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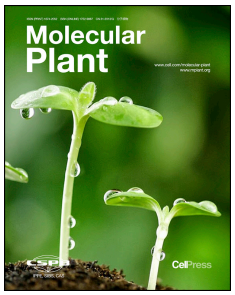

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Improving rice photosynthesis and yield through trehalose 6-phosphate signalling

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T6P/ SnRK1, a major regulator in plants

The biosynthetic route for the synthesis of trehalose in plants is through a low flux pathway that leads to the accumulation of micromolar amounts of trehalose 6-phosphate (T6P) and trehalose when sucrose is available. T6P accumulates in proportion to sucrose (Lunn et al., 2006) as an indispensable regulator of carbohydrate utilisation (Schluepmann et al., 2003). At least some of the indispensability of T6P and its profound and widespread effects on growth and development can be explained through T6P inhibition of the protein kinase, SnRK1 (Zhang et al., 2009) a central regulator of responses to carbon and energy status. Through T6P/ SnRK1 growth and development are regulated in line with sucrose availability through large-scale regulation of gene expression which includes derepression of anabolic growth processes when carbon is available (Zhang et al., 2009). In crops there are several emerging cases where T6P/ SnRK1 can be modified to alter carbon use and allocation to improve yield in field conditions (Paul et al., 2020). The most successful route through which T6P has been modified for yield and resilience so far genetically has been by altering trehalose phosphate phosphatase (TPP) activity. Ectopic expression of a TPP enzyme in maize reproductive tissue using a MADS6 promoter increased grain numbers in the field especially under drought (Nuccio et al., 2015). Altered distribution of sucrose away from pith towards developing grain was associated with altered expression of SWEETs and prevented abortion of grain under drought (Oszvald et al., 2018). In sorghum it was found through genetic crosses of sweet and grain sorghum that a bZIP transcription factor elevated TPP expression which underpinned the large differences in height of stems and accumulation of carbohydrates within stems of sweet and grain sorghum (Paul et al., 2020). A TPP gene in rice was found to underlie a QTL for germination under flooded conditions through better mobilisation of starch reserves likely through SnRK1 providing promise in the development of direct-seeded rice (Paul et al., 2020).

NAC23 transcription factor regulates T6P, sucrose levels and yield in rice

A recent study by Li et al. (2022) has found another way to modify T6P/ SnRK1 through TPPs. In rice the sugar-inducible transcription factor, NAC23, was found to repress TPP1 thereby increasing T6P levels. Interestingly, the paper found a feedforward loop whereby SnRK1, an antagonist of NAC23, was inhibited by the extra T6P accumulated resulting in even stronger repression of TPP1. Overexpressing NAC23 and hence driving the feedforward loop NAC23-TPP-T6P-SnRK1 increased allocation of sucrose away from leaves towards grain resulting in +13-17% higher yield due to more panicles and higher 1000-grain weight in the paddy-field-grown rice. There are several novel aspects of this work. Firstly, the paper provides new insight on the regulation of T6P levels through a sugar-inducible transcription factor, NAC23, that represses TPP1. NAC23 represents a new part of a previously unknown mechanism of regulating T6P levels, although a similar transcription factor in Arabidopsis, TAF1, has been implicated in altering T6P content but decreasing T6P seemingly through increasing trehalase rather than TPPs (Garapati et al., 2015). Detail has been lacking up until now of the mechanism through which sucrose induces T6P levels. The large effects exerted through TPP1 suggest a strong level of sugar control of T6P concentration at the TPP catalytic step. Expression of TPSs and other TPPs was also affected by NAC23 so there could be other effects of the pathway on T6P mediated by NAC23 other than through TPP1. But nevertheless TPP1 seems most affected by NAC23 in rice. Secondly, the work confirms large effects on whole plant sucrose allocation of modifying the T6P/ SnRK1 regulatory network. Thirdly, photosynthetic rate of leaves was increased. Whether this was a direct effect on photosynthesis or an effect of altered source-sink which increased

photosynthesis is unclear. However, it confirms Oszvald et al. (2018) that changing sucrose allocation through T6P increases leaf photosynthesis which further implicates T6P as a source-sink integrator. So far, modifying T6P has been a far more successful way of improving photosynthesis than direct targeting of photosynthesis itself, although ectopic expression of Rubisco has shown promise in paddy rice (Yoon et al., 2020). A fourth point is that trehalose levels were decreased in the higher yielding rice plants due to a decrease in TPP activity which does not appear to be detrimental. However, plants were grown in paddy field conditions with no exposure to drought where trehalose accumulation particularly in chloroplasts could function as a ROS scavenger. Nevertheless the change in sucrose allocation towards seeds could in itself protect against drought as observed in Nuccio et al. (2015) and a decrease in trehalose may not matter.

