Managing carbon for grain yield
Sustainable wheat yield improvements with T6P

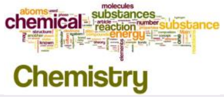
Matthew Paul

International Wheat Yield Partnership

Research to Deliver Wheat for the Future

ROTHAMSTED RESEARCH

- Field testing DMNB-T6P in agricultural environments
- Gene-trait association study for TPS and TPP genes in HiBAP



nature biotechnology

Article

https://doi.org/10.1038/s41587-025-02611-1

Membrane-permeable trehalose 6-phosphate precursor spray increases wheat yields in field trials

Received: 4 June 2024

Accepted: 21 February 2025

Published online: 29 April 2025

Check for updates

Cara A. Griffiths[1], Xiaochao Xue[2], Javier A. Miret[1], Fernando Salvagiotti[3,4], Liana G. Acevedo-Siaca[5,8], Jacinta Gimeno[5], Matthew P. Reynolds[5], Kirsty L. Hassall[1,9], Kirstie Halsey[1], Swati Puranik[1], Maria Oszvald[1], Smita Kurup[1], Benjamin G. Davis[2,6,7] & Matthew J. Paul[1]

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Revised: 12 April 2021 | Accepted: 12 April 2021

DOI: 10.1002/fes3.292

Food and Energy Security WILEY

ORIGINAL RESEARCH

Gene-based mapping of trehalose biosynthetic pathway genes reveals association with source- and sink-related yield traits in a spring wheat panel

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Abstract
Trehalose 6-phosphate (T6P) signalling regulates carbon use and allocation and is a target to improve crop yields. However, the specific contributions of trehalose phosphate synthase (TPS) and trehalose phosphate phosphatase (TPP) genes to source- and sink-related traits remain largely unknown. We used enrichment capture sequencing on TPS and TPP genes to estimate and partition the genetic variation of yield-related traits in a spring wheat (*Triticum aestivum*) breeding panel specifically built to capture the diversity across the 75,000 CIMMYT wheat cultivar collection. Twelve phenotypes were correlated to variation in TPS and TPP genes including plant height and biomass (source), spikelets per spike, spike growth and grain filling traits (sink) which showed indications of both positive and negative gene selection. Individual genes explained proportions of heritability for biomass and grain-related traits. Three *TPS1* homologues were particularly significant for trait variation. Epistatic interactions were found within and between the TPS and TPP gene families for both plant height and grain-related traits. Gene-based prediction improved predictive ability for

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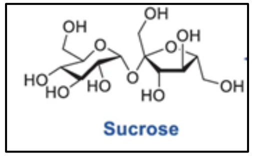
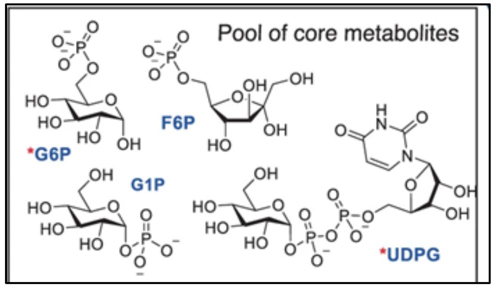
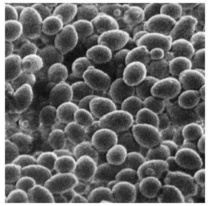
SNF1 related protein kinases (sucrose non fermenting 1)
AMPK/ SnRK1
Carbon and energy regulation





TPS
T6P
TPP
Trehalose

Activated by
low C, low energy AMP
(starvation)



SNF1
Yeast

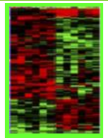
Mobilise energy reserves

AMPK
Mammals

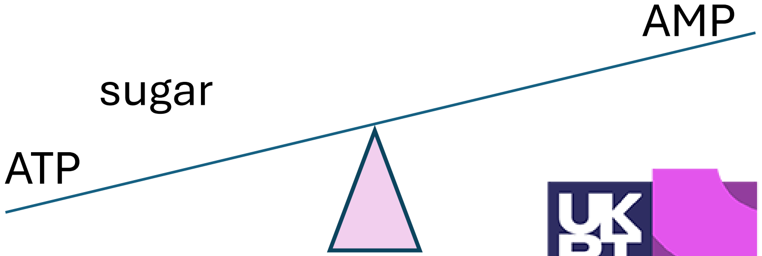
Mobilises energy reserves
Inhibits cholesterol synthesis
Regulates blood sugar/ diabetes
Drug target e.g. Metformin

T6P ⊣ SnRK1
Plants

signal of sucrose availability



Growth
Starch synthesis



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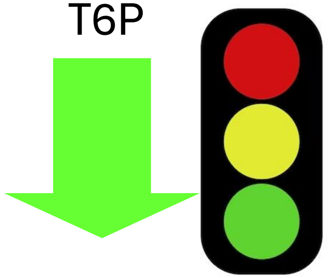
responsive mode

T6P inhibition of SnRK1 discovered by us at
ROTHAMSTED RESEARCH
Zhang et al. 2009

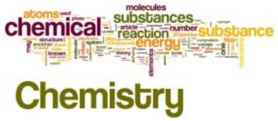
T6P

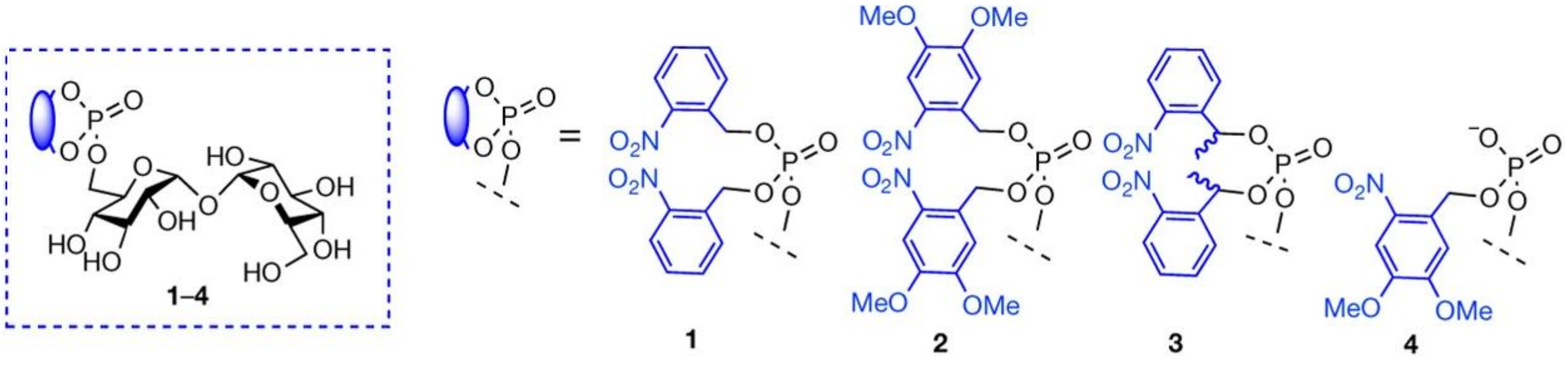


catabolism

anabolism

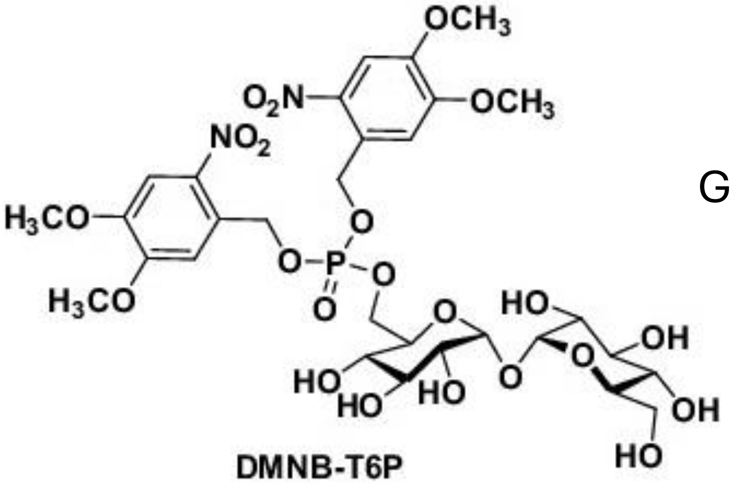
An alternative to complex genetics 2006-





T6P is plant-impermeable. Unnatural precursors mask the phosphate charge and increase hydrophobicity allowing uptake followed by subsequent photo-activated release of T6P in planta.

