

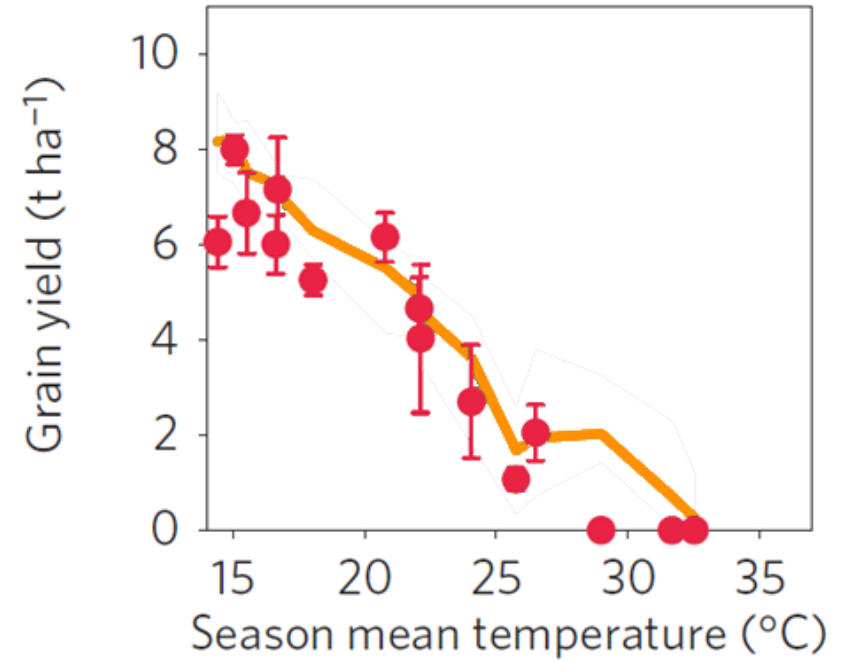
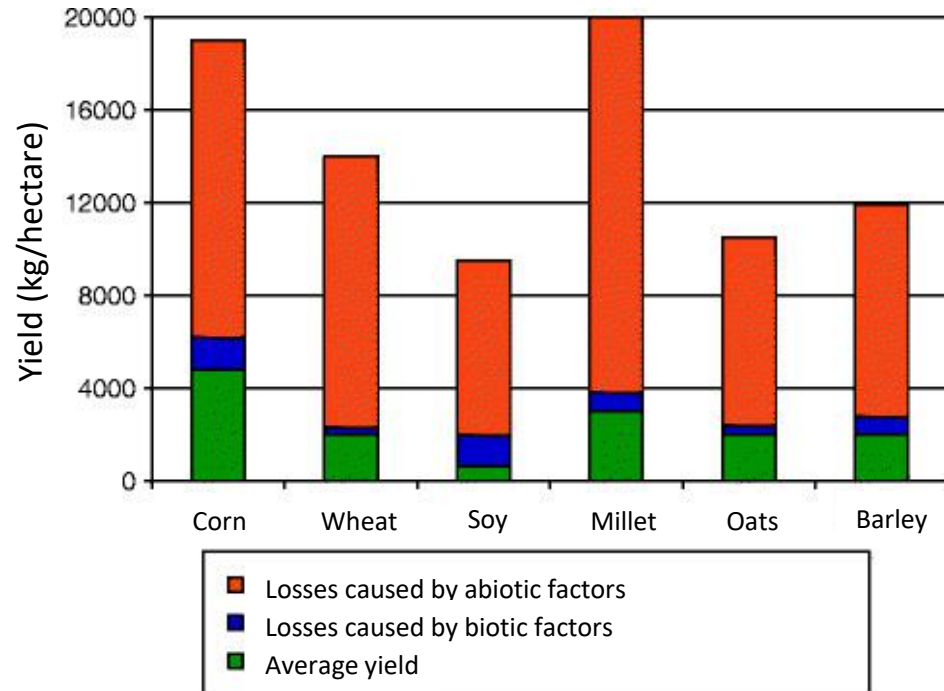


**Comparative wheat phosphoproteome
profiling pinpoints high temperature-
associated breeding markers**

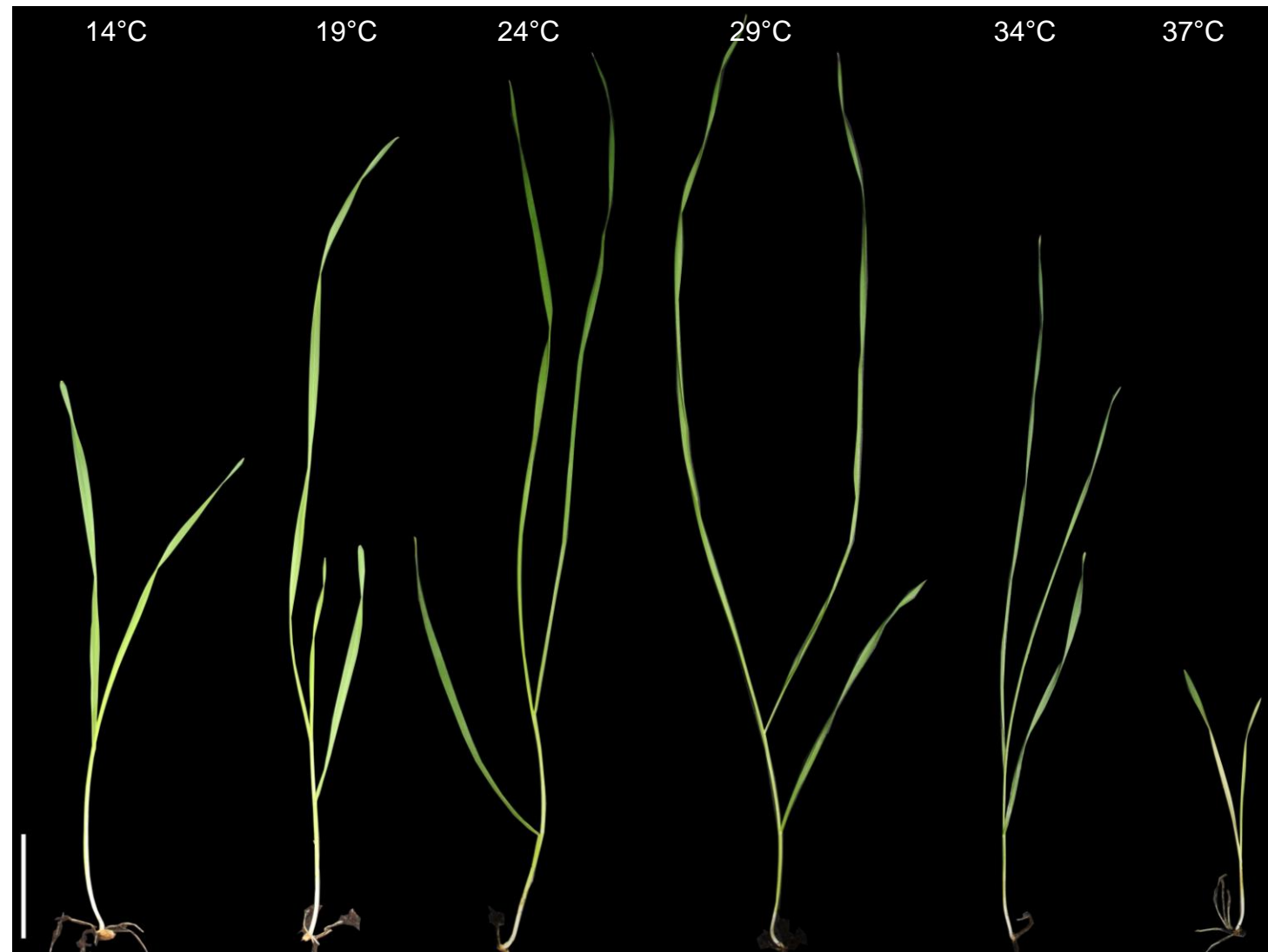
Ive De Smet

VIB-UGent Center for Plant Systems Biology

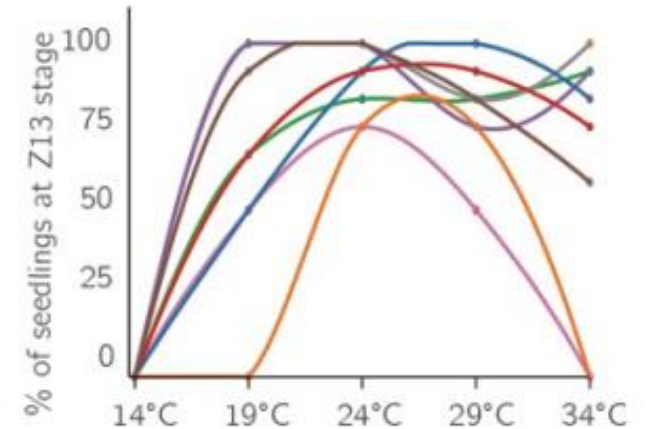
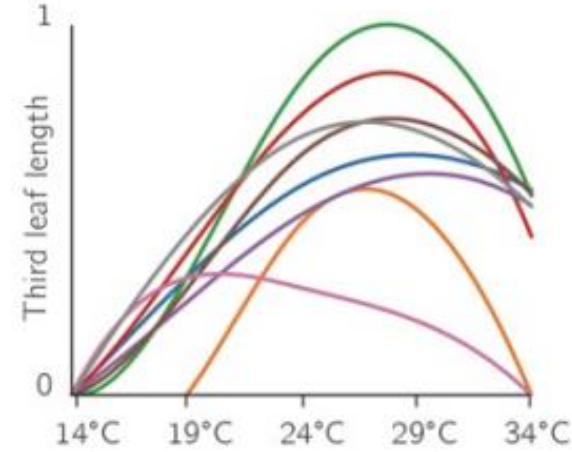
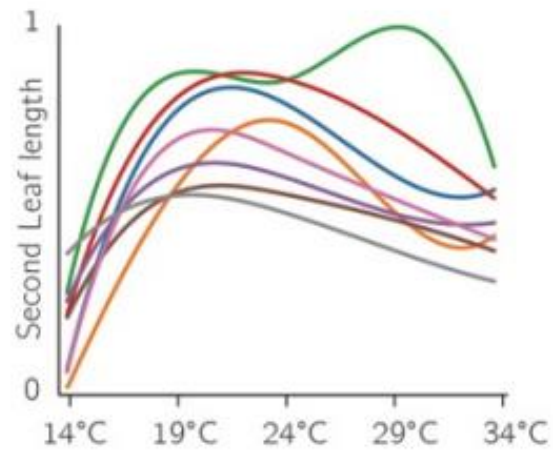
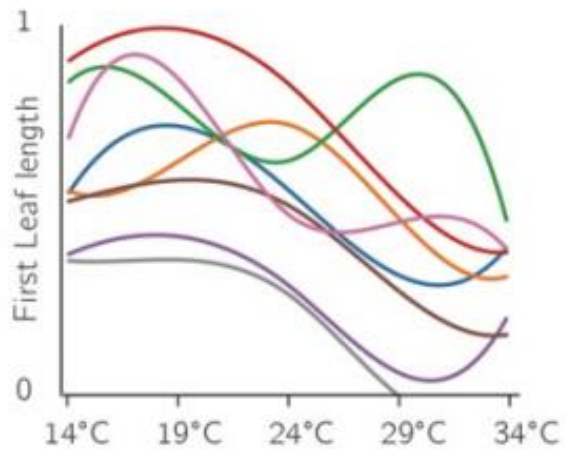
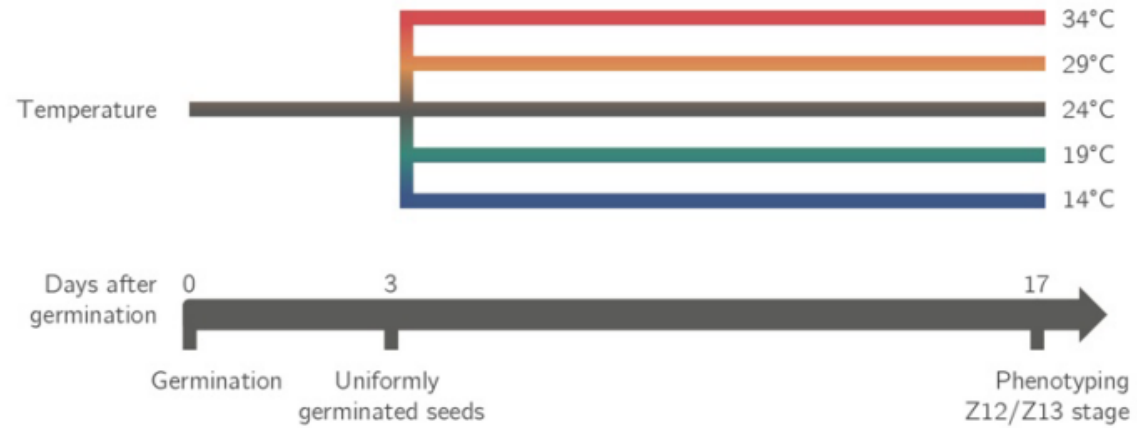
Abiotic stress impacts wheat yield



