yield.

One recent compelling piece of evidence that genetic modification of photosynthesis in wheat at least during grain filling would be ineffectual for yield without a more holistic appraisal of whole-plant processes was published recently (Borrill *et al.*, 2015). NAM RNAi plants with delayed senescence and higher rates of photosynthesis during grain filling accumulated fructan in stems rather than filling grain with the extra assimilate. This shows that capacity to fill grain rather than flag leaf photosynthesis in this instance almost completely limits yield during grain filling. Up-regulation

of grain-filling capacity in wheat should be the target for improving wheat yield at this developmental stage coupled to greater flag leaf photosynthesis.

Trehalose 6-phosphate regulates sucrose allocation which influences grain number and grain size, improving cereal yields

The example presented in Borrill *et al.*, (2015) shows that improving photosynthesis would benefit yield only if the extra assimilate produced ends up in the grain and that this does not automatically happen. In contrast to the fundamental understanding of the photosynthetic process and where the inefficiencies reside (Long *et al.*, 2015), little is known about processes that direct assimilate to stem storage or to grain. Recently, however, modification of the sugar signal T6P has shown great promise in modifying sucrose allocation in crops.

Heterologous expression of *Escherichia coli* trehalose pathway genes in tobacco (Goddijn *et al.*, 1997) gave the first indication of a novel regulatory pathway in plants. In the ensuing period, it has been shown that T6P is essential for carbohydrate utilization (Schluepmann *et al.*, 2003), as a signal of sucrose availability (Lunn *et al.*, 2006; Nunes *et al.*, 2013), regulating metabolism (Martins *et al.*, 2013; Figueroa *et al.*, 2016) and ensuring metabolic reprogramming in the light of sucrose availability through the feast–famine protein kinase (SnRK1) (Zhang *et al.*, 2009) linking sucrose supply with metabolism, growth, and development. Baena-González *et al.*, (2007) showed at least a 1000 genes to be regulated by SnRK1, with different sets being induced and repressed depending on SnRK1 activity. Catabolic processes and repression of growth and development were found with high SnRK1 activity. With low SnRK1 activity, activation of anabolic processes and growth and development were found. Careful targeting of SnRK1 activity through T6P regulation of SnRK1, in either the famine direction, through decreasing T6P contents to enhance stress responses, or in the feast direction through increasing T6P contents for yield potential enhancement, is showing great promise in cereal improvement, as outlined below. Cell and developmental specificity are key to successful modification for yield and yield resilience. This is not surprising given the strong tissue and developmental regulation of T6P, in addition to the regulation of T6P by sucrose. For example, wheat grain inner pericarp contains higher sucrose levels than endosperm 17 d after anthesis, but 60-fold lower T6P than endosperm. In contrast to 10 d earlier, T6P and sucrose levels are comparable in both tissues (Martínez-Barajas *et al.*, 2011). Hence the T6P–sucrose relationship is strongly tissue and development dependent.

Drought at flowering causes decreased allocation of sucrose to reproductive structures in maize, resulting in their abortion. The reduction in seed numbers greatly reduces yield. Kernel abortion due to drought was prevented by feeding sucrose (Boyer and Westgate, 2004; Zinselmeier, 1995*a*, *b*). Accordingly, a strategy was devised to prevent kernel abortion during drought at flowering through genetic engineering of T6P contents based on the regulation of the utilization of sucrose by T6P (Schluepmann *et al.*, 2003) through SnRK1, which had also been shown to regulate carbohydrate allocation in plants (Schwatchtje *et al.*, 2006). Targeting of a rice trehalose phosphate phosphatase (TPP) with a MADS6 promoter active in reproductive tissues during the flowering period increased maize yield with and without drought at flowering (Nuccio *et al.*, 2015). The success of this strategy appears to be because T6P, when targeted carefully, regulates sink strength and the amount of sucrose attracted to sinks. In this case, low T6P decreased by the TPP transgene acts as a starvation signal up-regulating sucrose movement to sinks. This is particularly effective during drought at flowering. However, it could also be a general means to increase sucrose allocation to sink tissues in all conditions to increase yield potential. In the transgenic progeny, sucrose levels were higher in female spikelets and there was a shift in biomass partitioning away from stems to grain, increasing the harvest index (Nuccio *et al.*, 2015). The reason why this trait has not been selected for already is probably because there is still some legacy of natural selection for survival rather than maximizing productivity in maize. Plants, unless adapted under the most climatically stable conditions, have no way of knowing if a drought will be short or long term. Natural selection adopts a safety-first strategy, which is seed abortion, to enable at least some seed to survive the drought. However, this abortion may be more than is necessary if the drought is short lived, compromising final grain yield. Interestingly, improved performance under drought in this example has been achieved by improving carbon allocation rather than targeting water use efficiency itself.