Professor Ben Davis FRS
Oxford University and
Rosalind Franklin Institute



DMNB-T6P

Gave best *in planta* T6P levels

6-O-Bis-(4,5-dimethoxy-2-nitrobenzyloxyphosphoryl)-d-trehalose
(DMNB-T6P)

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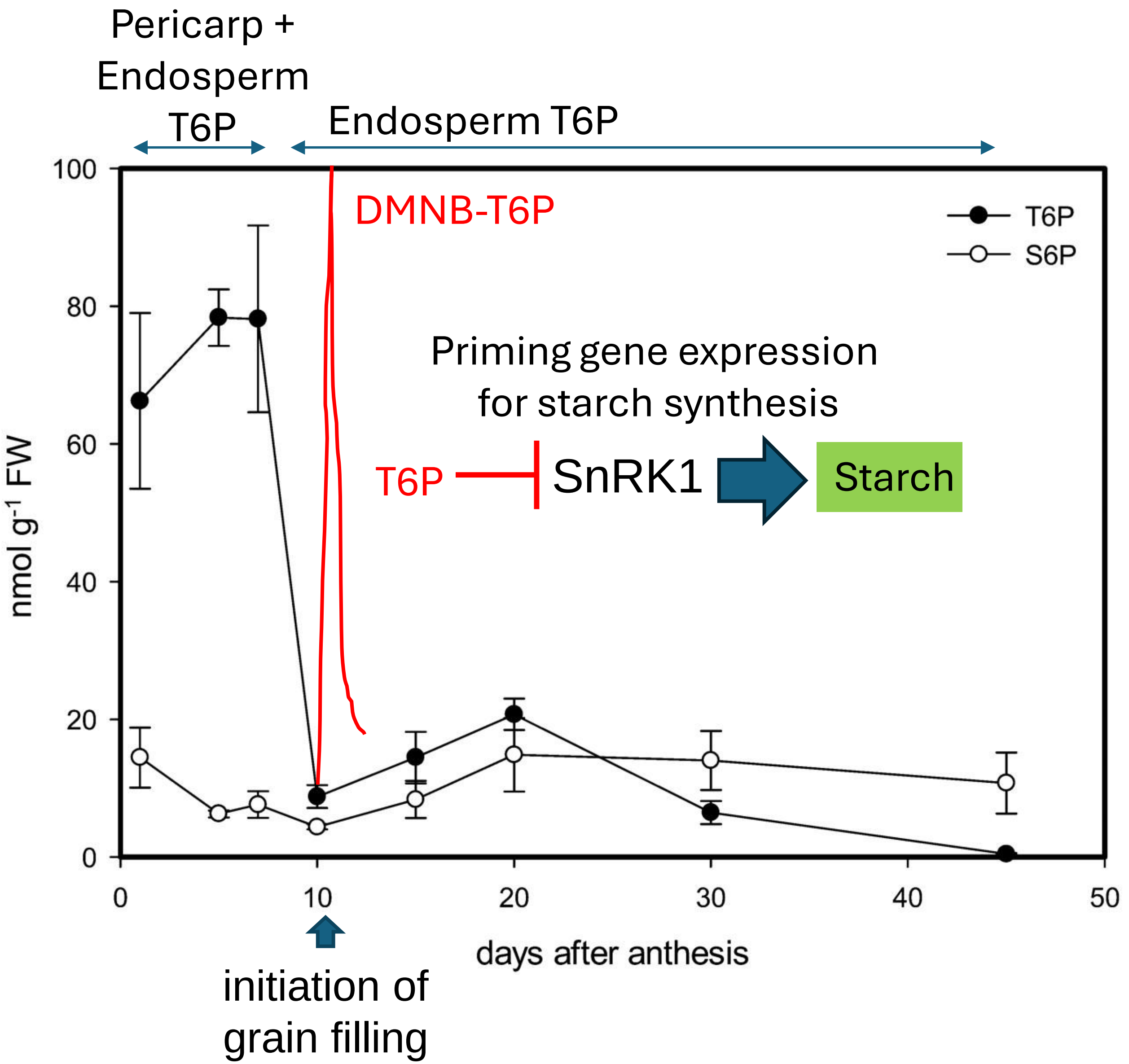
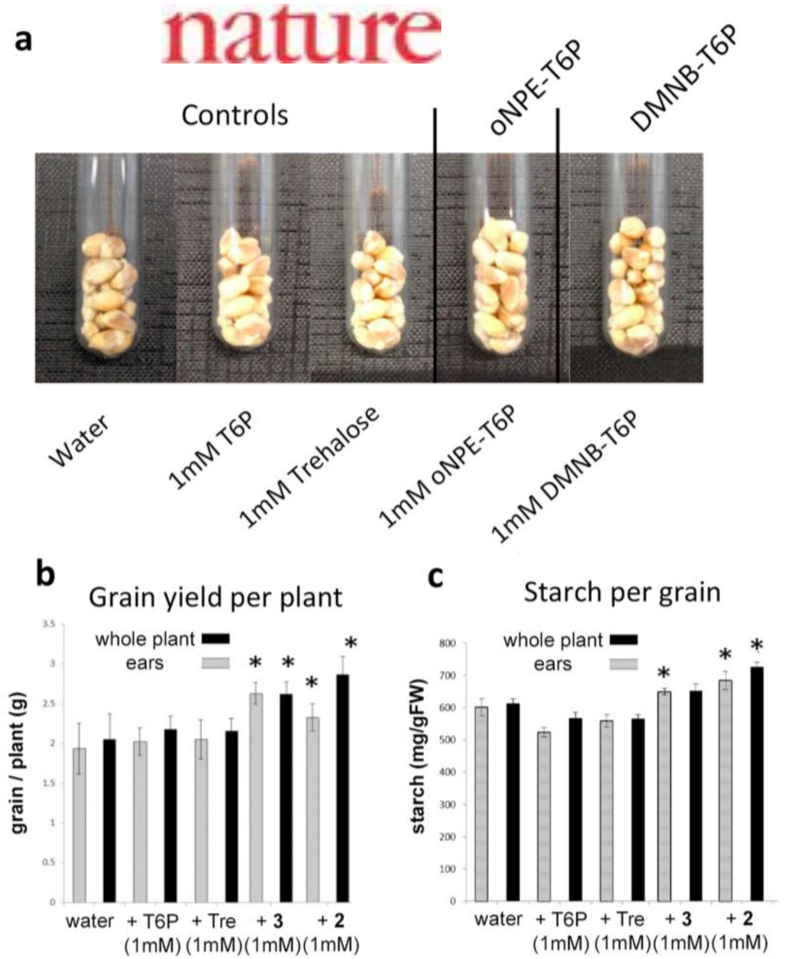
Increasing T6P at the start of grain filling 10 DAA

Cara Griffiths



Yield increased +18% in controlled environment



Plant Physiol, Volume 156, Issue 1, May 2011, Pages 373–381, https://doi.org/10.1104/pp.111.174524

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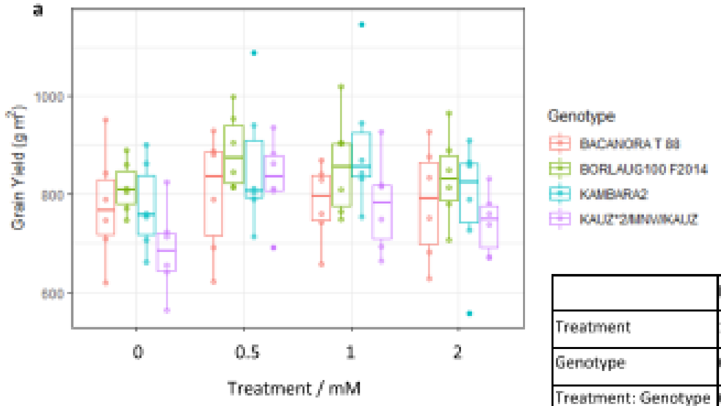
Follow on Fund

Field testing in agricultural environments at CIMMYT and at INTA Argentina

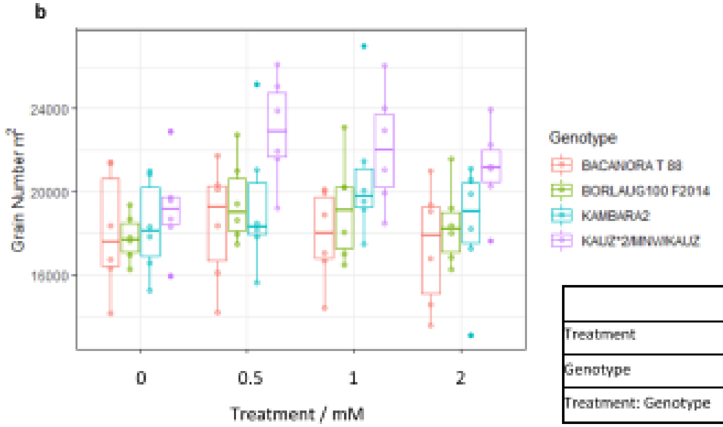
Table S1. Spraying treatments of DMNB-T6P spray used in Argentina and Mexico. In Argentina three volumes (dose rates) of 1 mM DMNB-T6P were used in 2021 and 2022; two volumes in 2018 and 2020, compared to control. In Mexico, dose 2 volume was used with 0.5, 1 or 2 mM DMNB-T6P.