High temperature impacts wheat growth and development

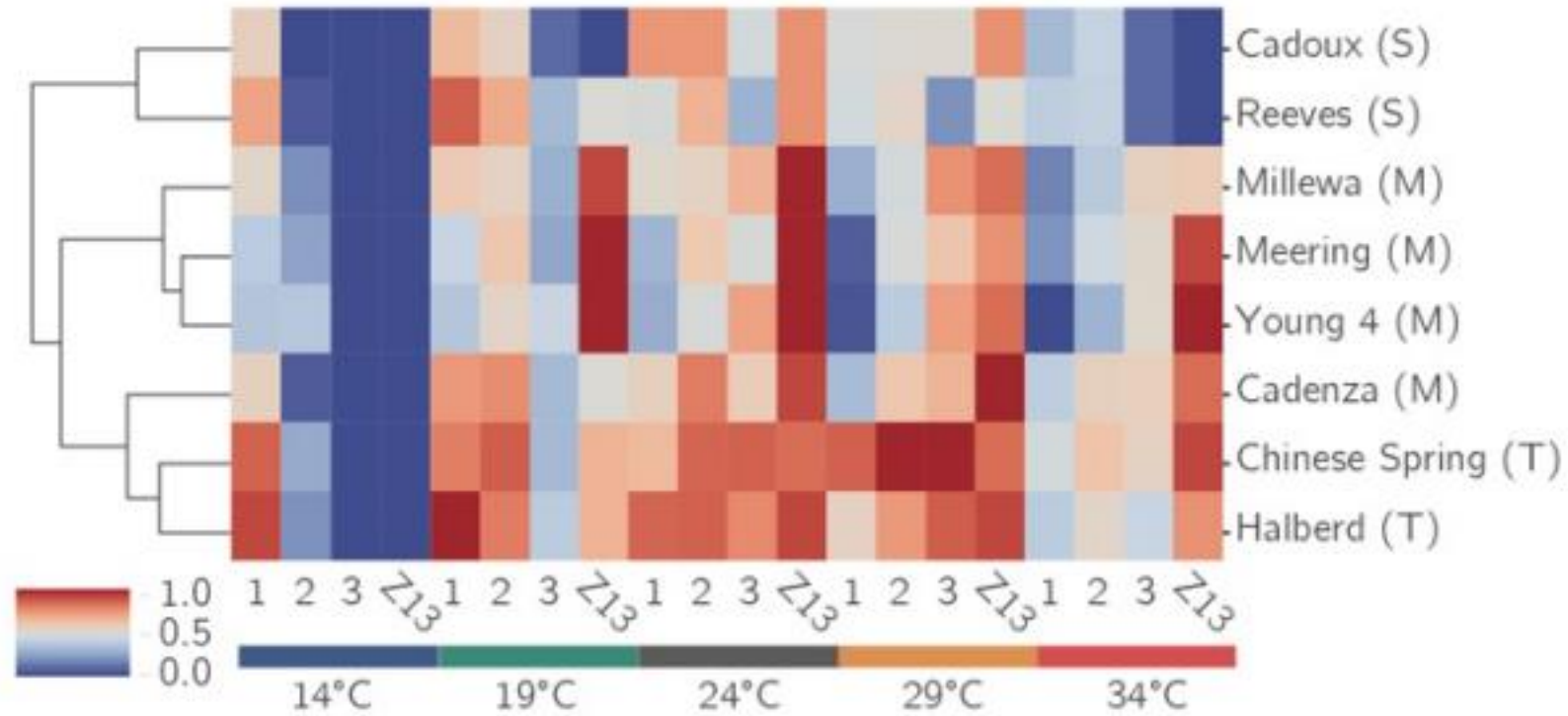


High temperature impacts wheat growth and development

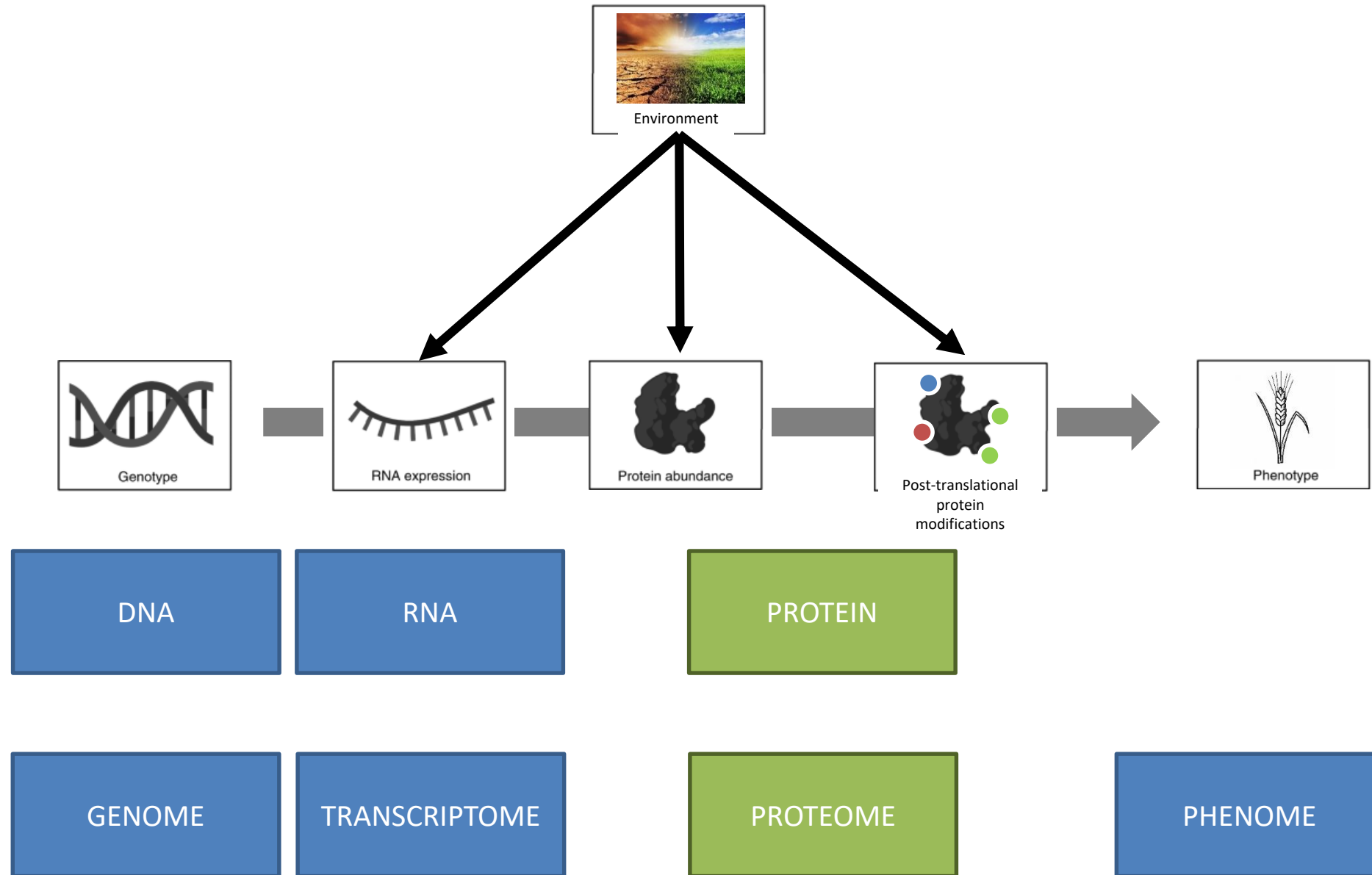


Chinese Spring (T) — Halberd (T) — Cadenza (M) — Meering (M) — Millewa (M) — Young 4 (M) — Cadoux (S) — Reeves (S)

High temperature impacts wheat growth and development



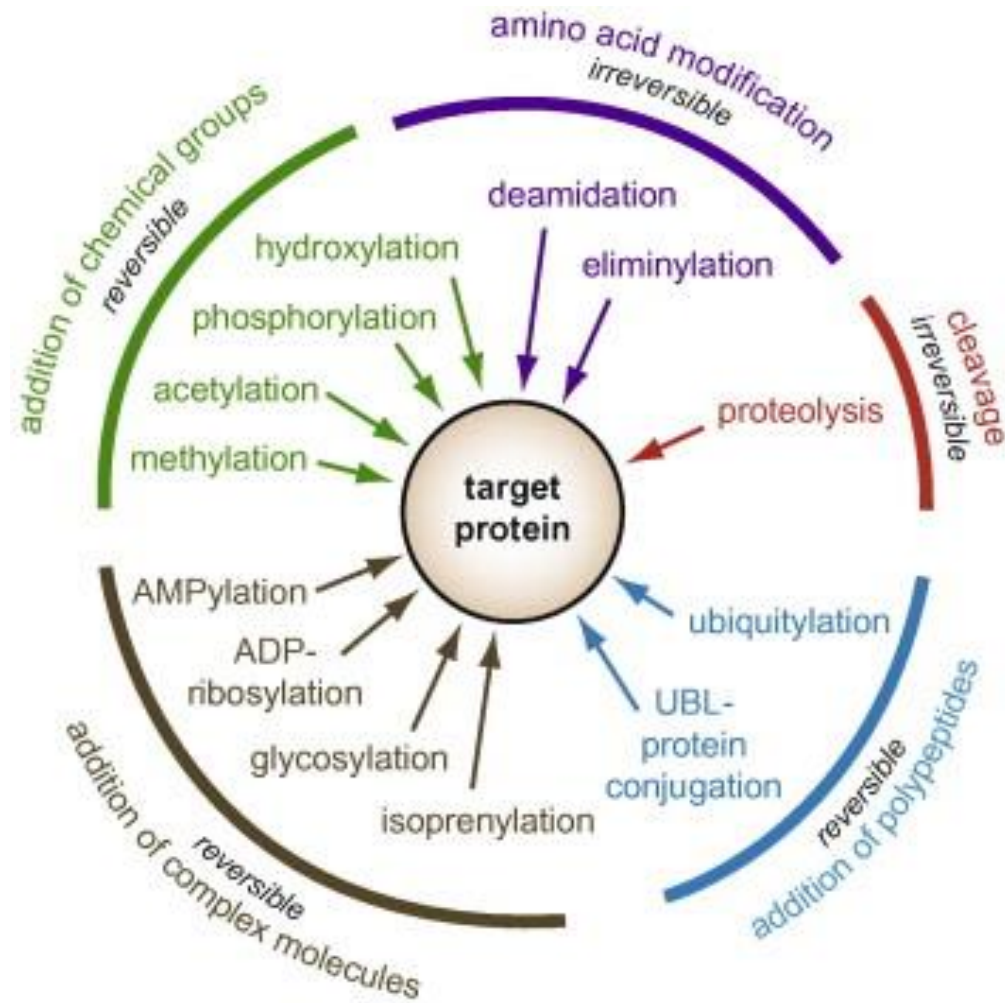
Focus on signalling in plants



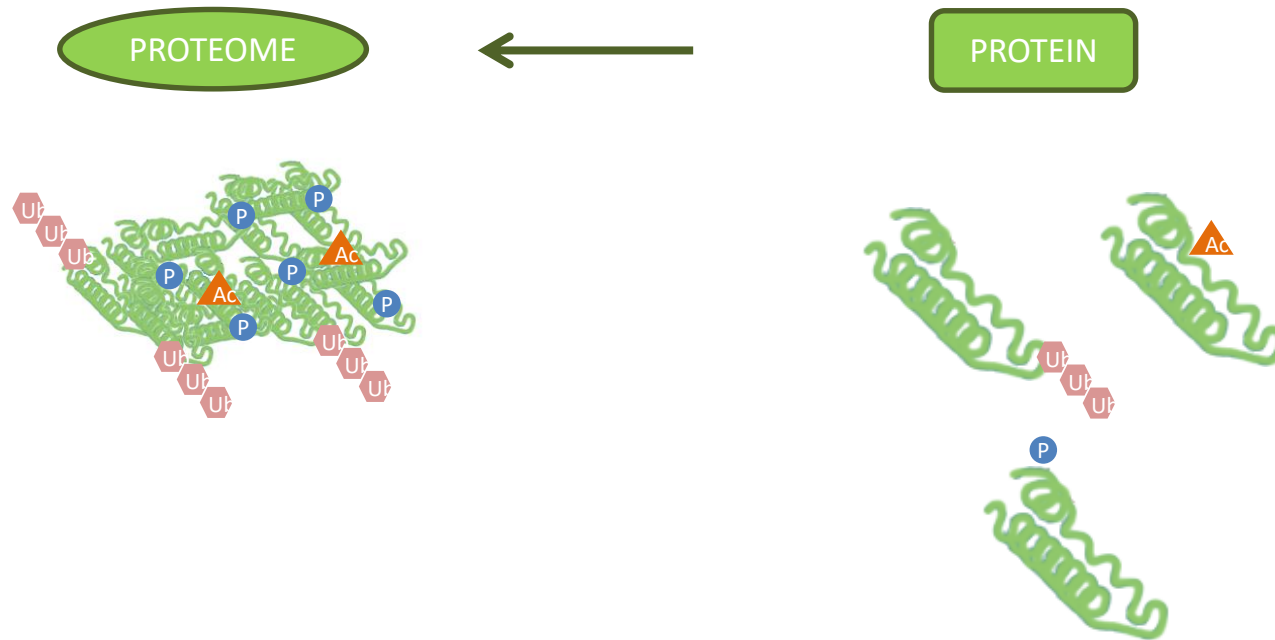
Protein pool expands through post-translational modifications



