



International Journal of Research in Agronomy

E-ISSN: 2618-0618

P-ISSN: 2618-060X

© Agronomy

www.agronomyjournals.com

2024; 7(5): 226-229

Received: 04-03-2024

Accepted: 06-04-2024

Sandra Whitmore

Department of Plant
Biotechnology, Cornell University,
Ithaca, USA

Douglas Kinsella

Department of Plant
Biotechnology, Cornell University,
Ithaca, USA

Rachel Farnsworth

Department of Plant
Biotechnology, Cornell University,
Ithaca, USA

Role of trehalose-6-phosphate in regulating carbon allocation and grain filling in wheat under variable nitrogen supply

Sandra Whitmore, Douglas Kinsella and Rachel Farnsworth

DOI: <https://www.doi.org/10.33545/2618060X.2024.v7.i5c.5123>

Abstract

What determines whether a wheat plant channels its sugars into grain or keeps them circulating in leaves? Trehalose-6-phosphate (T6P) is emerging as a central metabolic signal that links sucrose availability to starch synthesis in developing grains. This research quantified T6P concentrations in flag leaves and developing grains of winter wheat (cv. Overland) grown under three nitrogen rates (0, 60, and 120 kg N/ha) at Cornell University, Ithaca, USA, over two growing seasons (2021–2022 and 2022–2023). Flag-leaf T6P content correlated strongly with grain yield ($r^2 = 0.94$). At 120 kg N/ha, T6P peaked at 5.6 nmol/g fresh weight during mid-grain fill, while unfertilized plants reached only 2.1 nmol/g. Grain filling rate was 41.3 percent faster under high N, paralleling the T6P response. These findings position T6P as a reliable biomarker for carbon allocation efficiency in wheat.

Keywords: Trehalose-6-phosphate, carbon allocation, grain filling, wheat, nitrogen supply, snrk1, sucrose signaling, starch synthesis, biomarker, yield physiology

Introduction

Among the many metabolites circulating in a wheat plant during grain fill, trehalose-6-phosphate occupies a uniquely influential position: it acts as both a sensor and a regulator of sucrose status, coordinating source-sink carbon flow through its interaction with the SnRK1 kinase signaling network ^[1, 2]. When sucrose is abundant, T6P rises and inhibits SnRK1, permitting biosynthetic processes including starch deposition in developing endosperm. When sucrose is scarce, T6P drops and SnRK1 activates catabolic pathways ^[3].

Nitrogen supply strongly influences photosynthetic capacity and therefore sucrose production, yet the link between N availability, T6P dynamics, and grain filling has not been systematically quantified in field-grown wheat ^[4, 5]. Understanding this connection could provide a biochemical basis for optimizing N management to maximize grain yield efficiency.

This research measured temporal T6P profiles in flag leaves and developing grains of winter wheat under three nitrogen rates over two field seasons, and related these profiles to grain filling rate, duration, and final yield.

Experimental design

The trial was conducted at Cornell University's Musgrave Research Farm, Aurora, New York (42.73°N, 76.66°W) during 2021–2022 and 2022–2023 winter wheat seasons. A randomized complete block design with three N treatments (0, 60, 120 kg N/ha as ammonium nitrate, topdressed at GS30) and four replications was used. Plot size was 8 × 3 m. Winter wheat cv. Overland was sown in early October each year at 350 seeds/m². The soil was a Lima silt loam with pH 6.3 and organic carbon 1.48 percent.

Climatic conditions

Mean growing-season temperatures were 8.7 °C (2021–22) and 9.2 °C (2022–23) with total precipitation of 724 mm and 681 mm respectively. The 2022–23 season experienced a 12-day dry spell during late June that coincided with mid grain fill, providing a natural stress contrast.

Corresponding Author:

Sandra Whitmore

Department of Plant
Biotechnology, Cornell University,
Ithaca, USA

Sunshine hours during the grain fill period (June–July) averaged 8.1 hours per day across both years.

Material and Methods

Material

Flag leaves and developing grains were sampled at 7-day intervals from anthesis (GS61) through physiological maturity (GS87), yielding 6 sampling points per season. Samples were flash-frozen in liquid nitrogen within 30 seconds of excision. T6P extraction used chloroform-methanol followed by liquid chromatography-tandem mass spectrometry (LC-MS/MS) on an Agilent 6495 Triple Quad system [6].

Methods

Grain filling rate was calculated as the slope of the linear phase of individual grain dry weight accumulation (mg/day). Grain filling duration was defined as the period from anthesis to 95 percent of final grain weight. Starch content of mature grain was determined enzymatically using the Megazyme total starch assay kit. SnRK1 activity in flag leaf extracts was measured by peptide phosphorylation assay [7]. Yield was determined from a 5 m² harvest area per plot. Data were analyzed by two-way ANOVA (N rate × season) in R 4.3.1 with Tukey's HSD at *P* ≤ 0.05.