Per 7 m^2 plot	DMNB-T6P per plot (g) for 1 mM final concentration	DMSO per plot (ml)	Water per plot (ml)	Tween 20 per plot (ml)
Dose 1 (year 3, 4 only) Total volume 220 ml	0.167	3.60	216	0.22
Dose 2 (year 1-4) Total volume 438 ml	0.332	7.3	430	0.44
Dose 3 (year 1-4) Total volume 656 ml	0.50	10.9	644	0.66
Control (year 1-4) Total volume 547 ml	0	9.1	538	0.54

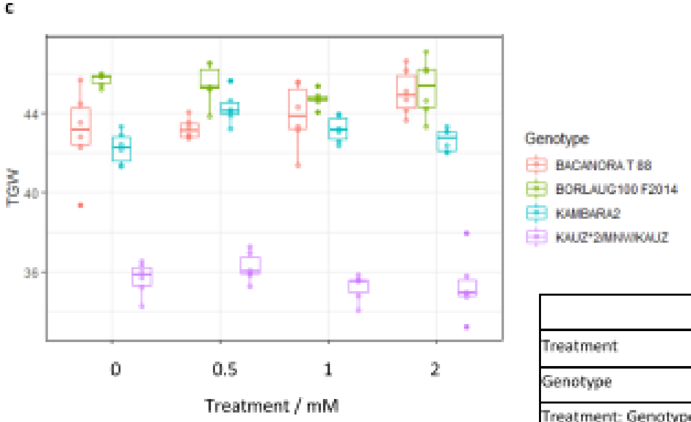




	Residual df	F statistic	p-value
Treatment	15	2.53	0.097
Genotype	60	4.21	0.009
Treatment: Genotype	60	0.57	0.814



	Residual df	F statistic	p-value
Treatment	15	2.5	0.099
Genotype	60	8.16	<0.001
Treatment: Genotype	60	0.58	0.811



	Residual df	F statistic	p-value
Treatment	15	1.81	0.189
Genotype	60	410.41	<0.0001
Treatment: Genotype	60	3.05	0.005

Irrigated plots

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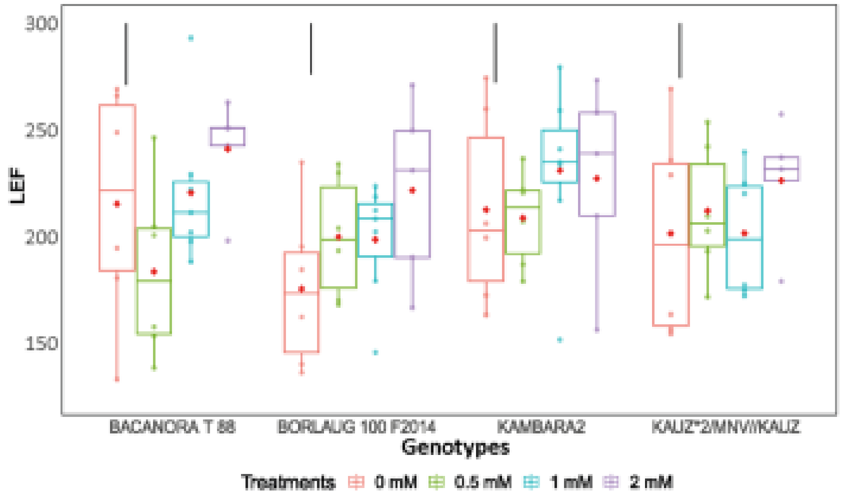


ANOVA p= 0.018

Linear election flow

Measured 3 days after DMNB-T6P application

DMNB-T6P applied 10 DAA

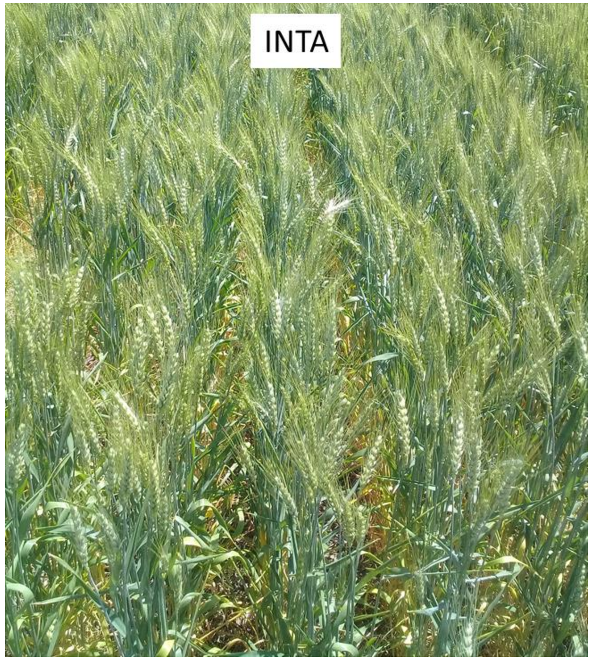
Fernando Salvagiotti

Plots 7 m long 7 rows spaced 20 cm. Central rows harvested for grain yield.

Randomised complete block design

Elite Argentinian spring wheat bread-making varieties Buck Saeta, DM Ceibo and MSINTA 415.

Phosphorus, sulphur and nitrogen fertilisation was performed using super triple phosphate (20% P), calcium sulphate (18% S) and urea were applied at planting at a rate of 100 kg ha^{-1}; N fertilization was estimated by summing pre-plant soil N test as nitrates at 0-60 cm depth (PPNT) plus N added as fertilizer to reach 140 kg N ha^{-1} as urea-ammonium-nitrate (32% N). N rates were 130, 119, 77 and 101 for year 1, 2, 3 and 4 respectively. Soil organic matter was 2.3% in year 1, 2.5% in year 2, 2.6% in year 3 and 1.9% in year 4 whereas pH was 5.5, 6.1, 5.9 and 5.8 in the four years, respectively.



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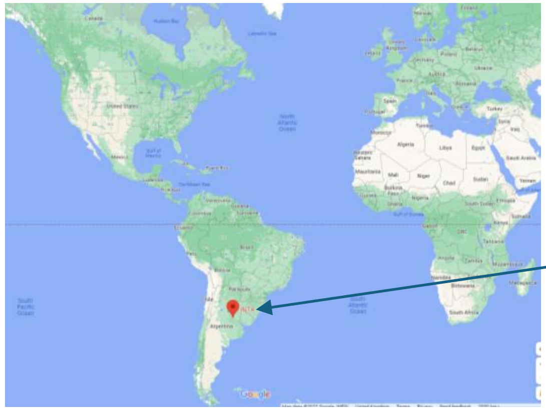
Rain-fed plots

DMNB-T6P applied 10 DAA
Except in 2021 at 16 DAA
(late delivery due to covid)

Over 4 years



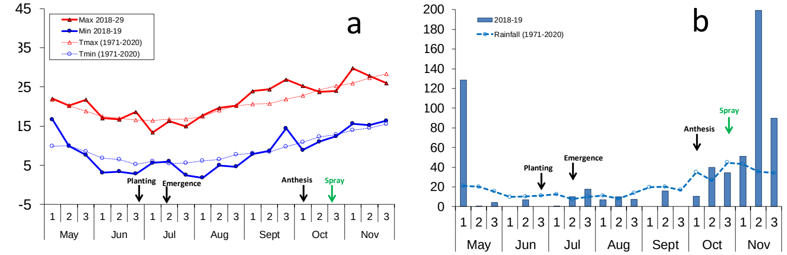
Field trials were conducted at National Institute of Agricultural Research (INTA) Oliveros Research Station, Santa Fe Argentina

Argentina has variable rainfall
(wet and dry years)
with wheat yields close
to global average
of 3.6 tonnes per ha