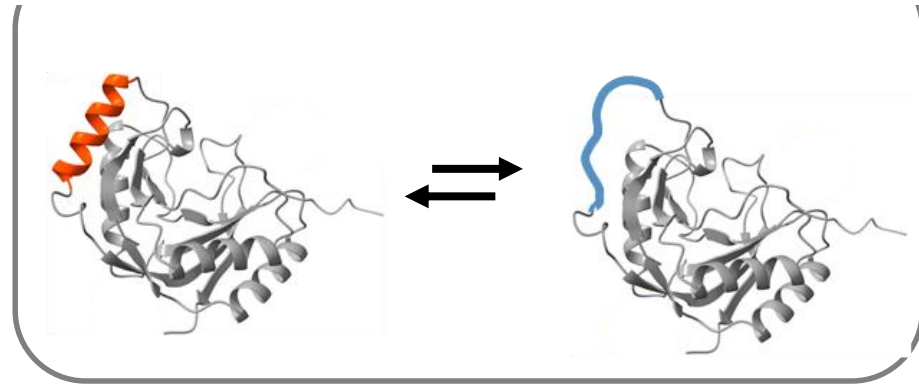
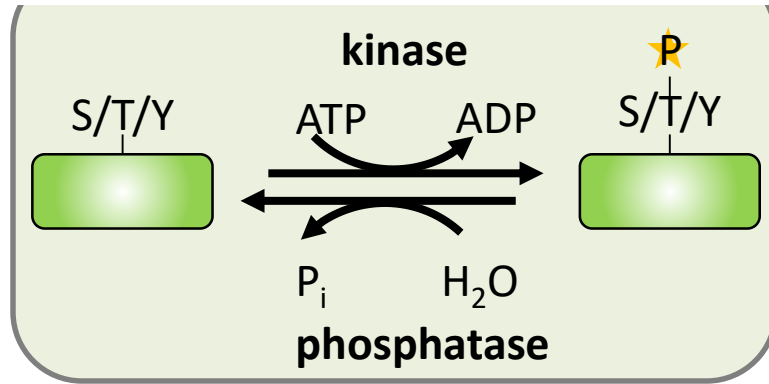
Protein pool expands through post-translational modifications



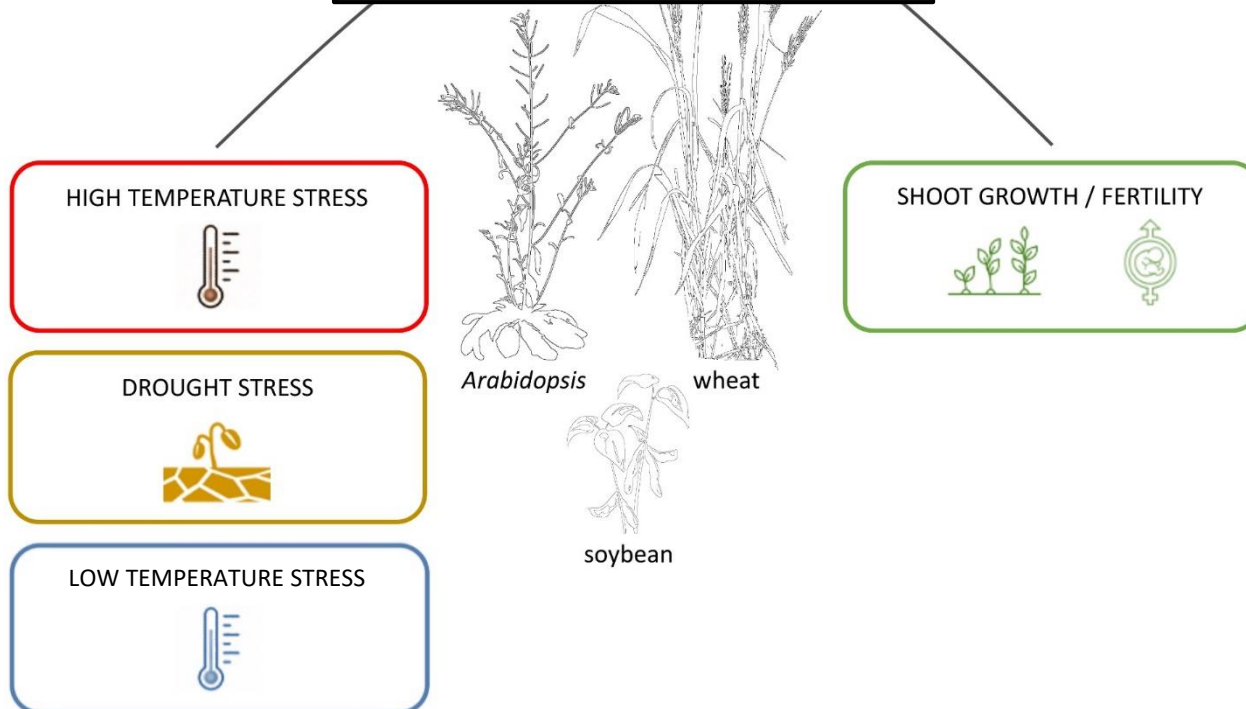
Protein pool expands through post-translational modifications



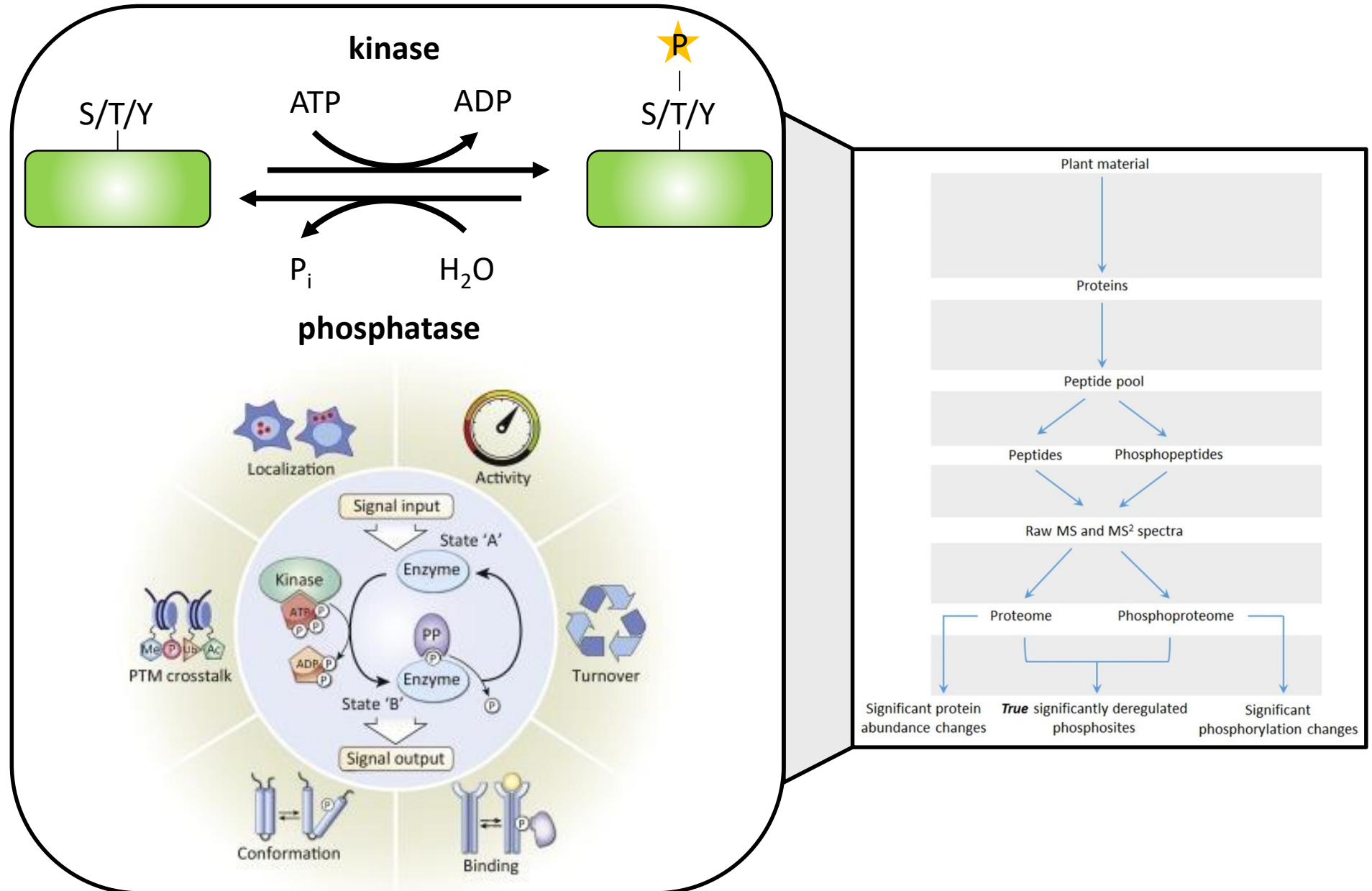
Focus on signalling in plants



POST TRANSLATIONAL PROTEIN MODIFICATIONS
(MAINLY PHOSPHORYLATION)
PROTEIN STRUCTURE



Phosphorylation is involved in everything



A resource for the community



VIB **Plant PTM Viewer**
From site-seeing to protein function

Navigation menu: N-terminus proteolysis, Methionine Oxidation, Lys-Sumoylation, Phosphorylation, Lys-Malonylation, Lys-2-Hydroxyisobutyrylation, Lys-Methylation, N-Glycosylation, N-Acetylation, Lys-Acetylation, Lys-Succinylation, Nitroxylation, Myristoylation, S-Glutathionylation, N-Ubiquitination, O-GlcNAc, Lys-Ubiquitination, Reversible Cys-Oxidation, Carbonylation

Plant PTM Viewer 2.0 From site-seeing to protein function

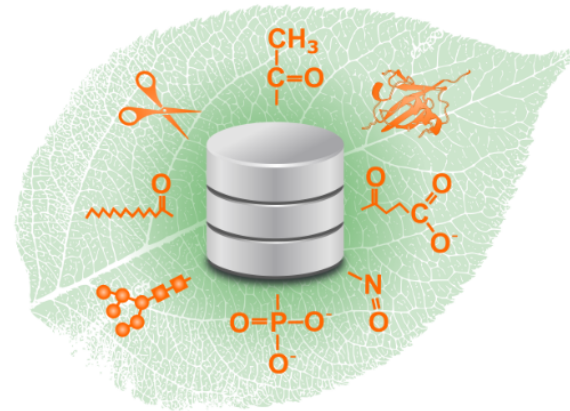
Home Search Analysis Browse Submit Data About



Peer-reviewed MS/MS studies

From site-seeing to protein function

Plant PTM Viewer



Plant PTM Viewer is a centralized resource for plant post-translational modifications (PTMs) intuitive for wet- and dry-lab scientists.

Plant PTM Viewer provides innovative tools to analyze the potential role of PTMs for specific proteins or in a broader systems biology context.

Plant PTM Viewer is an open repository and accepts newly peer-reviewed plant PTM data - for more information click [here](#)

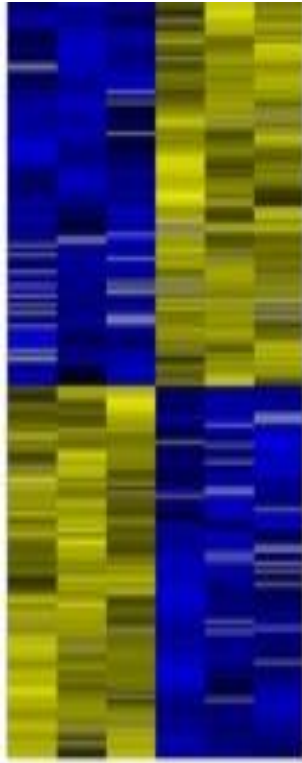
Below we provide a brief outline of Plant PTM Viewer [features](#) and current PTM data [statistics](#).