In a similar vein, a new chemical approach to increase T6P levels may enable modification of sucrose allocation and use. Owing to its polarity, T6P is not readily taken across plant membranes. Synthesis of T6P precursors with chemical groups attached to change the molecular charge to enable uptake by the plant has been pioneered recently in sugar signalling (Griffiths *et al.*, 2016). The chemical groups attached to T6P are cleaved off in sunlight once the compound has been taken up by the plant to release T6P endogenously (Griffiths *et al.*, 2016). A pulse of T6P can be delivered far in excess of what has been possible with genetic tools and sufficient to deliver up to 20% higher grain yield of wheat plants. The full mechanistic details await elucidation, but it is likely that T6P primes gene expression as a signal of sucrose availability for the utilization of sucrose. In wheat grain, a major sink for sucrose is starch synthesis. T6P up-regulates gene expression for starch synthesis (Griffiths *et al.*, 2016), improving grain size and yield, providing strong evidence that the capacity for starch synthesis is a major limitation to wheat yield which can be improved quite simply through application of T6P spray. In the same study, it was found that the treatment of vegetative wheat plants before rewatering after drought enabled better growth recovery of both existing vegetative material and new growth (Griffiths *et al.*, 2016). Interestingly, this bears a striking similarity to resurrection plants where accumulation of large amounts of trehalose was thought to be causally related to the strong growth recovery following rewatering after desiccation (Bianchi *et al.*, 1993).