temperature — rainfall

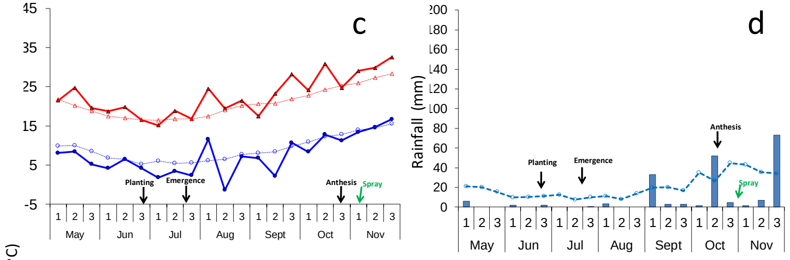
Rainfall compared to historical average

2018



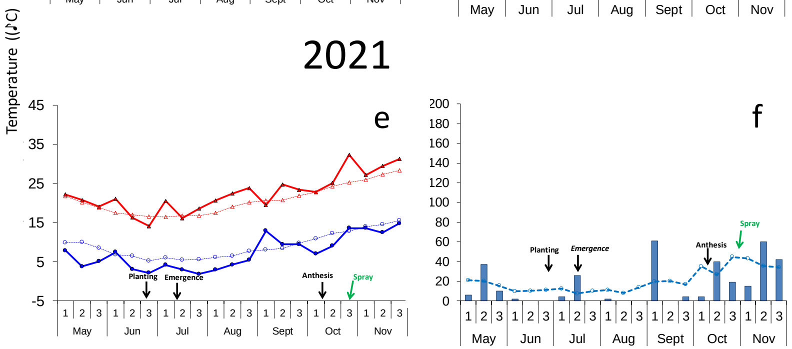
40% above

2020



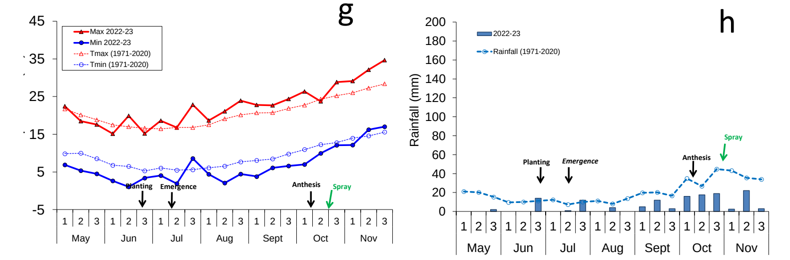
70% below

2021



26% below

2022

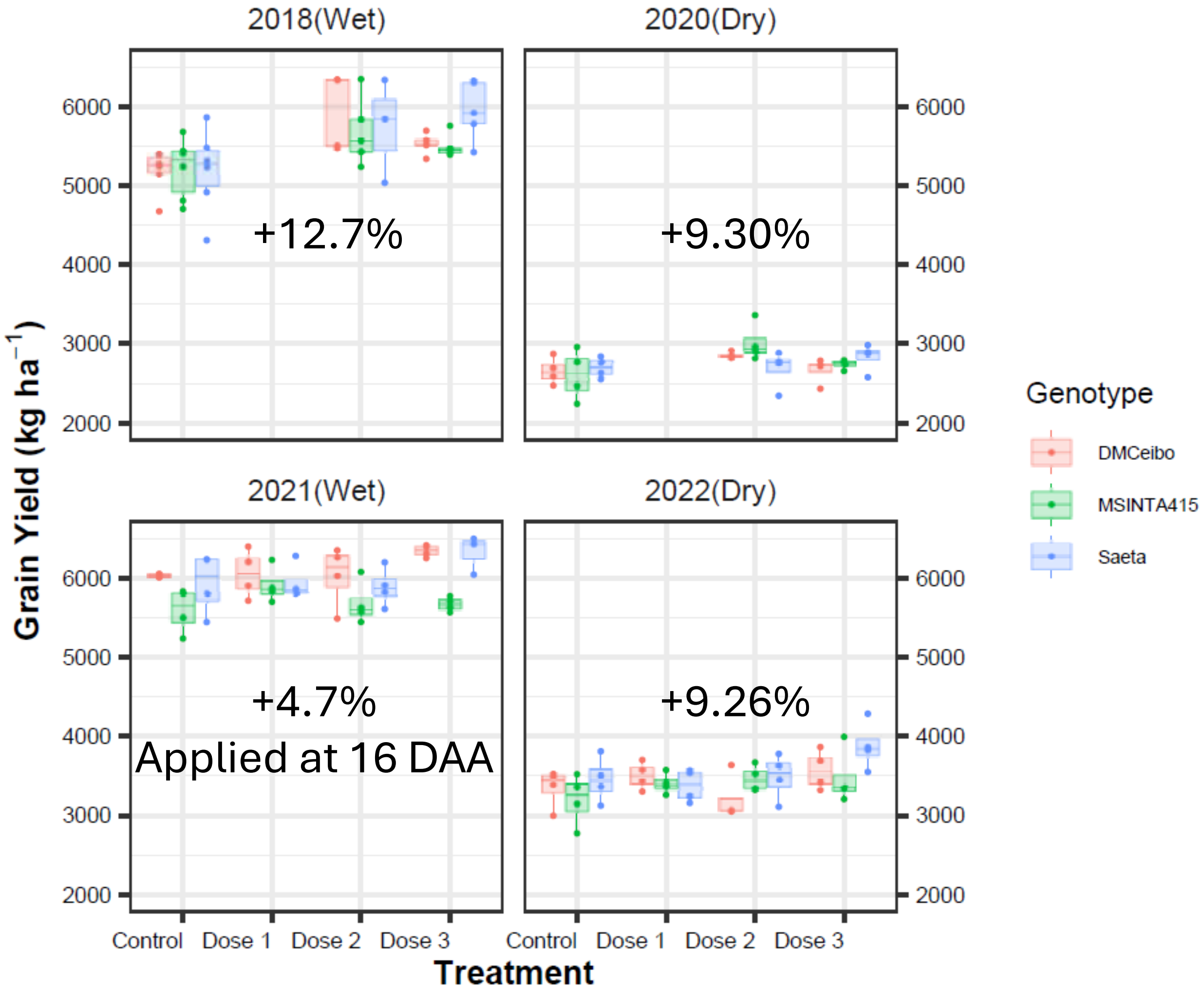


67% below

1 mM DMNB-T6P spray
applied at 10 DAA
Except in 2021 (16 DAA)
At different volumes (doses)

Window of 10-16 DAA
for application
of DMNB-T6P

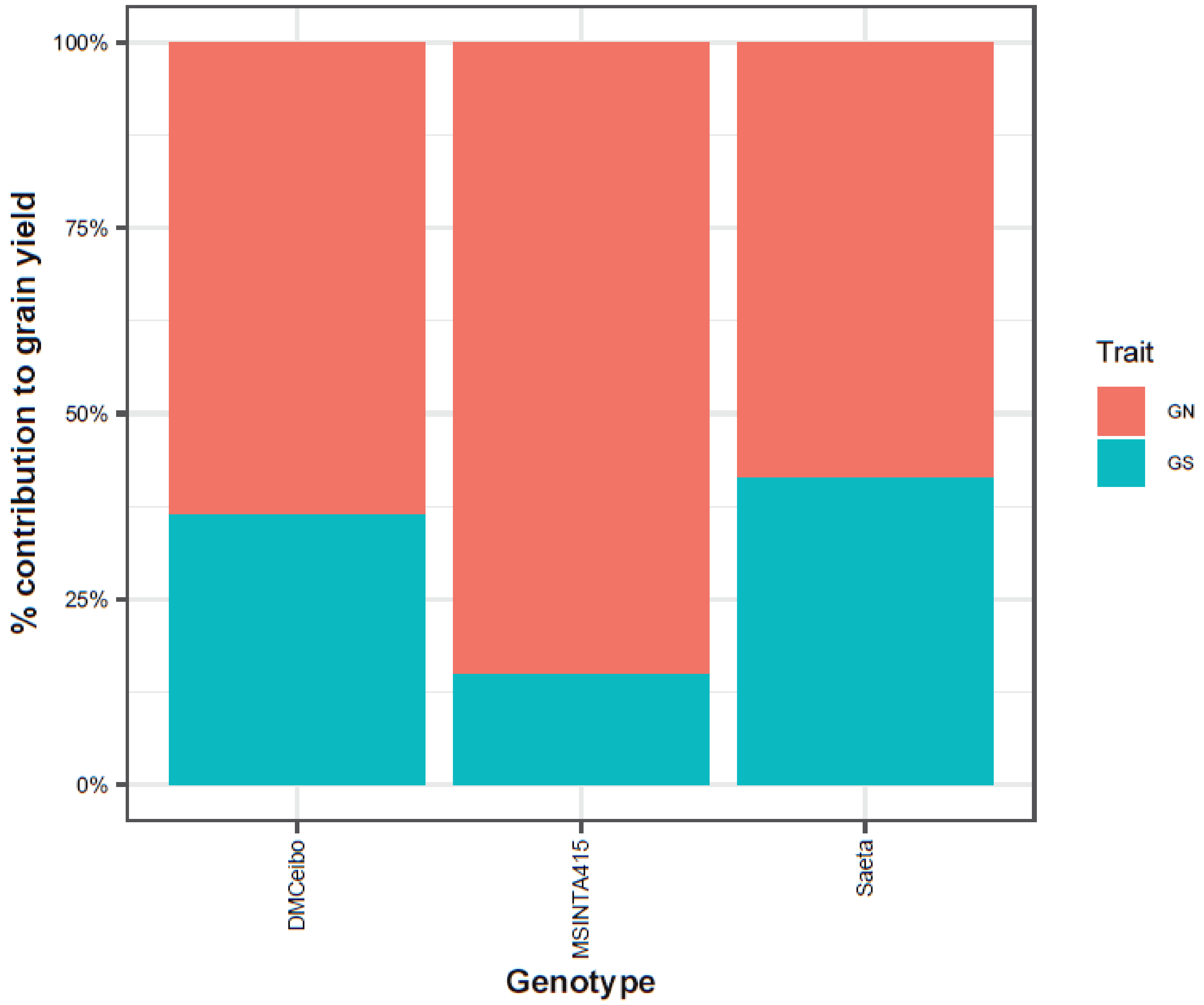
Average for 10 DAA treatment
= +10.4% yield increase



Year	Residual df	Treatment	Genotype	Genotype:Treatment
2018	34	$p = 0.00011$	$p > 0.1$	$p > 0.1$
2020	24	$p = 0.065$	$p > 0.1$	$p > 0.1$
2021	33	$p = 0.035$	$p = 0.00014$	$p > 0.1$
2022	33	$p = 0.010$	$p = 0.092$	$p > 0.1$

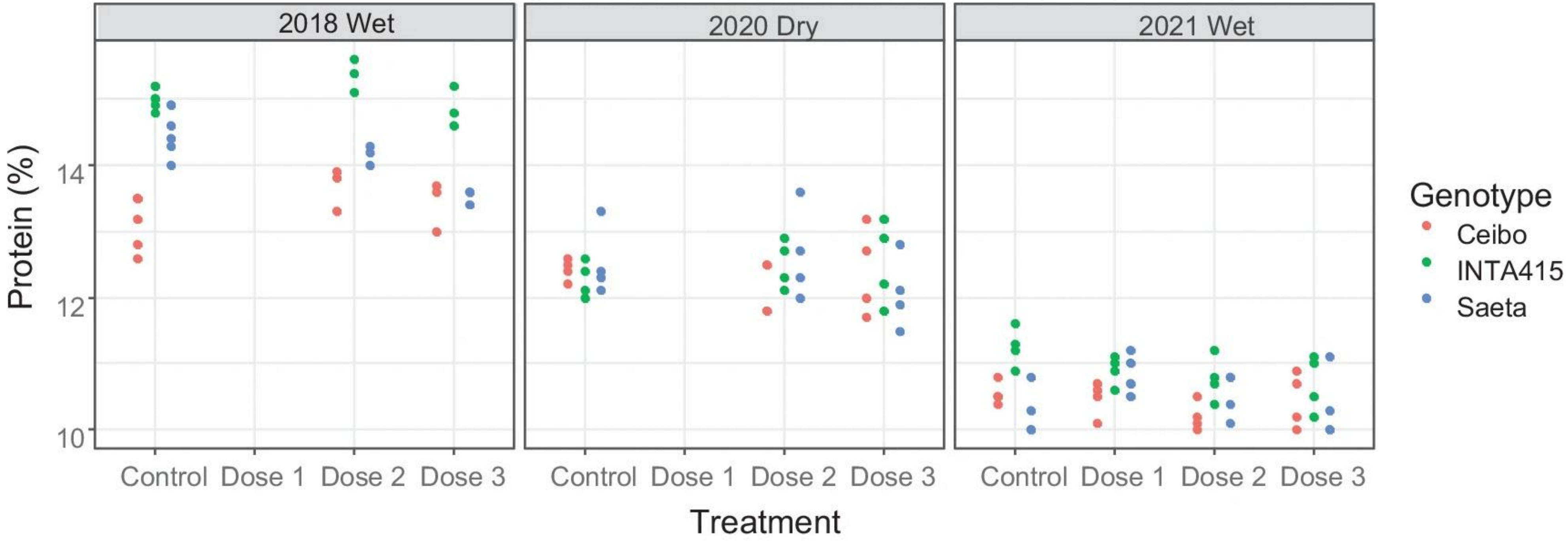
Combined analysis over all 4 years
using a mixed model framework fitted
with restricted maximum likelihood
$p<0.001$