Strategies to modify T6P signalling for yield

Li et al. (2022) highlights that there may be multiple ways to modify T6P signalling for yield. Targeting NAC23 may be particularly successful because it results in promotion of a feedforward loop to alter whole plant sucrose allocation driving T6P inhibition of SnRK1 which also regulates NAC23. Interestingly expression of NAC23 is driven constitutively, a blunt instrument, but works in this case. An earlier study (Garg et al., 2002) using constitutive expression to drive a dual TPS-TPP construct also worked in rice, but this work does not appear to have been taken further. For such a powerful signal as T6P genetic modification has had the challenge of effective targeting of transgene with the right promoter e.g. Nuccio et al. (2015) where only one was successful out of many different constructs tested. Whether NAC23 provides an easier route to transgenic development of T6P modification more widely remains to be seen. Currently it is difficult to predict what will happen when T6P levels are changed. As can be seen in Figure 1 an increase in T6P levels as a consequence of overexpressing NAC23 changes sucrose allocation towards grain in rice, but so does decreasing T6P in maize as a consequence of overexpressing TPP1, where sucrose allocation is changed away from pith tissue towards developing seeds. Spatial and developmental context are key for T6P. It may be possible to work this out in different transgenic strategies.

Natural genetic variation linked to traits is another way forward for where a TPP gene underpinned a QTL for anaerobic germination (Paul et al., 2020). Lyra et al. (2021) showed both historic and ongoing selection of TPS and TPP genes in wheat and links to 12 traits. TPPs crossed from exotic germplasm could provide opportunity to improve grain number and filling (Lyra et al., 2021) as a way to continue successes from targeting TPP genes through transgenic approaches. Additionally analysis of SNPs and genetic variation and their link to traits can direct strategies for gene editing of selected TPS and TPP genes and combinations as there are epistatic interactions between TPSs and TPPs (Lyra et al., 2021).

Another approach is through chemical intervention where T6P precursors are applied to crops. This shows promising results in glasshouse (Griffiths et al., 2016) and field (unpublished). Additionally such compounds can be used to interrogate mechanism and downstream T6P response networks that could be selected for yield.

In summary, Li et al. (2022) adds to the ever growing success story of altering T6P to improve sucrose allocation for yield in crops. This is because T6P orchestrates changes in sucrose allocation that link source and sink to improve yield. Future prospects for T6P appear excellent as the T6P mechanism has not yet been optimised for yield exemplified in several studies (Paul et al., 2020; Lyra et al., 2021) and here again in Li et al. (2022) through a novel mechanism. Given the emerging successes in modifying T6P for yield beyond what has already been achieved through domestication and breeding future prospects targeting T6P through different strategies to improve yield and food security under a range of environments are very promising.

Author contributions

MJP wrote the manuscript which all authors planned, discussed and agreed content in advance. All authors edited and approved the final version.

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References

Garapati P, Feil R, Lunn JE, Van Dijck P, Balazadeh S, Mueller-Roeber B. (2015) Transcription Factor Arabidopsis Activating Factor1 Integrates Carbon Starvation Responses with Trehalose Metabolism. Plant Physiol. 169, 379-90. doi: 10.1104/pp.15.00917.

Garg, A.K., Kim, J-K., Owens, T.G., Ranwala, A.P., Do Choi, Y. Kochian, L.V. et al. (2002) Trehalose accumulation in rice plants confers high tolerance to different abiotic stresses. *Proc. Natl. Acad. Sci. USA* 99, 15898-15903.

Griffiths, C.A., Sagar, R., Geng, Y., Primavesi, L.F., Patel, M.K., Passarelli, M.K. et al. (2016) Chemical intervention in plant sugar signalling increases yield and resilience. *Nature* doi:10.1038/nature20591.

Li Z., Wei X., Tong X., Zhao J., Liu X., Wang H., Tang L., Shu Y., Li G., Wang Y., Ying J., Jiao G., Hu H., Hu P., and Zhang J. (2022). The OsNAC23-Tre6P-SnRK1a feedforward loop regulates sugar homeostasis and grain yield in rice. Mol. Plant. doi: https://doi.org/10.1016/ j.molp.2022.01.016.

Lunn, J.E., Feil, R., Hendriks, J.H., Gibon, Y., Morcuende, R., Osuna, D., Scheible, W.R., Carillo, P., Hajirezaei, M.R., Stitt, M. (2006) Sugar-induced increases in trehalose 6-phosphate are correlated with redox activation of ADP-glucose pyrophosphorylase and higher rates of starch synthesis in Arabidopsis thaliana. Biochem J. 397, 139-148.

Lyra DH, Griffiths CA, Watson A, Joynson R, Molero G, Igna AA, Hassani-Pak K, Reynolds MP, Hall A, Paul MJ (2021) Gene-based mapping of trehalose biosynthetic pathway genes reveals association with source- and sink-related traits in a spring wheat panel. Food and Energy Security 10.1002/fes3.292.