Downloadable tutorials can be found [here](#).

<https://www.psb.ugent.be/webtools/ptm-viewer/>

Discovery (and validation) in wheat / mode-of-action in *Arabidopsis*

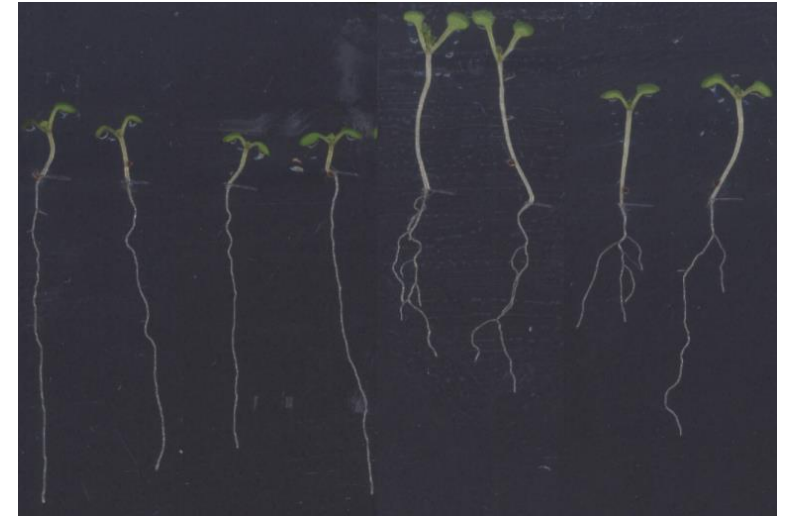
Relevant candidates in wheat



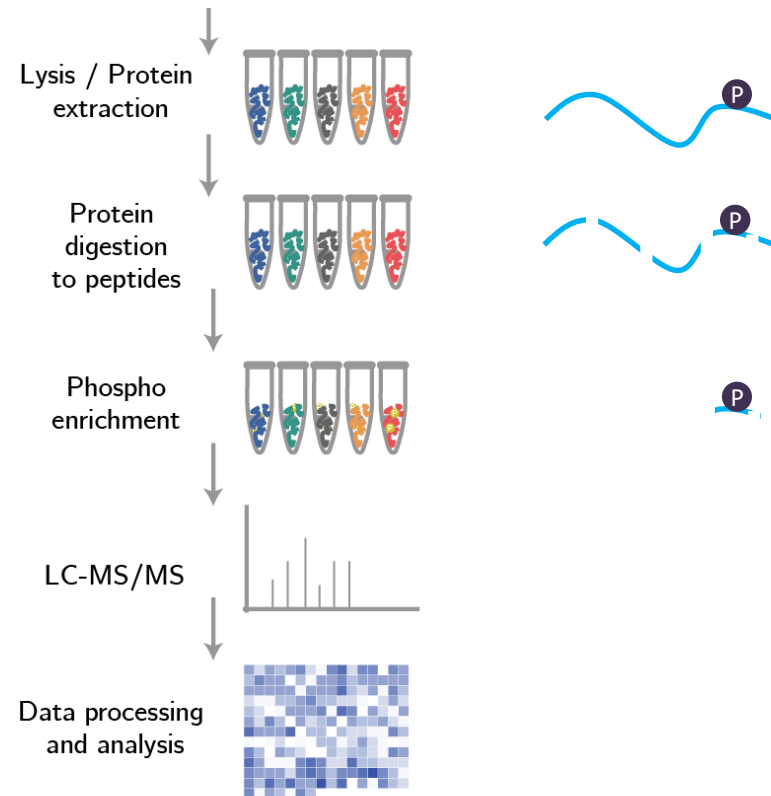
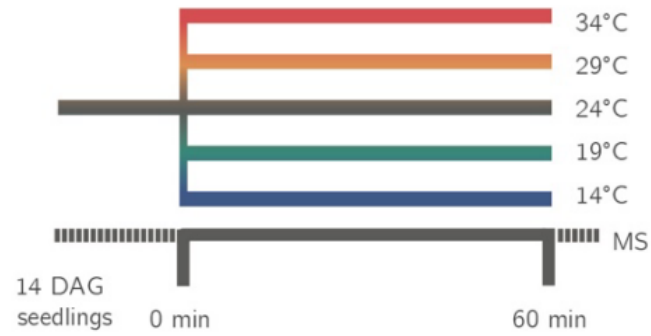
Validation in wheat



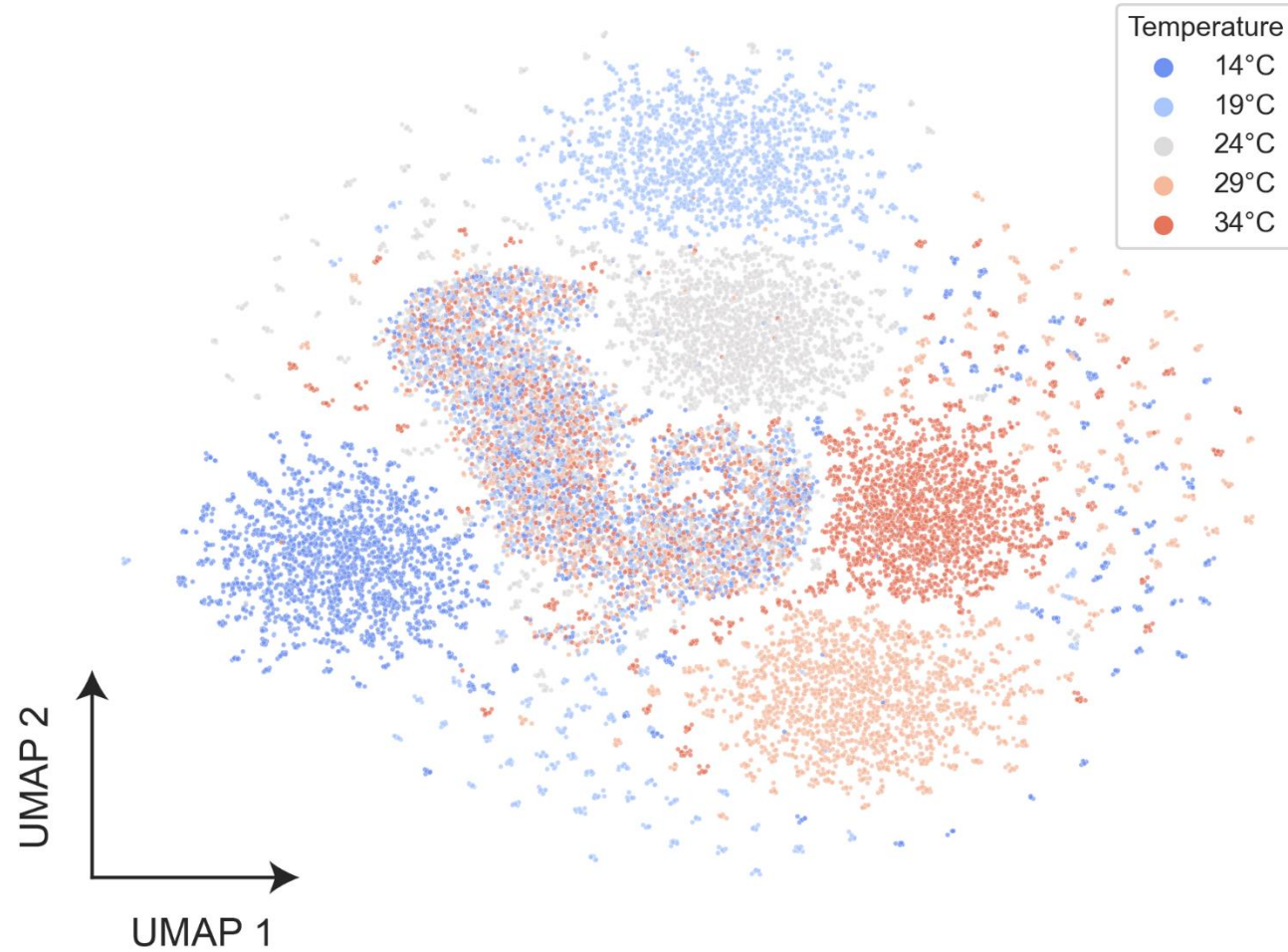
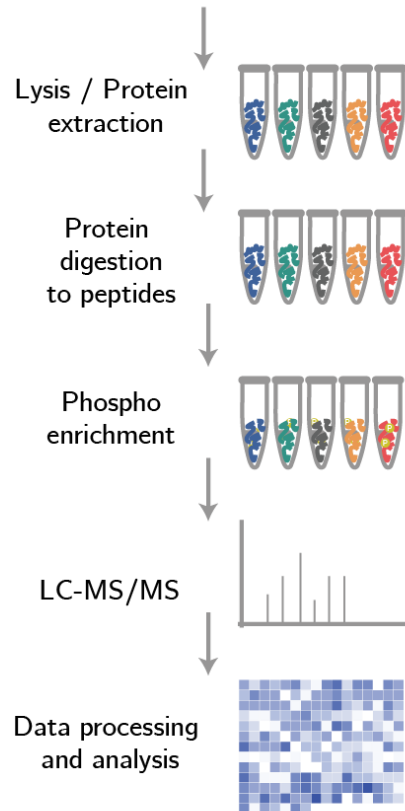
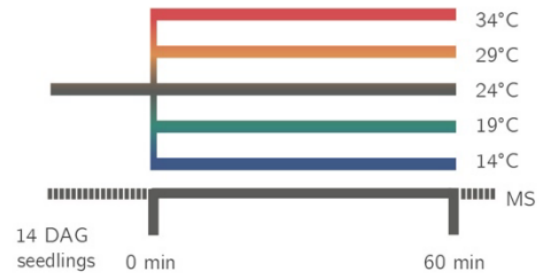
Mode-of-action in *Arabidopsis*



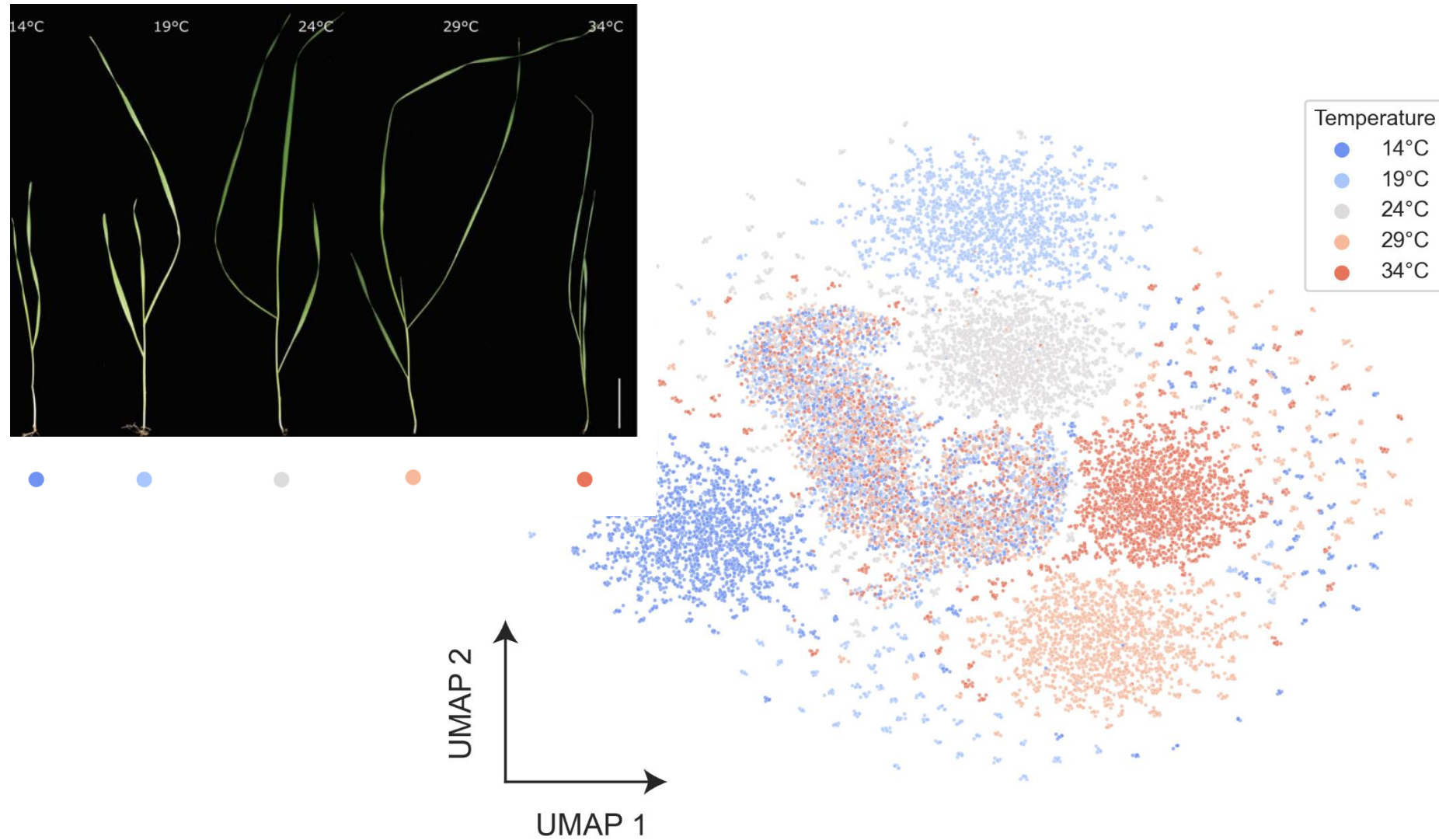
Temperature gradient phosphoproteomics in wheat