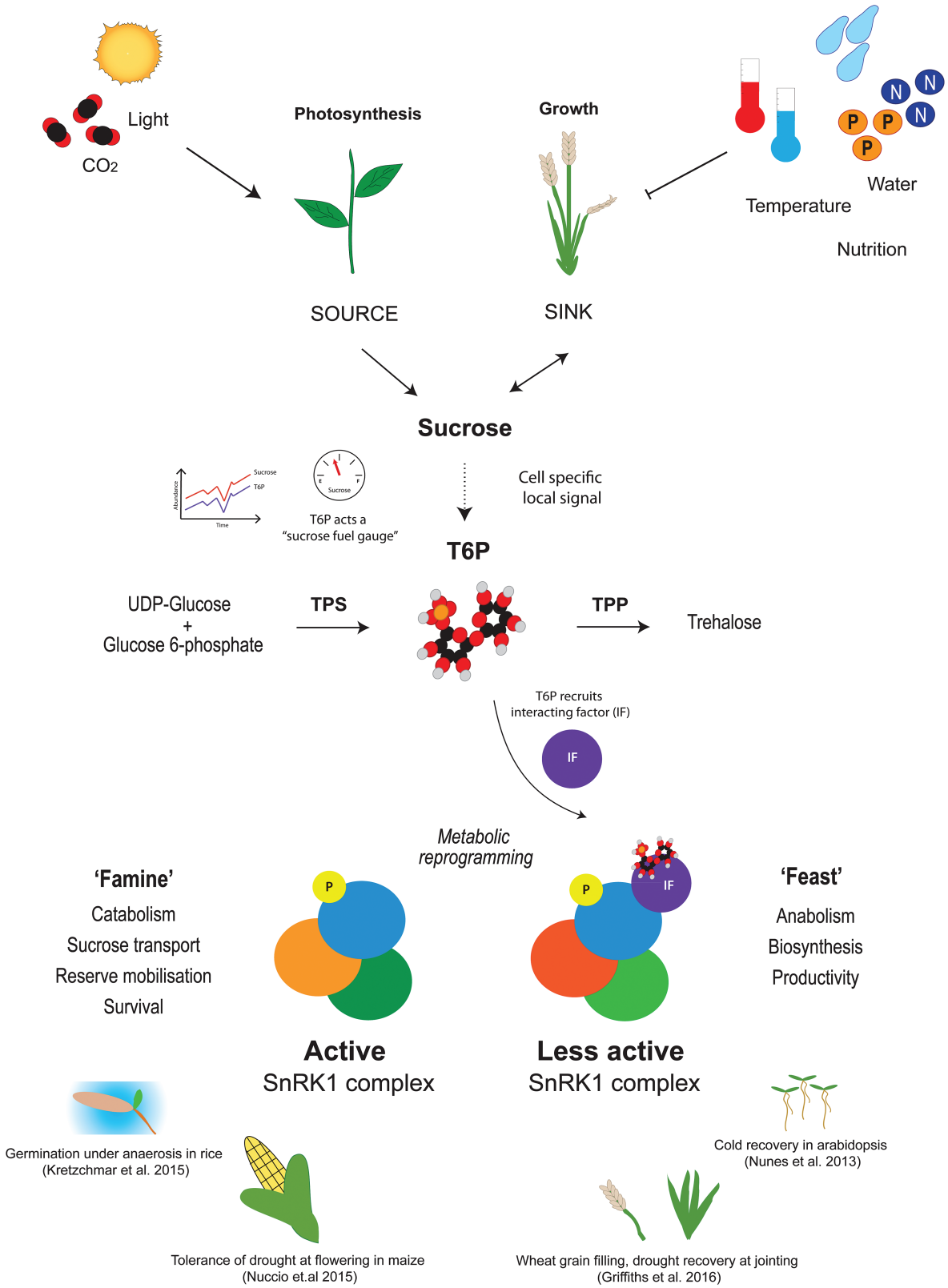

Fig. 1. Trehalose 6-phosphate (T6P), synthesized by trehalose phosphate synthase (TPS) and subsequently catalysed to trehalose by trehalose phosphate phosphatase (TPP), signals sucrose availability through the feast–famine protein kinase, SnRK1, which regulates genes involved in metabolism, growth, and development. An intermediary factor (IF) is necessary for inhibition of SnRK1 by T6P (Zhang *et al.*, 2009). Low T6P results in activation of genes for famine responses; high T6P results in activation of genes for feast responses. Decreases in T6P through genetic modification (Nuccio *et al.*, 2015) and marker-assisted selection (Kretzschmar *et al.*, 2015), or increases in T6P through chemical intervention (Griffiths *et al.*, 2016), have resulted in improved performance and large yield improvements in maize, rice, and wheat.