Nuccio, M.L., Wu, J., Mowers, R., Zhou, H-P., Meghji, M., Primavesi, L.F., Paul, M.J., Chen, X., Gao, Y., Haque, E. (2015) Expression of trehalose 6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. Nat. Biotech. 33, 862-869.

Oszvald M., Primavesi, L.F., Griffiths, C.A., Cohn, C., Basu, S.S., Nuccio, M.L., Paul, M.J. (2018) Trehlaose 6-phosphate in maize reproductive tissue regulates assimilate partitioning and photosynthesis. Plant Physiol. 176, 2623-2638.

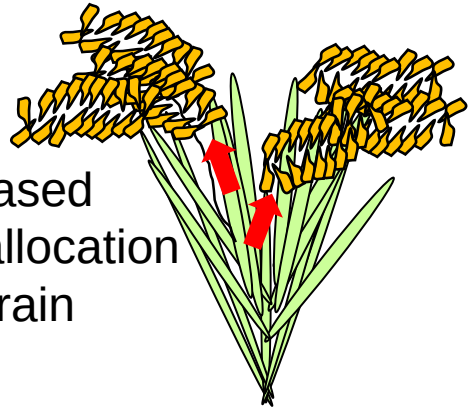
Paul MJ, Watson A, Griffiths CA (2020) Trehalose 6-phosphate signalling and impact on crop yield. Biochemical Society Transactions doi 10.1042/BST20200286.

Schluepmann H, Pellny T, van Dijken A, Smeekens S, Paul MJ (2003) Trehalose 6-phosphate is indispensable for carbohydrate utilisation and growth in Arabidopsis thaliana. Proceedings of National Academy of Sciences 100, 6849-6854.

Yoon, D-K., Ishiyama, K., Suganami, M., Tazoe, Y., Watanabe, M., Imaruoka, S., et al. (2020) Transgenic rice overproducing Rubisco exhibits increased yields with improved nitrogen-use efficiency in an experimental paddy field. *Nature Food* 1, 134-139.

Zhang Y, Primavesi LF, Jhurreea D, Mitchell R, Powers S, Schluepmann H, Delatte T, Wingler A, Paul MJ (2009) Inhibition of Snf1-related protein kinase (SnRK1) activity and regulation of metabolic pathways by trehalose 6-phosphate. Plant Physiology 149, 1860-1871.

Figure 1. Targeting trehalose phosphate phosphatase (TPP) through different strategies. (A) Expression of NAC23 transcription factor in rice decreases TPP expression and increases T6P in leaves increasing whole plant carbon allocation towards grain increasing yield in field conditions. Leaf sections showing iodine staining for starch which is decreased relative to wild type reflecting increased carbon allocation to grain. **(B)** Ectopic expression of TPP with MADS6 promoter in maize decreases T6P in pith and developing florets which results in more sucrose movement into developing florets from pith tissue increasing photosynthesis of leaves. A cross-section of the cob shows expression of MAD6-TPP in vasculature of pith and grain.



Increased
sucrose allocation
to grain

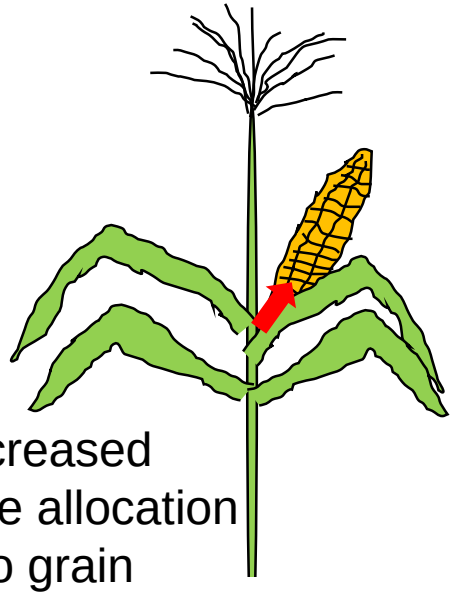
A. Rice

(Li et al. 2022)

constitutive overexpression of NAC23 transcription factor
to downregulate TPP1

NAC23 ↑	→	TPP1 ↓	T6P ↑	Trehalose ↓

movement of sucrose from leaves to grain
higher photosynthesis
more panicles per plant and higher 1000-grain weight
giving higher yield in paddy field
(full hydration)



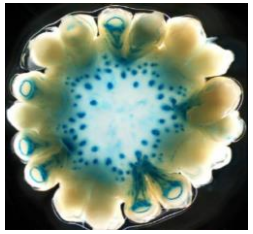
Increased
sucrose allocation
to grain

B. Maize

(Oszvald et al. 2018)

cob vasculature (MADS6) overexpression of TPP1

MADS6-TPP1 ↑	T6P ↓	Trehalose ↔



movement of sucrose from pith to grain
higher photosynthesis
more seed per cob giving higher yield in rainfed field
(with and without drought)