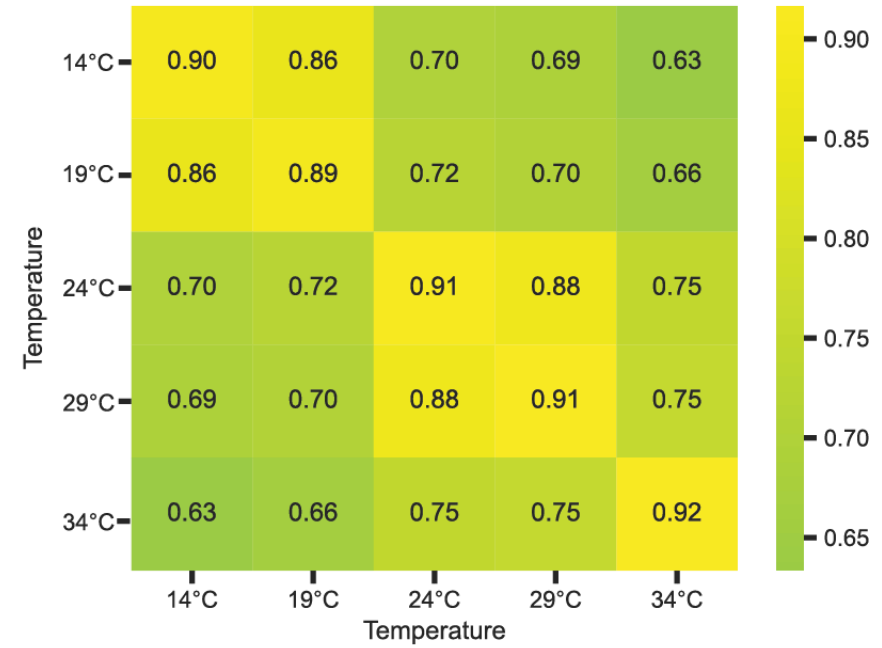
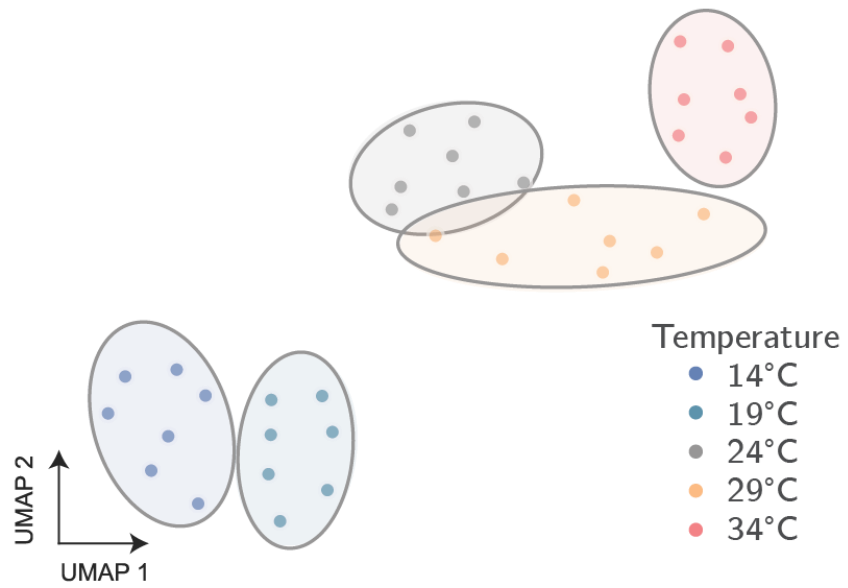
Temperature gradient phosphoproteomics in wheat



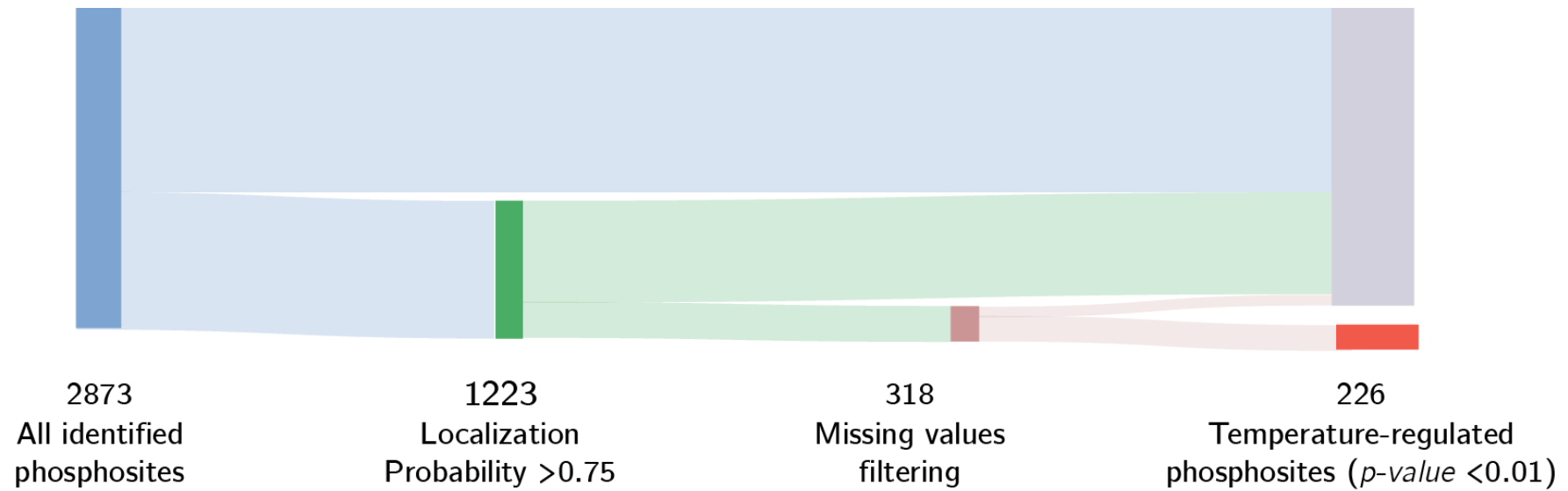
Temperature gradient phosphoprofiling in wheat



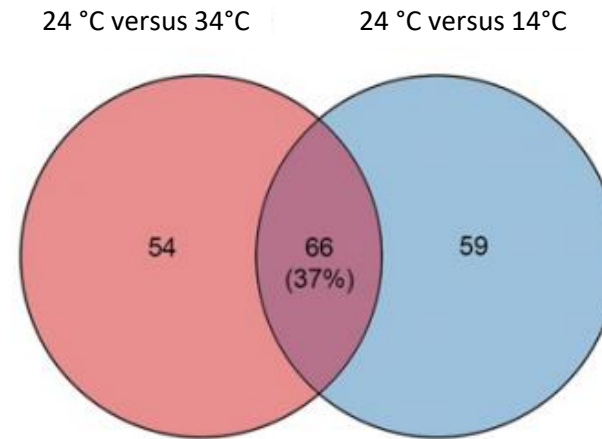
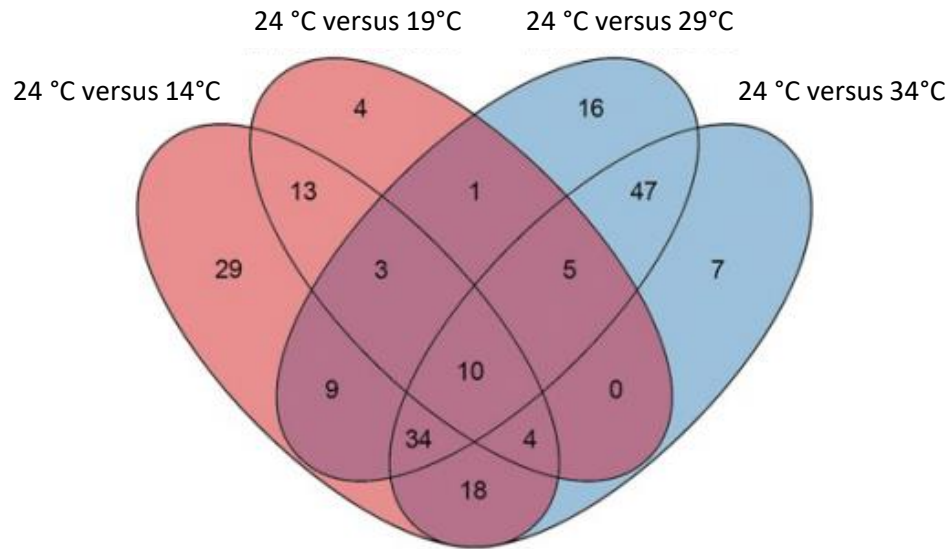
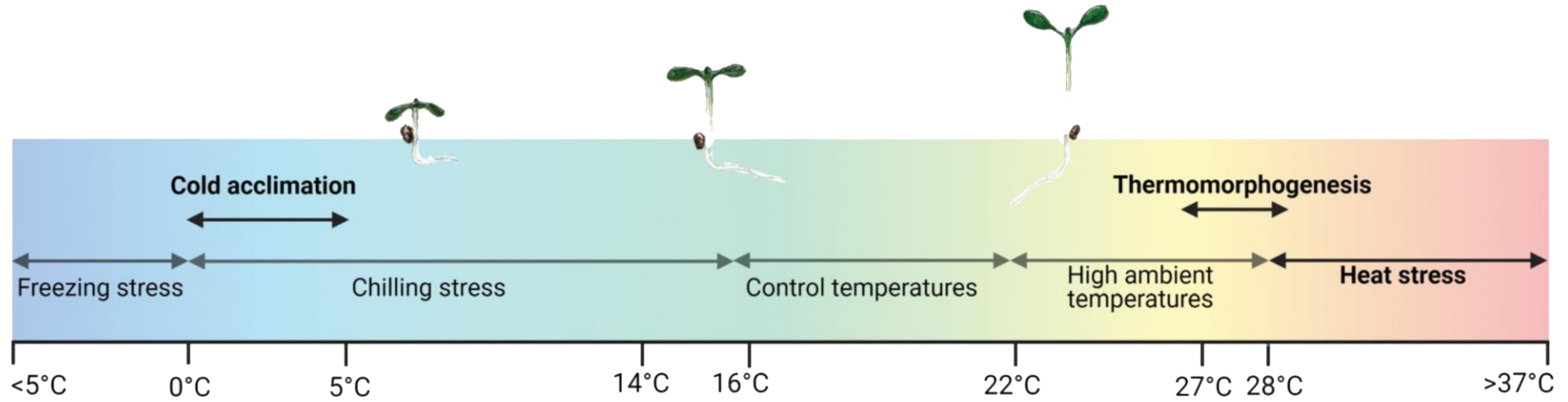
Temperature gradient phosphoprofiling in wheat