Both grain number and grain size contribute to yield increase with DMNB-T6P



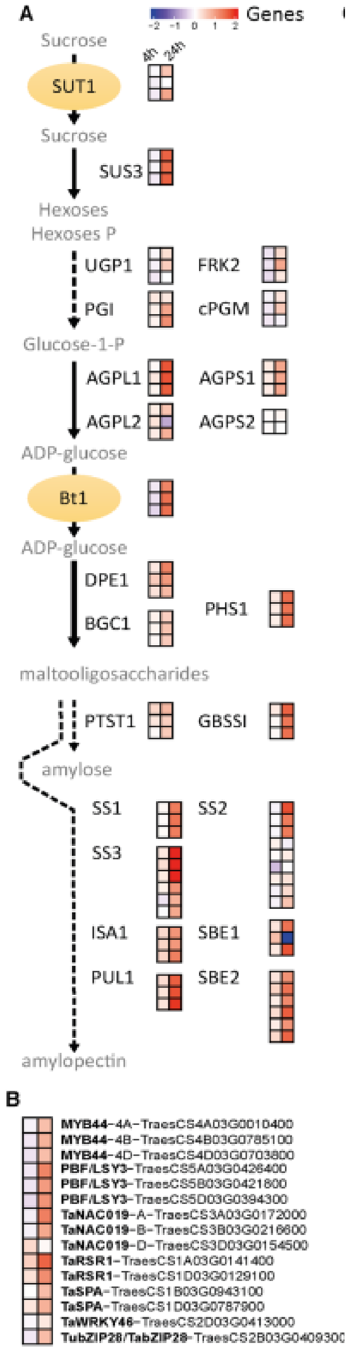

control
+DMNB-T6P
control
+DMNB-T6P
+16%
more yield
Grain number
+13%
more yield
Grain size

Grain protein was not diluted in the higher yielding crop



RNAseq

Starch and protein biosynthetic pathways massively upregulated by DMNB-T6P in grain