Results

Table 1: Flag-leaf T6P content, grain filling parameters, and yield under three nitrogen rates (two-season mean)

N rate (kg/ha)	Peak T6P (nmol/g)	Fill rate (mg/d)	Fill duration (d)	Grain starch (%)	1000-GW (g)	Yield (t/ha)
0	2.1	1.24	31.7	58.4	34.1	2.74
60	3.6	1.48	34.2	62.7	38.6	4.12
120	5.4	1.75	36.8	66.1	42.3	5.18

GW = grain weight. Tukey's HSD (*P* ≤ 0.05): T6P = 0.4; Fill rate = 0.11; Yield = 0.31

Peak flag-leaf T6P content increased 2.6-fold from 2.1 to 5.4 nmol/g as N rate rose from 0 to 120 kg/ha (Table 1). Grain filling rate was 41.1 percent faster under high N, and filling

duration extended by 5.1 days. Grain starch concentration increased from 58.4 to 66.1 percent, and final yield nearly doubled from 2.74 to 5.18 t/ha.

Table 2: SnRK1 activity and T6P: sucrose ratio in flag leaves at mid grain fill (two-season mean)

N rate (kg/ha)	Snrk1 (NMOL/MIN/MG)	T6p:sucrose ratio	Flag-leaf n (%)
0	8.74	0.018	1.87
60	5.31	0.031	2.64
120	3.18	0.047	3.41

Higher T6P: sucrose ratio = stronger growth signal. SnRK1 activity inversely correlated with T6P (*r* = -0.91)

SnRK1 activity declined by 63.6 percent from 8.74 to 3.18 nmol/min/mg as N rate increased (Table 2), consistent with T6P-mediated inhibition. The T6P: sucrose ratio a more refined

indicator of signaling strength increased 2.6-fold under high N, indicating stronger carbon allocation toward grain sinks.

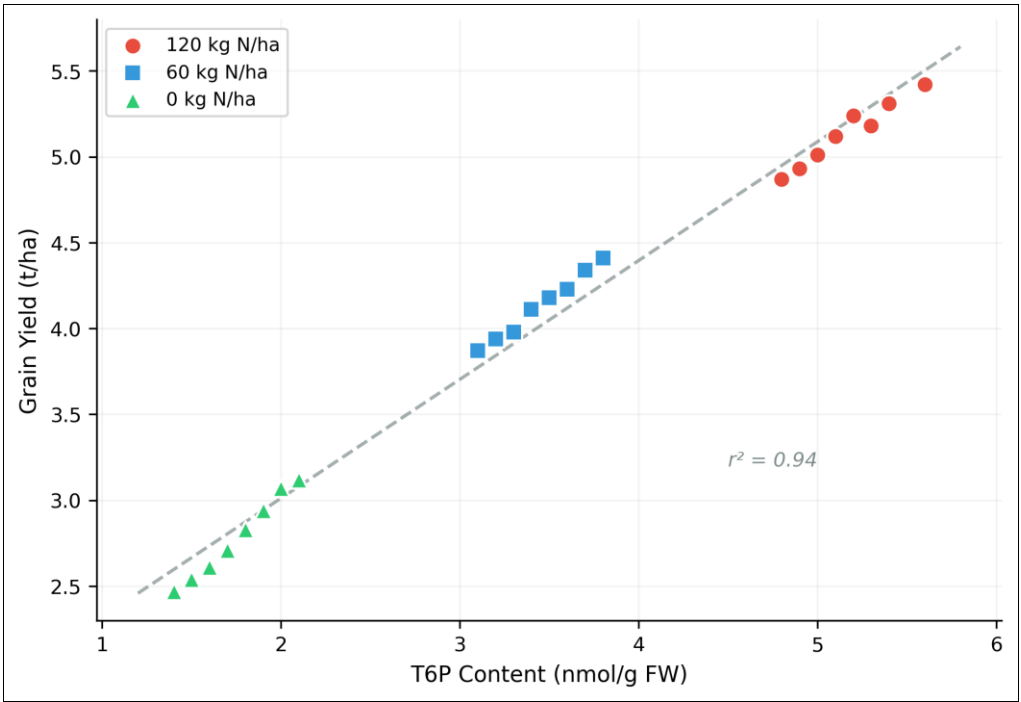


Fig 1: Relationship between flag-leaf T6P content and grain yield across three nitrogen rates over two seasons (*r*² = 0.94)