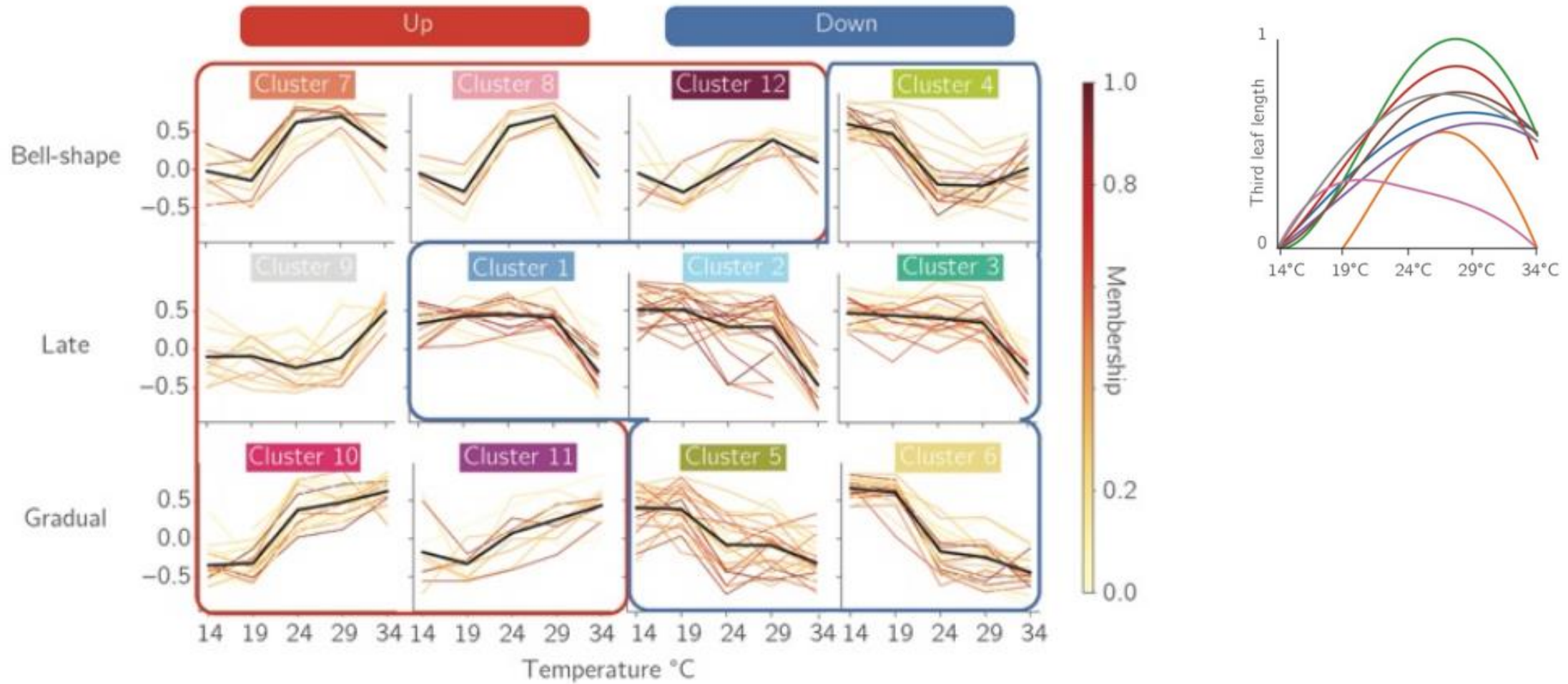
Temperature gradient phosphoproteomics in wheat



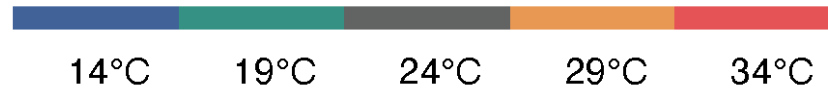
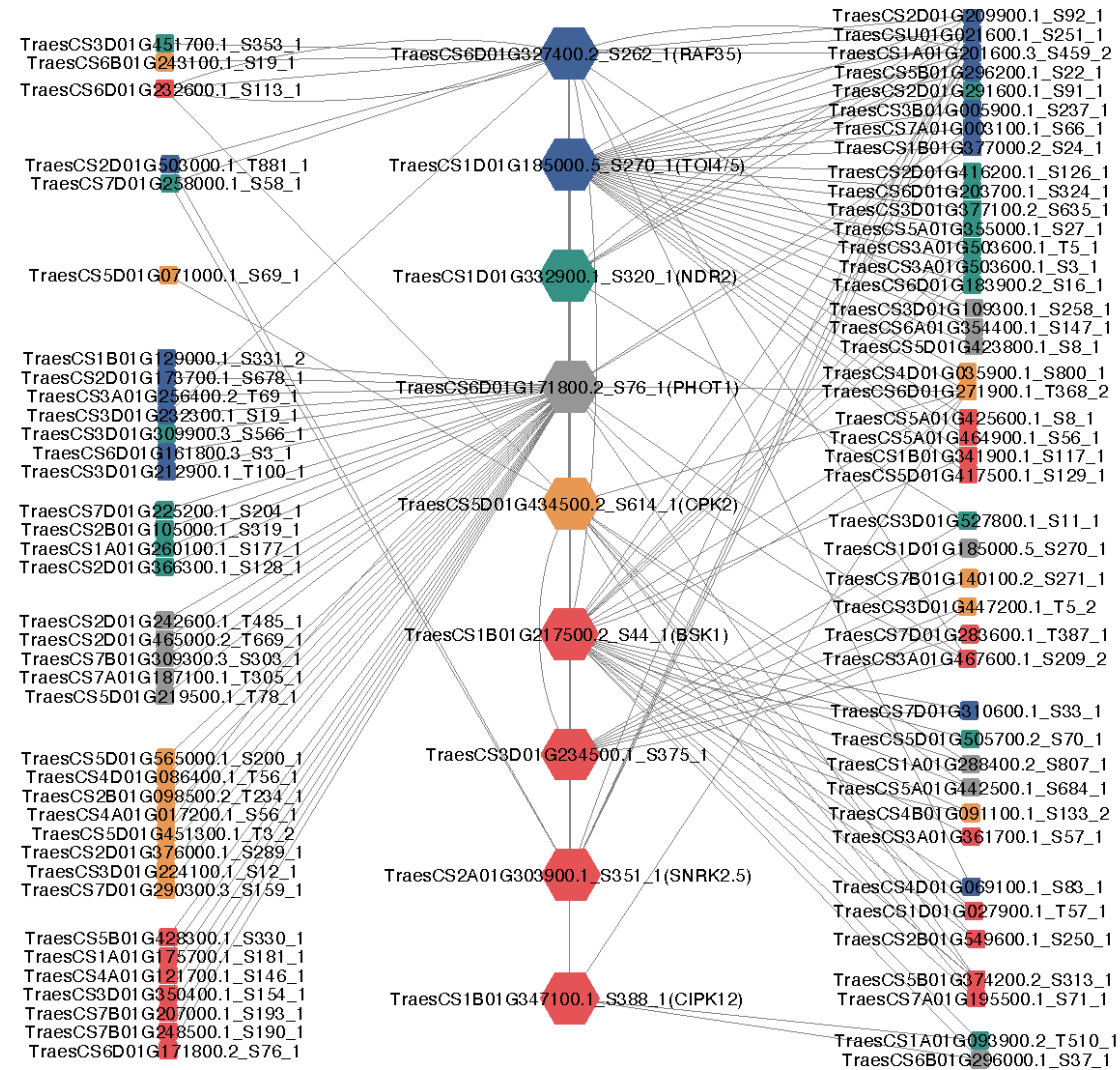
Temperature gradient phosphoprofiling in wheat



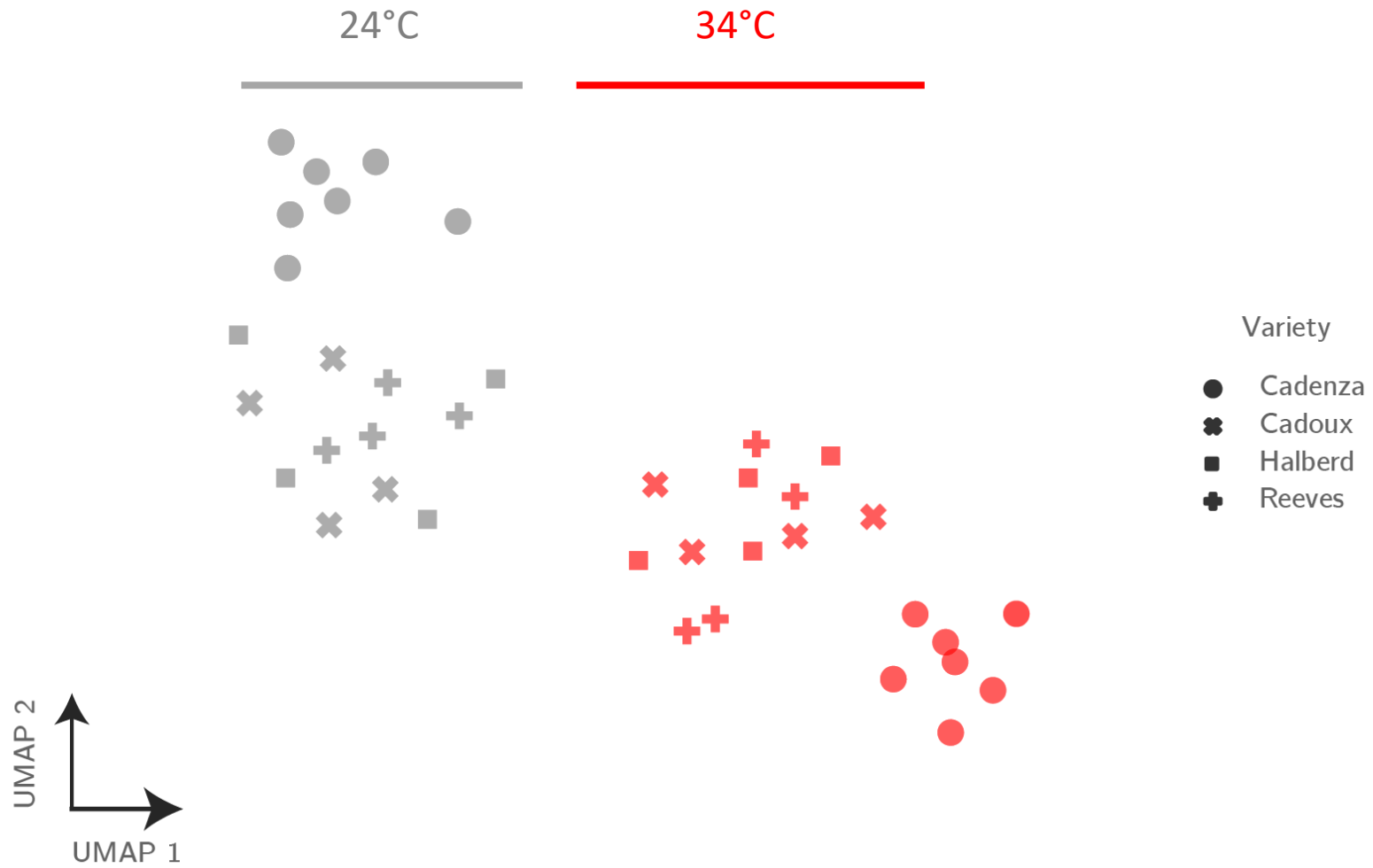
Temperature gradient phosphoprofiling in wheat



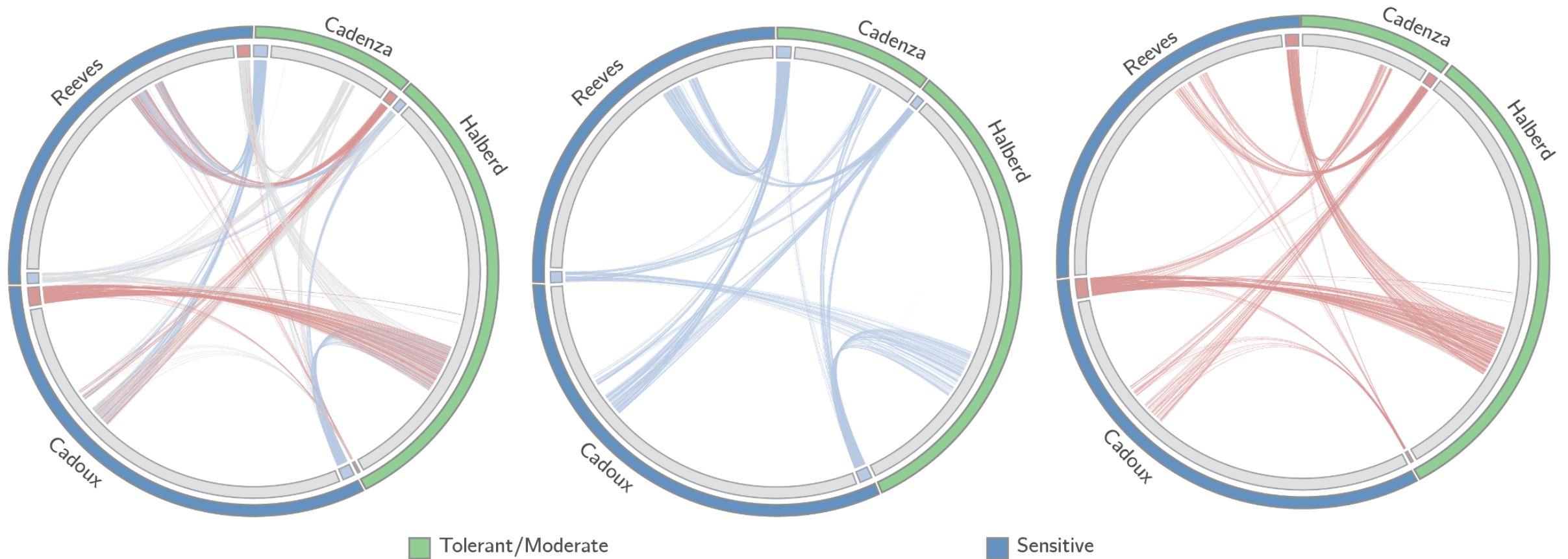
Signalling network from temperature gradient phosphoproteomics in wheat