However, the significance of trehalose as a necessary component of the resurrection process has been called into question. Drought-susceptible *Selaginella* species have been found to accumulate more trehalose than drought-tolerant *Selaginella* (Pampurova and Van Dijck, 2014). In fact, very little is known about the function of trehalose in plants. In the vast majority of plants, the trehalose pathway is a low flux pathway, typical of signalling pathways rather than pathways involved in the synthesis of higher abundance protection molecules. Priming gene expression for growth recovery after a period of cold also occurs in Arabidopsis, shown to be dependent on T6P (Nunes *et al.*, 2013). It is likely that T6P can act as a master regulator of growth and biosynthetic processes in a range of tissues and growth conditions. The T6P precursors provide the possibility of being able to treat different crops, tissues, and developmental stages without the need to select varieties or genes, or develop promoter–gene constructs for each crop, tissue, or environment, but rather the greater simplicity and flexibility of adjusting chemical configurations and formulations, application rates, and timings to suit different crops and conditions. The ability to push physiological boundaries with T6P precursors may be the kind of thing necessary for large yield improvements. Further benefit may come from use of T6P precursors in unravelling fundamental science in addition to increasing crop yields. In spite of progress in the T6P signalling area (Figueroa *et al.*, 2016) and in elucidating SnRK1 as a credible target (Zhang *et al.*, 2009), it has been quite difficult to separate primary from secondary effects of T6P because of strong pleiotropy using constitutive promoter systems (Pellny *et al.*, 2004). Additionally, small effects on T6P levels achieved with inducible promoters make it quite hard to perturb the system, although informative effects have been observed (Lunn *et al.*, 2014). In contrast, T6P precursors can provide a large immediate burst sufficient to increase wheat yields but also to enable tracking of large temporary perturbations in metabolism and gene expression within minutes of T6P release (Griffiths *et al.*, 2016). This may prove invaluable in dissecting the fundamental T6P signalling process and primary sites of action, particularly in sink regions where T6P modification is likely to have the most profound effects on crop yields. All of the benefits of modifying T6P so far in crops have come from alterations in sink tissues. There may also be benefits in leaves, perhaps in direct stimulation of photosynthesis (Pellny *et al.*, 2004) and in modifying leaf senescence (Wingler *et al.*, 2012).

Timing changes in T6P to different stages of development during the reproductive period and in different cells would be a strategy to increase sucrose allocation to increase both grain number (early reproductive development around anthesis, Nuccio *et al.*, 2015) and grain size (grain filling period, Griffiths *et al.*, 2016). Decreasing T6P can be used as a starvation signal to up-regulate sucrose transport processes to increase grain number. Increasing T6P can be used as a feast signal in cells that are synthesizing end-products such as starch to activate gene expression for the synthesis of starch. Elevated T6P can also promote growth recovery from drought (Griffiths *et al.*, 2016) and cold (Nunes *et al.*, 2013). Allocation and utilization of sucrose can be directed towards growth and yield formation, respectively, in these cases through regulation of SnRK1 by T6P (Zhang *et al.*, 2009; Nunes *et al.*, 2013).

Interestingly, it is not only modification of T6P in vegetative and reproductive tissues where benefits are likely to be found. A TPP gene was found underlying a quantitative trait locus (QTL) for germination of rice under anaerobic conditions produced during flooding (Kretzschmar *et al.*, 2015). Although not yet definitively proven, it is likely that low T6P as a starvation signal results in more active SnRK1 which enables better mobilization of starch reserves for germination under anaerobis. Thus, targeting T6P in different tissues can benefit crops at extremes of water availability and stages of development, from germination under flooding to drought at flowering. Significantly, overall, T6P appears to be at the centre of a sucrose homeostatic mechanism that determines the allocation and use of sucrose and other carbohydrates not yet optimized for yield potential itself and yield resilience in different environments (Fig. 1).

Conclusion

Targeting carbohydrate allocation in favour of sinks may seem a circuitous way to increase photosynthetic CO_2 uptake. It also may seem a less obvious way to combat stresses of water deficit or flooding instead of selecting for water use efficiency or anaerobis tolerance mechanisms directly. Nevertheless, targeting the mechanistic basis of source–sink balance for carbohydrate use and allocation appears to have extensive utility in crop improvement shown already for the three major global crops, maize, wheat, and rice in different environments. In terms of environmental challenges, it is water availability that most limits cereal yields globally, and, in terms of intrinsic processes, the determination of grain numbers and grain size that most limits yields. Once such limitations are addressed, photosynthesis may then respond to fill extra sink capacity through multiple ways of regulating photosynthesis at the whole-plant level. Engineering of photosynthesis beyond this sink regulation of photosynthesis could provide a further boost to crops yields.

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