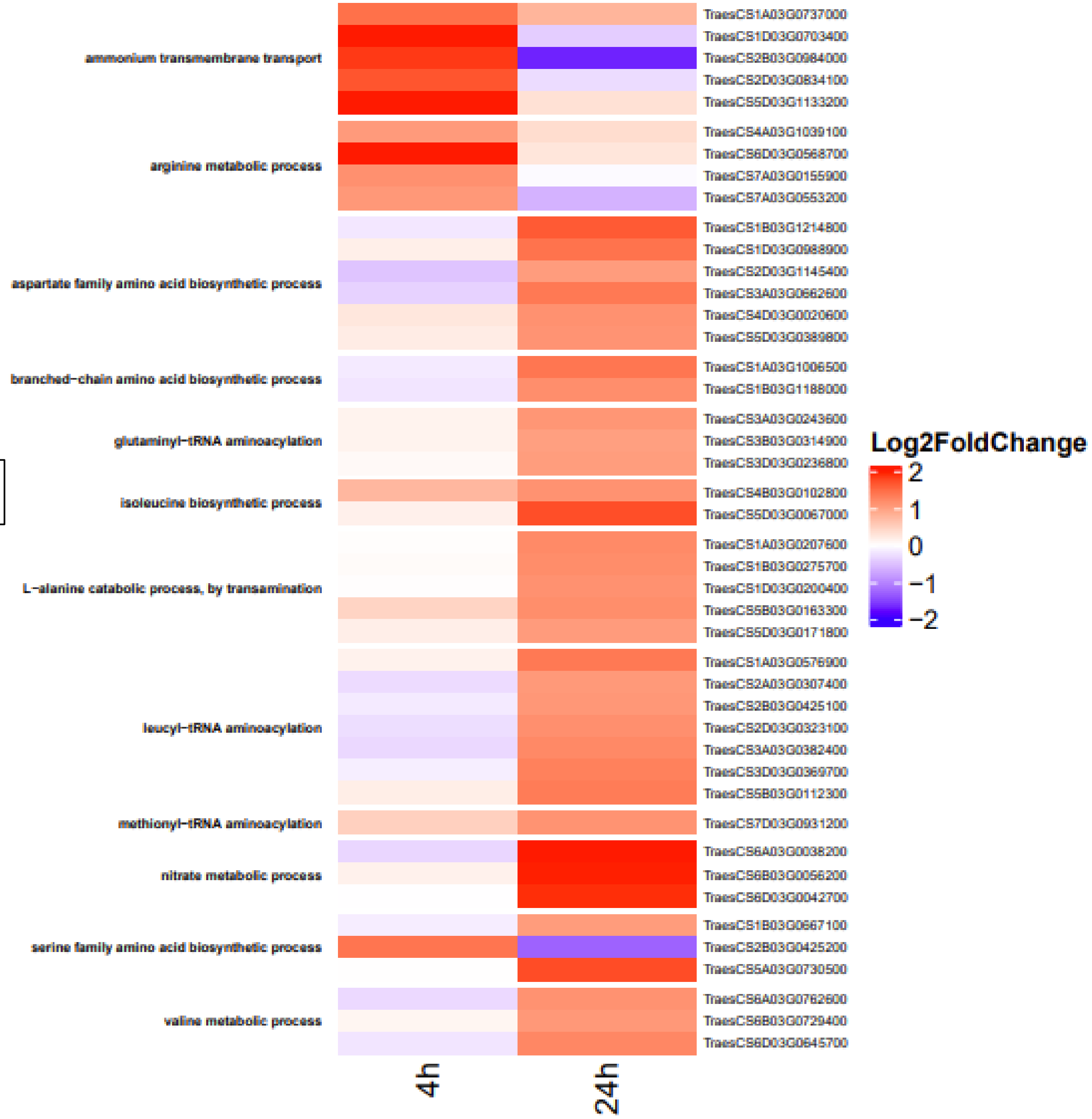


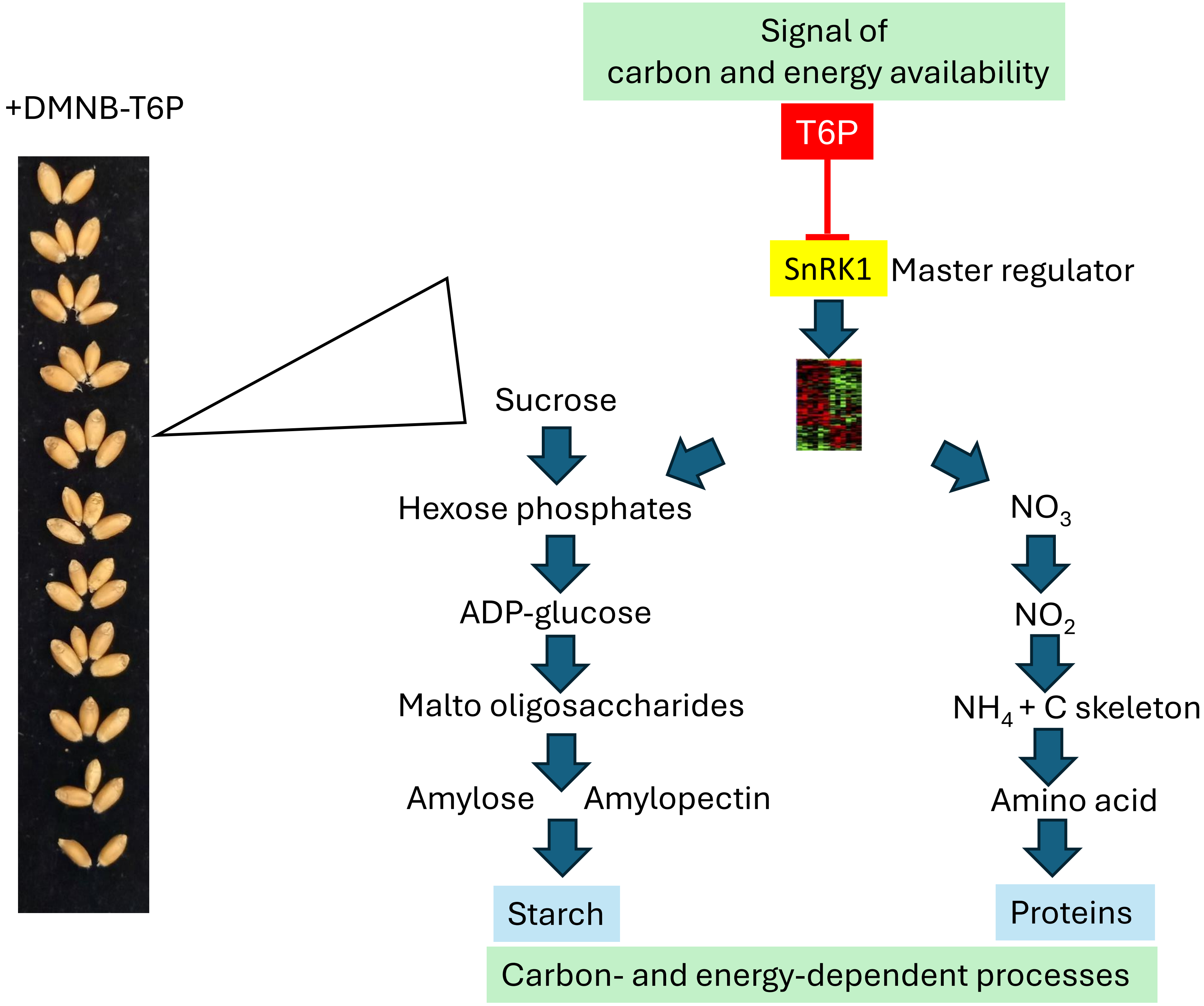
80 genes
15 TFs

34 genes


Javier Miret

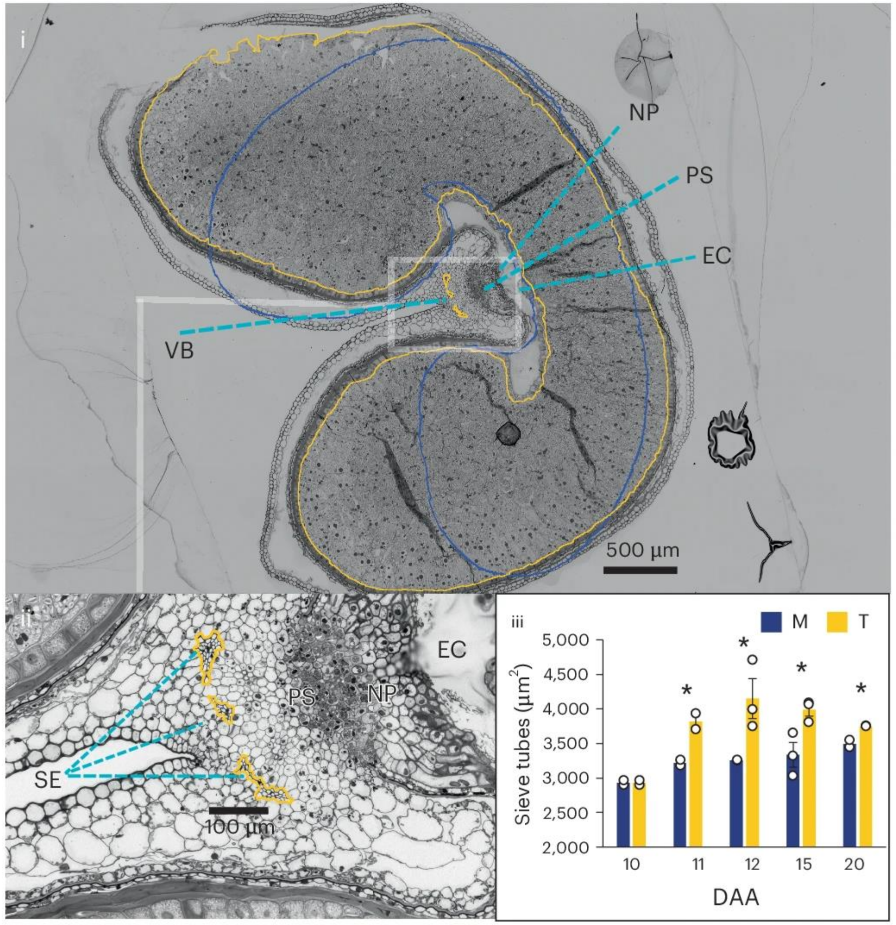

Swati Puranik

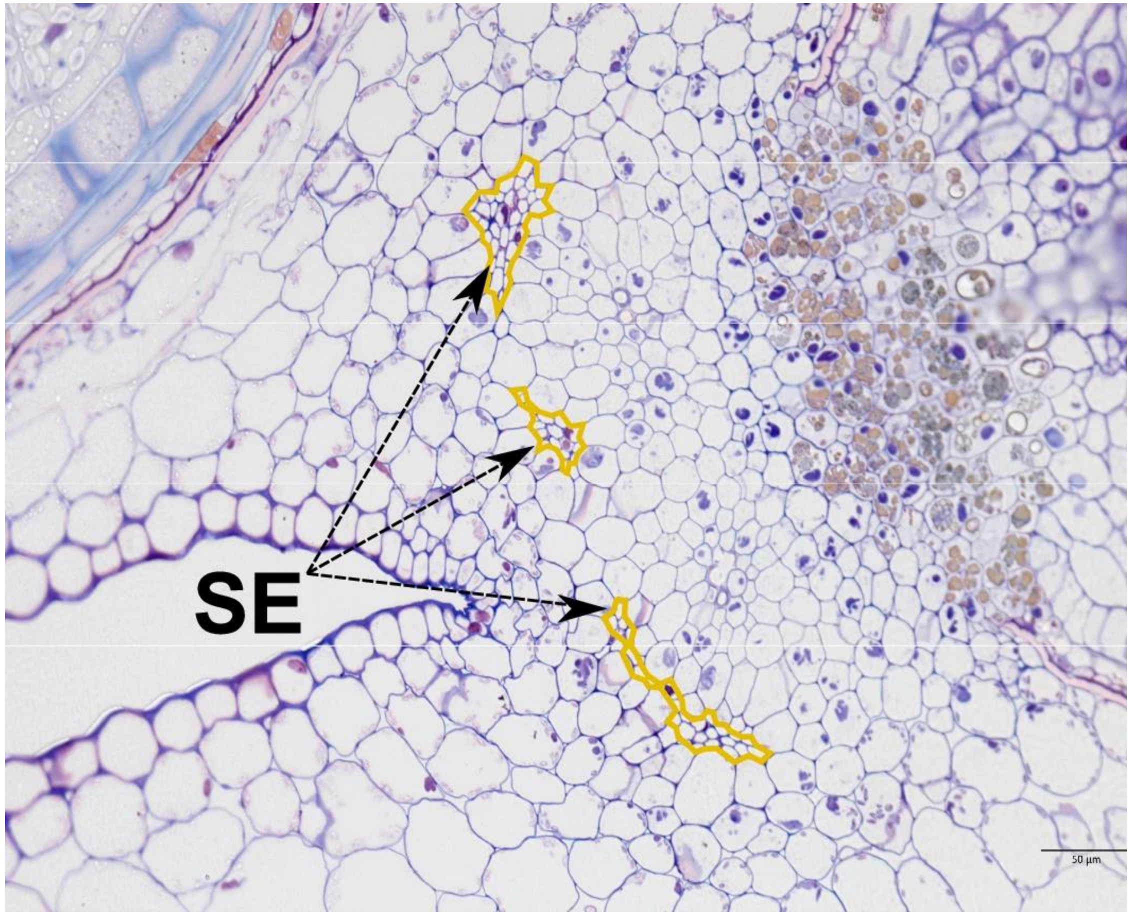




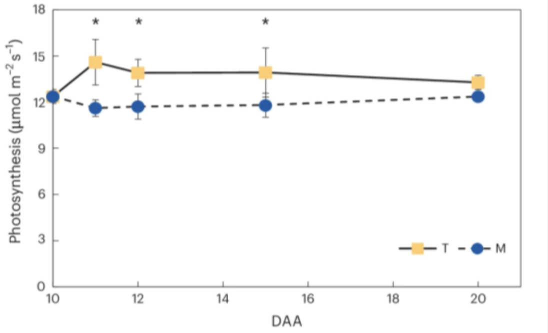
+DMNB-T6P
Signal of
carbon and energy availability
T6P
SnRK1
Master regulator
Sucrose
Hexose phosphates
ADP-glucose
Malto oligosaccharides
Amylose
Amylopectin
Starch
NO_3
NO_2
NH_4 + C skeleton
Amino acid
Proteins
Carbon- and energy-dependent processes

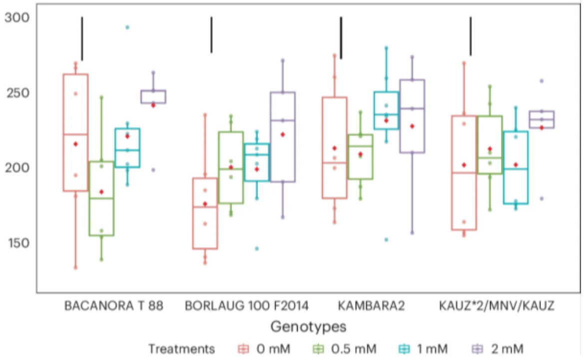
T6P enhances sieve tube development in grain



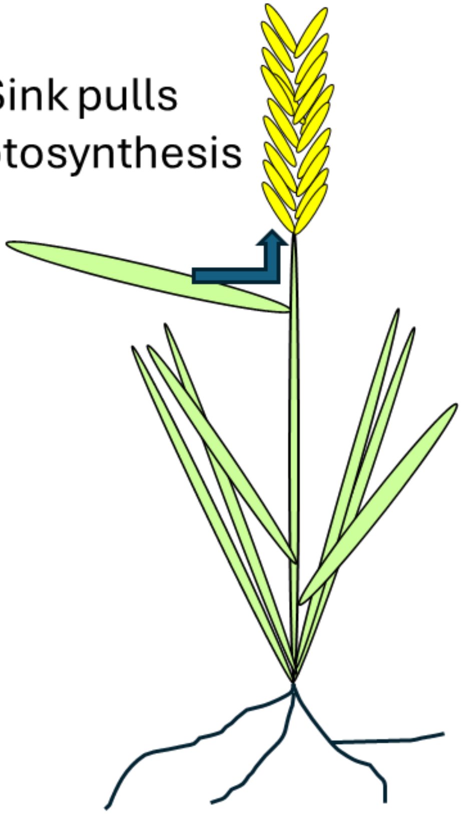


Increase in photosynthesis 10-20 DAA
Enough to increase C flow to starch and yield
Short enough period to restrict water loss through open stomata





Sink pulls photosynthesis



Summary of main findings

- Wheat yield increased in wet and dry years, average +10.4%

(Wheat yield change globally over course of study (2018-2022) +0.49% per year)

- Yield potential and resilience combined
- No need for extra fertiliser

Sustainable yield increases per unit water and fertiliser

Overcoming recalcitrant trade-offs

- Protein content of grain not diluted in higher yielding grain
- Both grain size and number increased

Points of interest

- T6P can deliver more yield by regulating genes already there in elite varieties
- What currently holds back starch and protein biosynthesis?
- What is currently holds back photosynthesis?