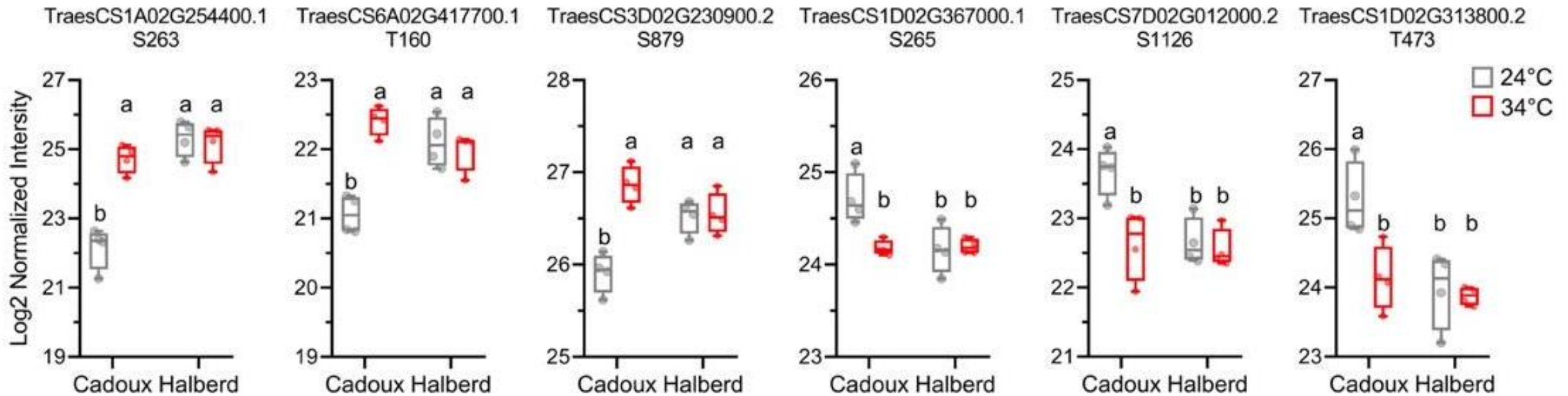
Temperature-regulated phosphoproteome: variety-dependent molecular responses



Temperature-regulated phosphoproteome: variety-dependent molecular responses



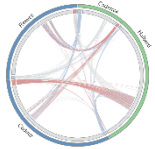
Temperature-regulated phosphoproteome: variety-dependent molecular responses



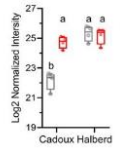
Predictive for breeding?



Capture environment-regulated phosphoproteome
in
sensitive and tolerant varieties
in
a growth chamber



Comparative analysis of phosphoproteome data



Identification
of
putative phosphoprotein-based biomarkers

Predictive for breeding?

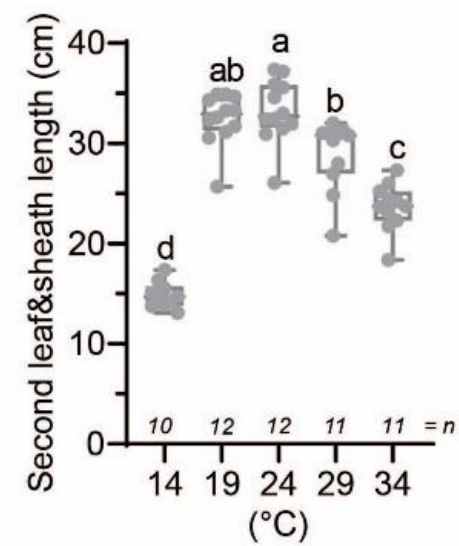
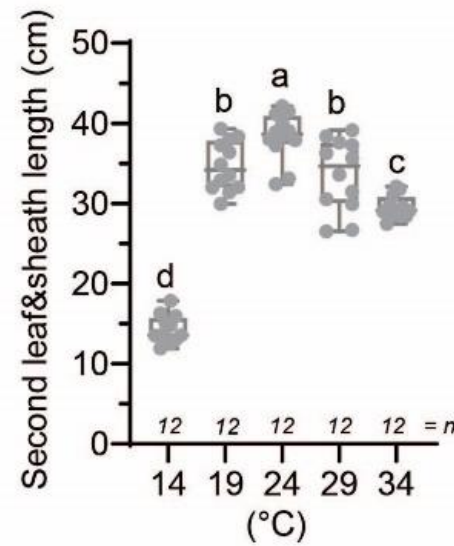
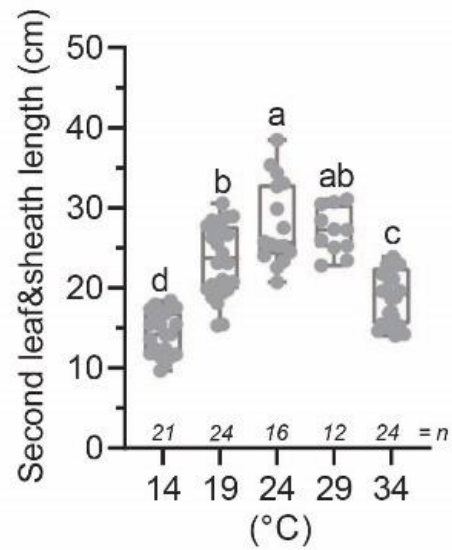
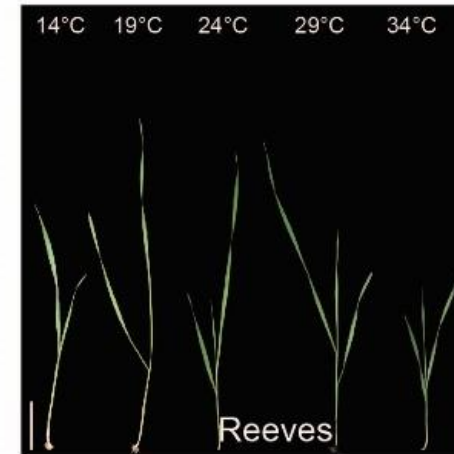
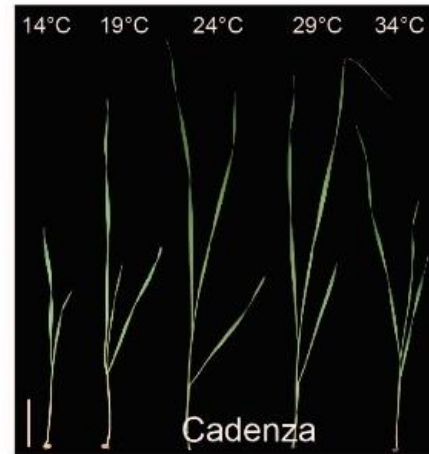
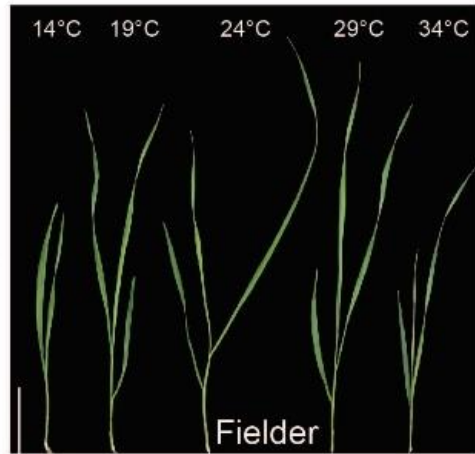
Breeding population

Physiological and genomic data
(sensitive versus tolerant variety)

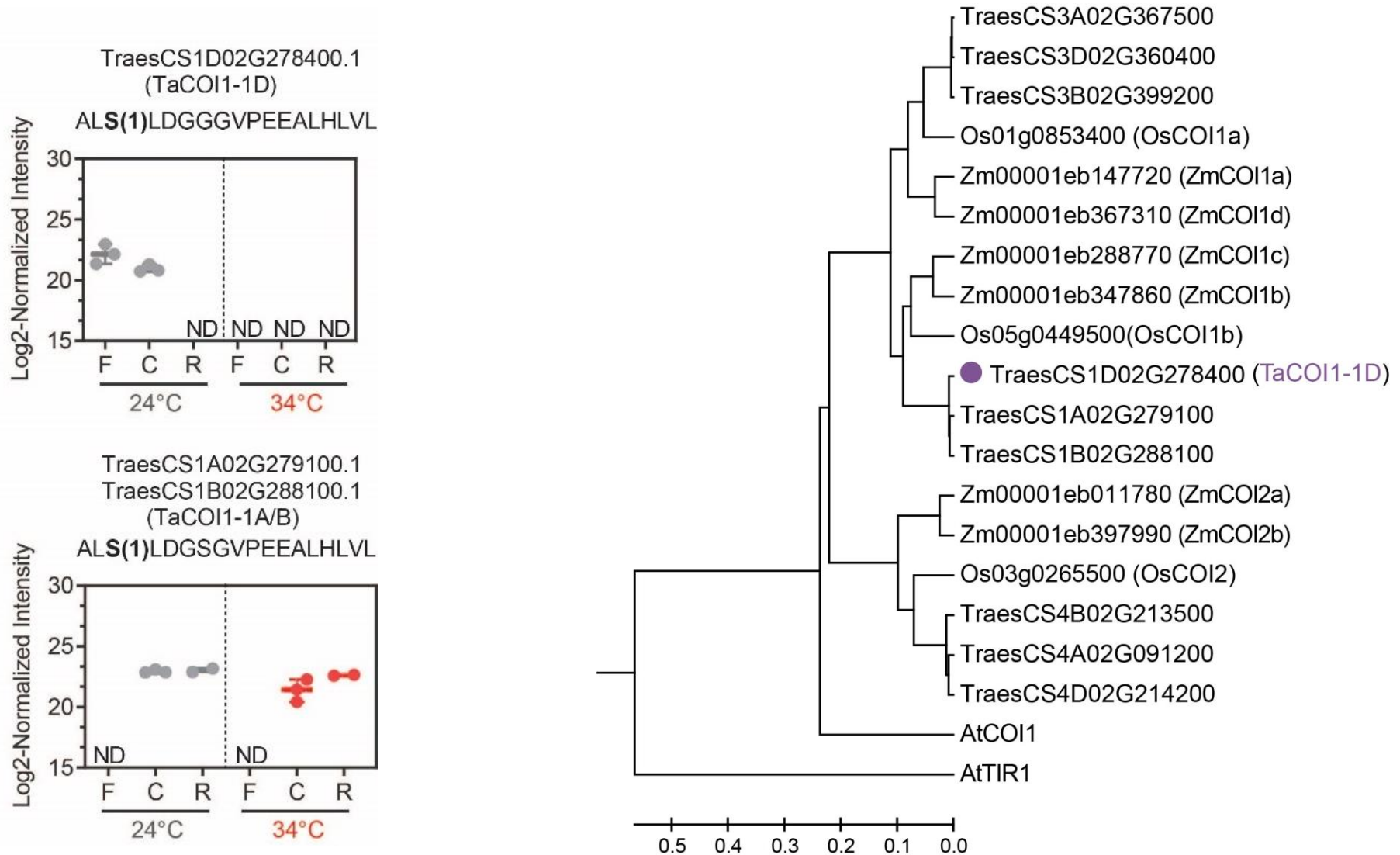
(Samples of) selected lines

Phosphoproteomics
(or phosphoantibody-based approach)