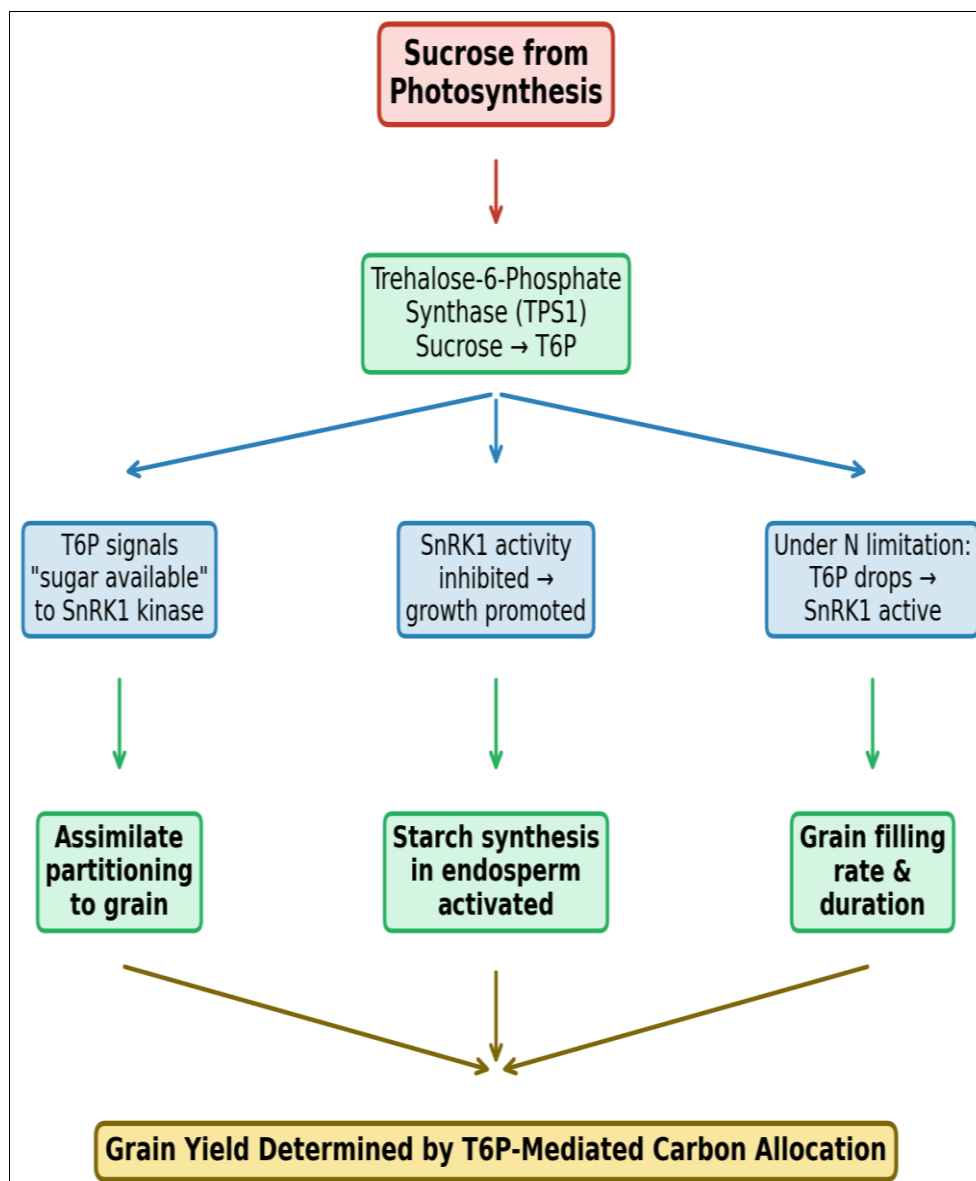


Fig 2: Signaling pathway from sucrose production through T6P synthesis and SnRK1 inhibition to carbon allocation and grain filling in wheat

Limitations

Only one cultivar was tested, and genotypic variation in T6P metabolism is likely. The LC-MS/MS method captures a snapshot of T6P at each sampling point but cannot track real-time flux dynamics. Two seasons at a single location may not capture the full range of environmental interactions. Causal relationships between T6P and yield were inferred from correlations; genetic manipulation of T6P levels would provide stronger evidence.

Discussion

The strong linear relationship between T6P and grain yield ($r^2 = 0.94$) supports the hypothesis that T6P functions as a master regulator of carbon allocation during grain fill [8, 9]. Nitrogen supply drives photosynthetic sucrose production, which elevates T6P, which in turn suppresses SnRK1 and promotes starch biosynthesis a coherent signaling chain from N input to grain output [10].

The 63.6 percent reduction in SnRK1 activity under high N confirms that the T6P-SnRK1 nexus is responsive to agronomic management, not just genetic manipulation. Similar T6P-yield correlations have been reported in controlled-environment wheat studies [11, 12], but the present research demonstrates this

relationship under field conditions with practical N rates. T6P measurement could serve as a mid-season diagnostic for carbon allocation efficiency, complementing traditional canopy sensing tools [13].