Need to understand source-sink regulation better

And regulation of starch and protein synthesis.

Starch synthesis previously thought to not limit yield

- No tradeoff between starch and protein grain number and size because T6P is a metabolic regulator that links sink with source to deliver more photosynthate to avoid competition for C between pathways within and between grain
- Resilience comes from increasing sink strength in grain and increased photosynthesis over a limited period during grain filling
- T6P pathway not yet optimized through breeding

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Article

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Food and Energy Security

WILEY

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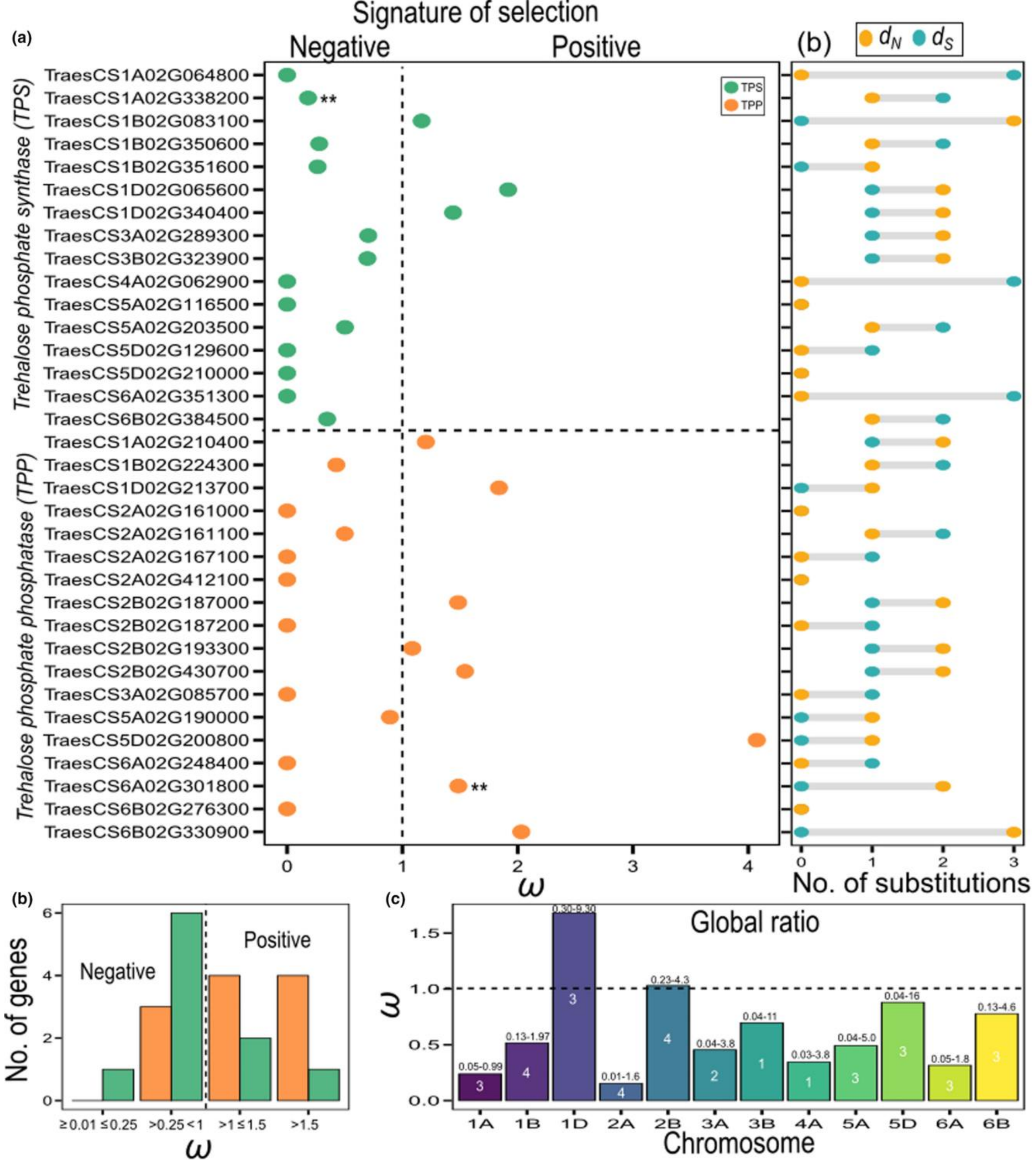


T6P pathway not yet optimised through breeding

The term dN/dS refers to the ratio of non-synonymous (changes amino acid) to synonymous substitution (no change in amino acid) in DNA sequences. It's a widely used metric in evolutionary biology/ crop breeding to understand selection acting on protein-coding genes. It helps determine if a gene is under purifying selection (where mutations are removed), or under positive selection (where mutations are favoured).

IWYP International Wheat Yield Partnership

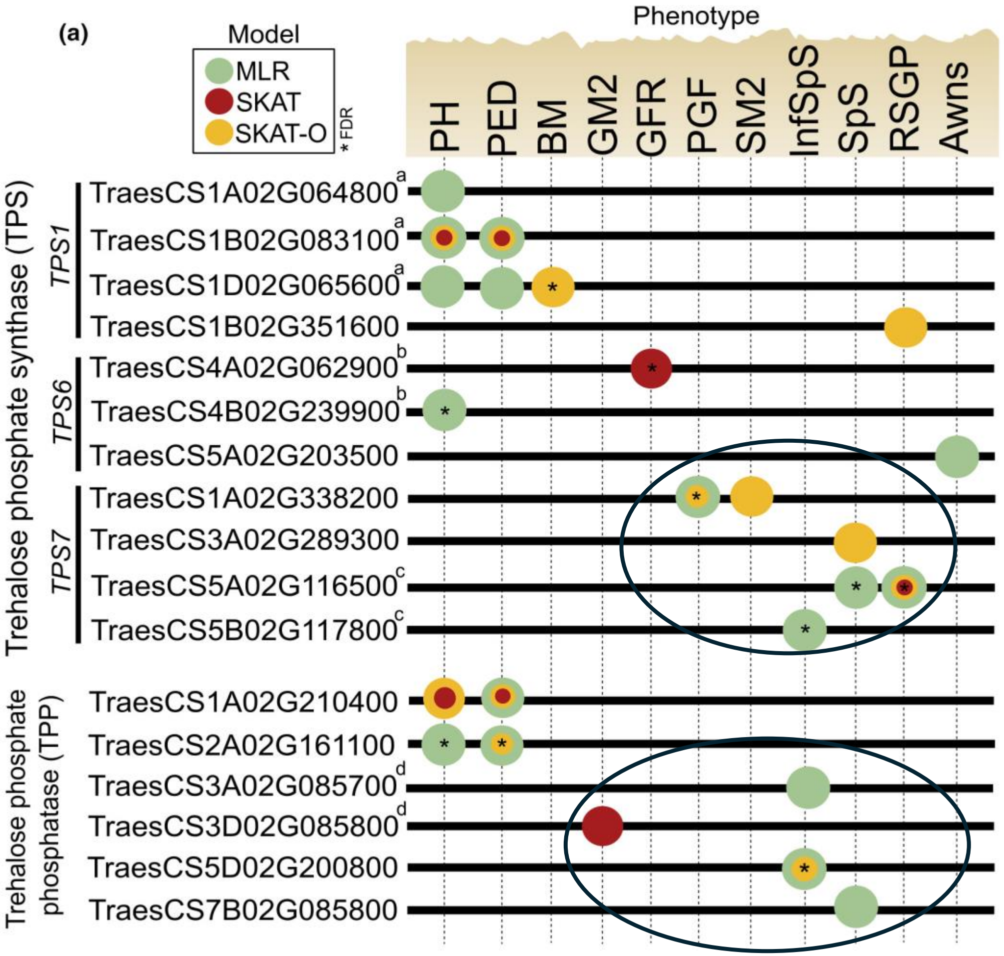
Research to Deliver Wheat for the Future

HiBAP
Represents a broad range of CIMMYT germplasm

Gene-based mapping of trehalose biosynthetic pathway genes reveals association with source- and sink-related yield traits in a spring wheat panel

Food and Energy Security, Volume: 10, Issue: 3, 07 May 2021, DOI: (10.1002/fes3.292)

Circle forms encoded by different colours represent genes detected by sequence kernel association test (SKAT), optimized SKAT (SKAT-O) and multiple linear regression (MLR) models. Gene-trait association detected using minor allele frequency (MAF) ≥ 0.01. Significance level used is Bonferroni correction (no asterisk, α = 0.05) and False Discovery Rate (with asterisk, α = 0.05). Circles overlapping each other represent multiple models detecting the same gene. Only genes and traits on which significant associations were detected are shown in the figure.



International Wheat Yield Partnership (IWYP)

Research to Deliver Wheat for the Future

T6P pathway associated with reproductive development

Gene trait associations

Reproductive traits

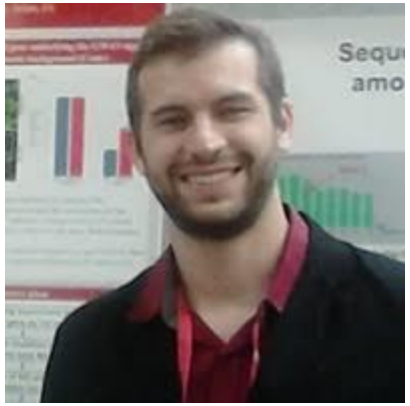
Spikelets per spike

Spikelet fertility

Spikes m^{-2}

Rapid spike growth phase (determines final grain number)

Percentage of grain filling PGF (number of days between anthesis and physiological maturity)



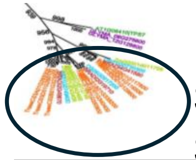
Danilo Lyra

Gene editing and Watkins collection confirm links of TPS genes to grain number determination

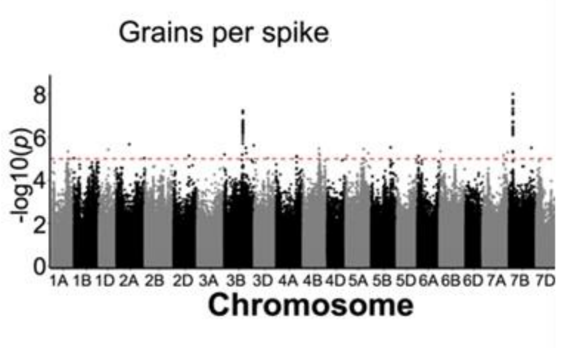
Class II TPSs spike architecture and grain number

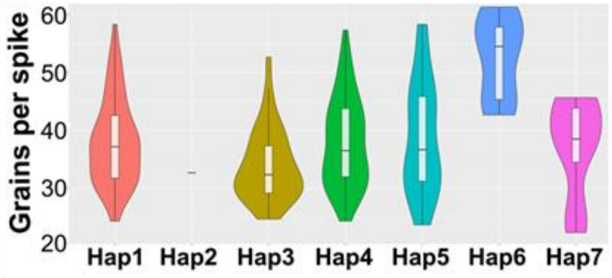
Gene editing

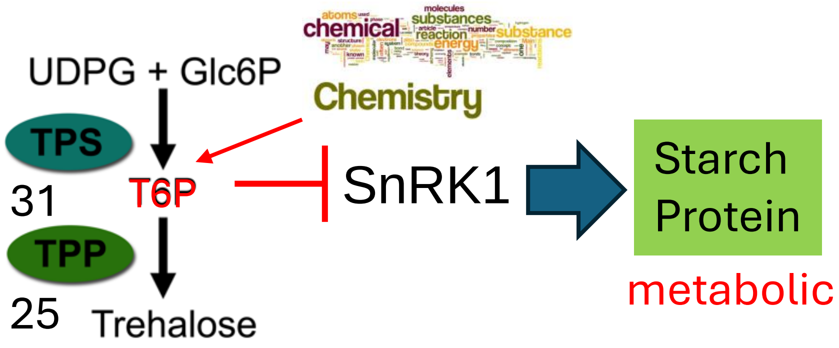
Genetic variation
Watkins TPS haplotypes

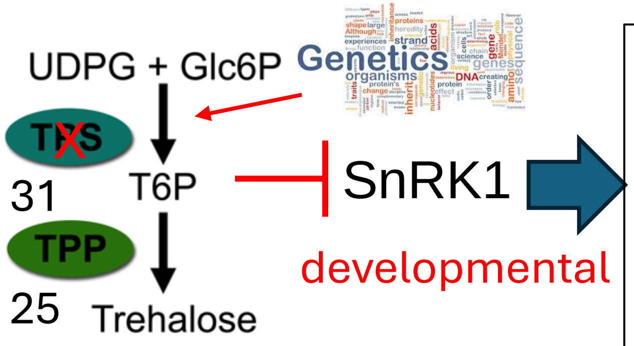


Simon Griffiths
Shifeng Cheng





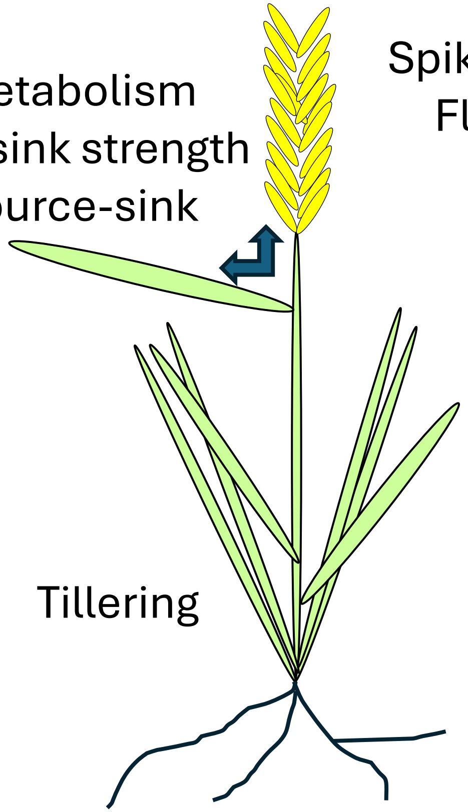




T6P regulates major crop traits from top to bottom of plant related to C allocation

Metabolism and sink strength
Source-sink

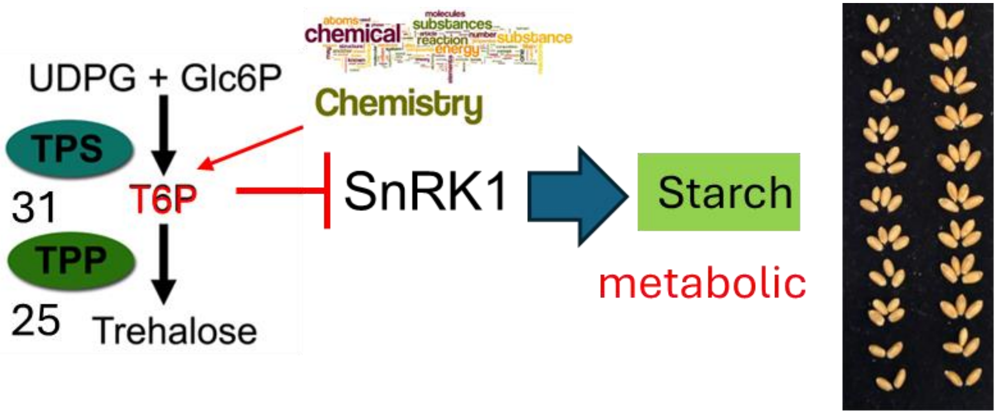
Spike development
Flowering time

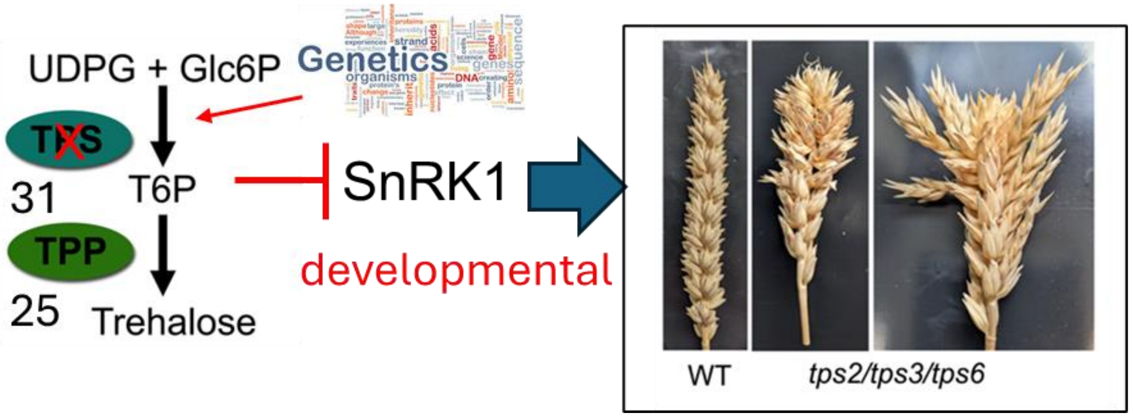


Tillering

Lateral root growth

Germination





Future goals
Understanding T6P-dependent mechanisms and how to modify crop traits chemically or genetically

Thank you to a great team!

ROTHAMSTED RESEARCH

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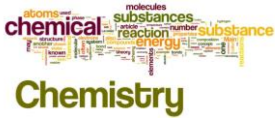


Matthew Paul


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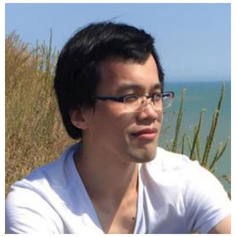
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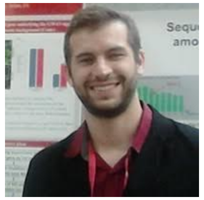
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