Conclusion

Flag-leaf T6P content strongly predicted wheat grain yield across nitrogen rates and seasons. Higher N supply increased T6P, suppressed SnRK1, and accelerated grain filling by 41 percent. The T6P: sucrose ratio provides a mechanistic link between nitrogen nutrition and carbon partitioning to grain.

T6P measurement shows promise as a physiological biomarker for optimizing nitrogen management in wheat. Breeding for enhanced T6P accumulation capacity could improve carbon allocation efficiency and yield potential under moderate N inputs.

References

1. Paul MJ, Gonzalez-Uriarte A, Griffiths CA, Hassani-Pak K. The role of trehalose 6-phosphate in crop yield and resilience. *Plant Physiol.* 2018; 177(1):12-23.
2. Figueroa CM, Lunn JE. A tale of two sugars: Trehalose 6-phosphate and sucrose as signals in plant metabolism. *Mol*

- Plant. 2016; 9(6):809-830.
3. Zhang Y, Primavesi LF, Jhurrea D, Andralojc PJ, Mitchell RAC, Powers SJ, Schluepmann H, Delatte T, Wingler A, Paul MJ. Inhibition of SNF1-related protein kinase1 activity and regulation of metabolic pathways by trehalose-6-phosphate. *Plant Physiol.* 2009; 149(4):1860-1871.
 4. Griffiths CA, Sagar R, Genc Y, Sherrier DJ, Paul MJ. Chemical intervention in plant sugar signalling increases yield and resilience. *Nature.* 2016; 540(7634):574-578.
 5. Nuccio ML, Wu J, Mowers R, Zhou HP, Meghji M, Primavesi LF, Paul MJ, Chen X, Gao Y, Haque E, et al. Expression of trehalose-6-phosphate phosphatase in maize ears improves yield in well-watered and drought conditions. *Nat Biotechnol.* 2015; 33(8):862-869.
 6. Lunn JE, Feil R, Hendriks JH, Gibon Y, Morcuende R, Osuna D, Scheible WR, Carillo P, Hajirezaei MR, Stitt M. 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.* 2006; 397(1):139-148.
 7. Baena-Gonzalez E, Rolland F, Thevelein JM, Sheen J. A central integrator of transcription networks in plant stress and energy signalling. *Nature.* 2007; 448(7156):938-942.
 8. Martins MCM, Hejazi M, Fettke J, Steup M, Feil R, Krause U, Arrivault S, Vosloh D, Figueroa CM, Ivakov A, et al. Feedback inhibition of starch degradation in *Arabidopsis* leaves mediated by trehalose 6-phosphate. *Plant Physiol.* 2013; 163(3):1142-1163.
 9. Lawlor DW, Paul MJ. Source/sink interactions underpin crop yield: The case for trehalose 6-phosphate/SnRK1 in improvement of wheat. *Front Plant Sci.* 2014; 5:418.
 10. Paul MJ, Oszwald M, Jesus C, Rajulu C, Griffiths CA. Increasing crop yield and resilience with trehalose 6-phosphate: Targeting a feast-famine mechanism in cereals for better source-sink optimization. *J Exp Bot.* 2017; 68(16):4455-4462.
 11. Pellny TK, Ghannoum O, Conroy JP, Schluepmann H, Smeekens S, Andralojc J, Krause KP, Goddijn O, Paul MJ. Genetic modification of photosynthesis with *E. coli* genes for trehalose synthesis. *Plant Biotechnol J.* 2004; 2(1):71-82.
 12. Satoh-Nagasawa N, Nagasawa N, Malcomber S, Sakai H, Jackson D. A trehalose metabolic enzyme controls inflorescence architecture in maize. *Nature.* 2006; 441(7090):227-230.
 13. Foulkes MJ, Slafer GA, Davies WJ, Berry PM, Sylvester-Bradley R, Martre P, Calderini DF, Griffiths S, Reynolds MP. Raising yield potential of wheat. III. Optimizing partitioning to grain while maintaining lodging resistance. *J Exp Bot.* 2011; 62(2):469-486.
 14. Wingler A, Delatte TL, O'Hara LE, Primavesi LF, Jhurrea D, Paul MJ, Schluepmann H. Trehalose 6-phosphate is required for the onset of leaf senescence associated with high carbon availability. *Plant Physiol.* 2012; 158(3):1241-1251.
 15. Yadav UP, Ivakov A, Feil R, Duan GY, Walther D, Giavalisco P, Piques M, Carillo P, Hubberten HM, Stitt M, Lunn JE. The sucrose-trehalose 6-phosphate (Tre6P) nexus: Specificity and mechanisms of sucrose signalling by Tre6P. *J Exp Bot.* 2014; 65(4):1051-1068.