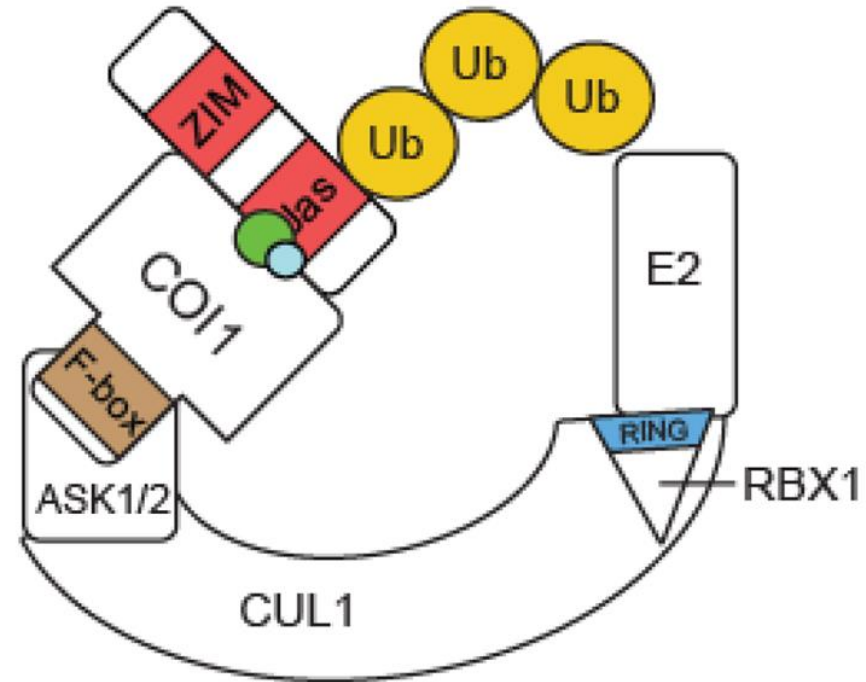
High temperature impacts wheat growth



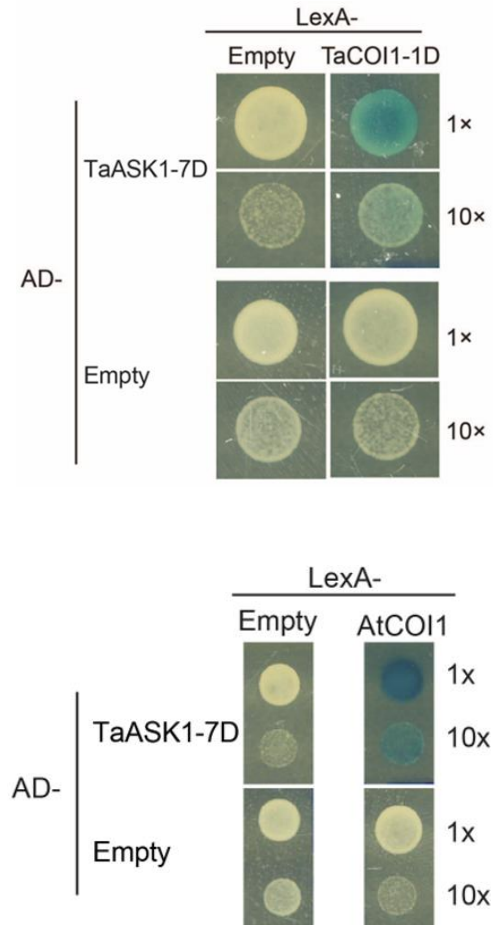
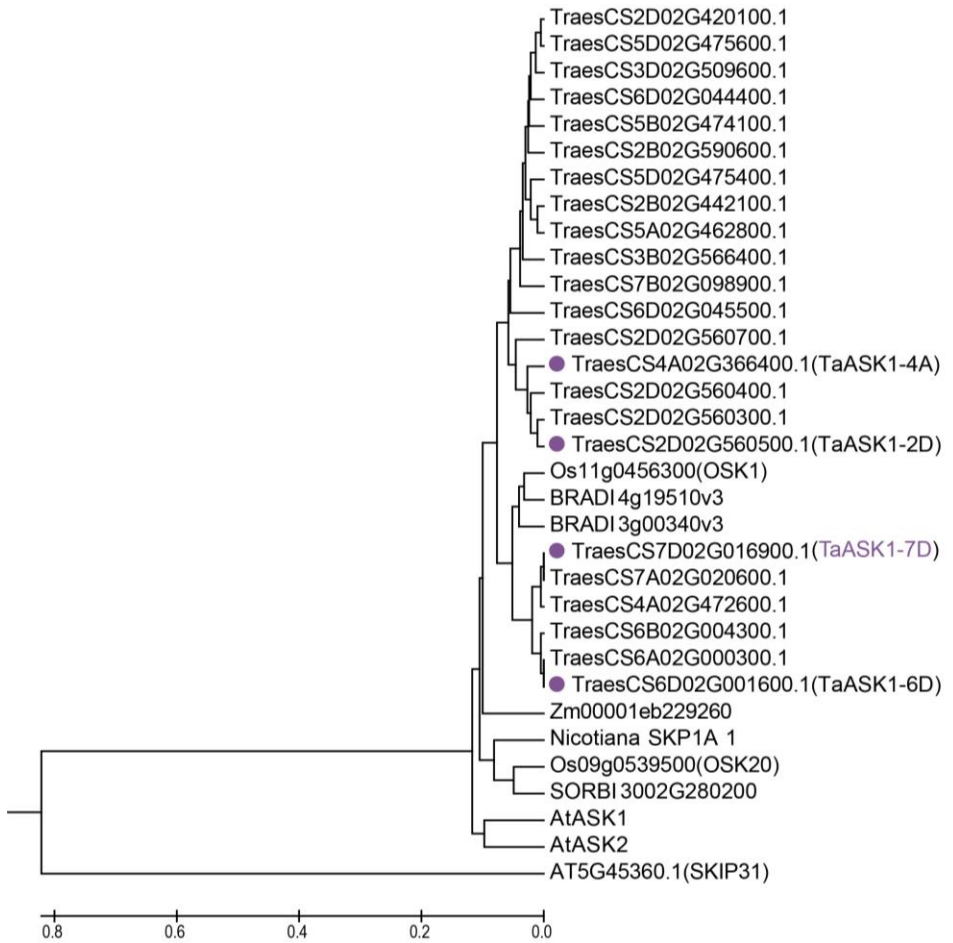
TaCOI11-D – A high temperature-regulated wheat (phospho)protein



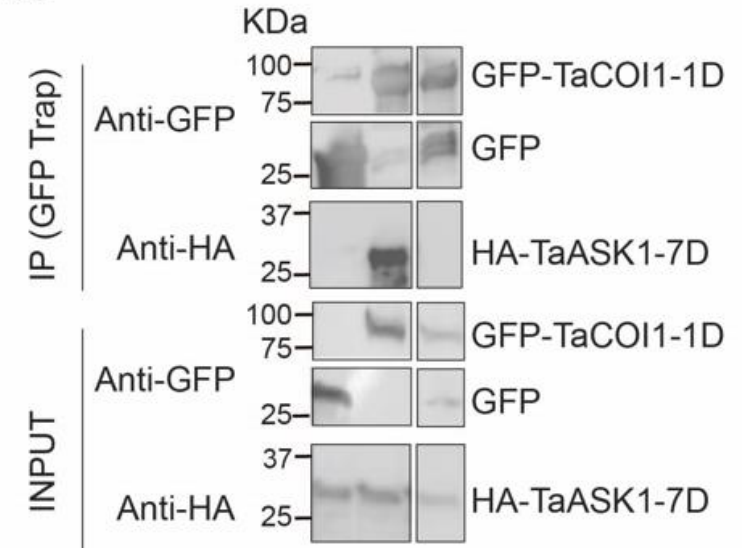
TaCOI11-D – A high temperature-regulated wheat JA receptor?



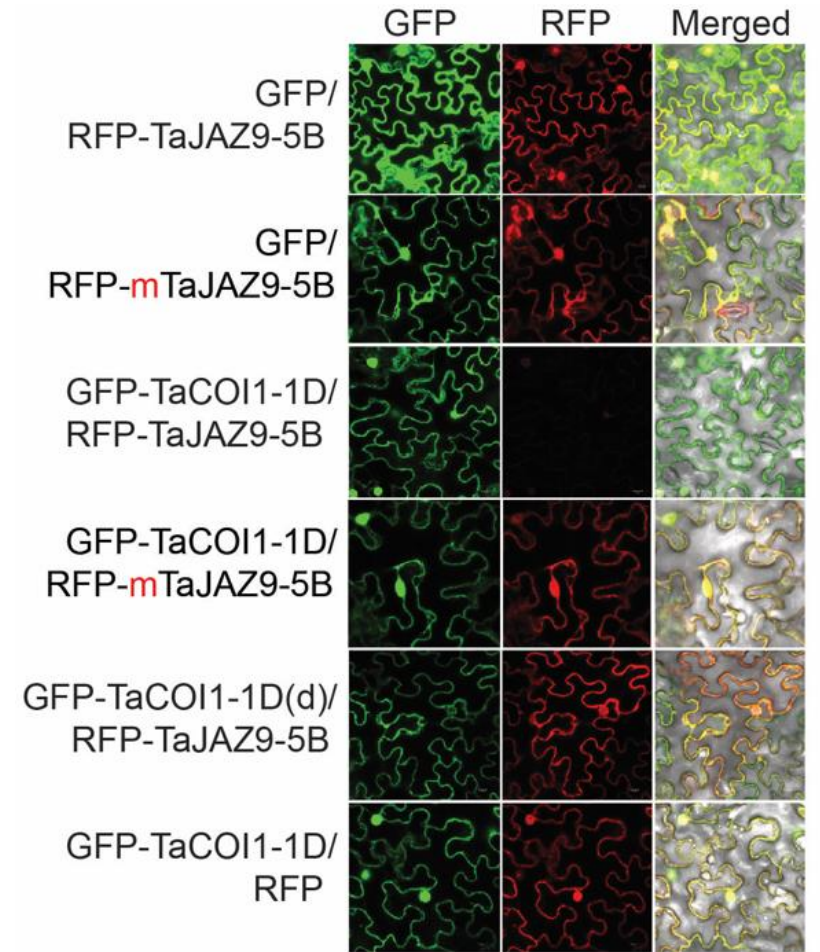
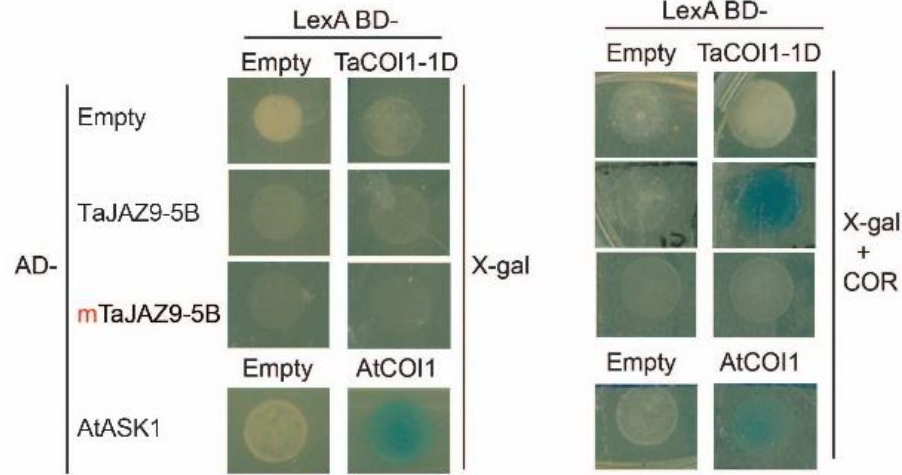
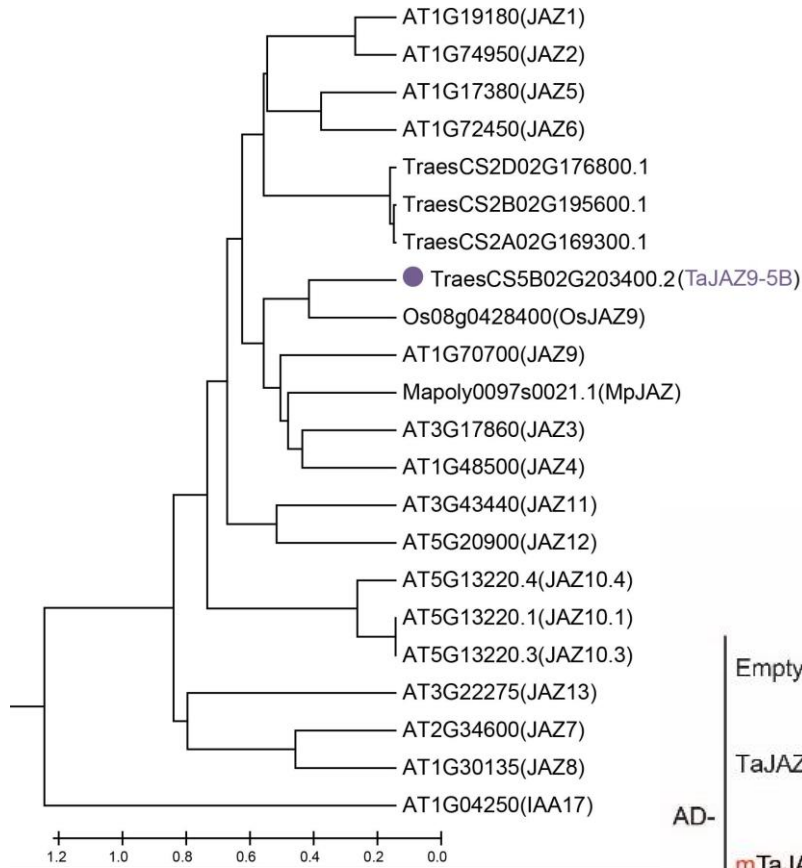
Wheat TaCOI1-1D interacts with TaASK orthologues



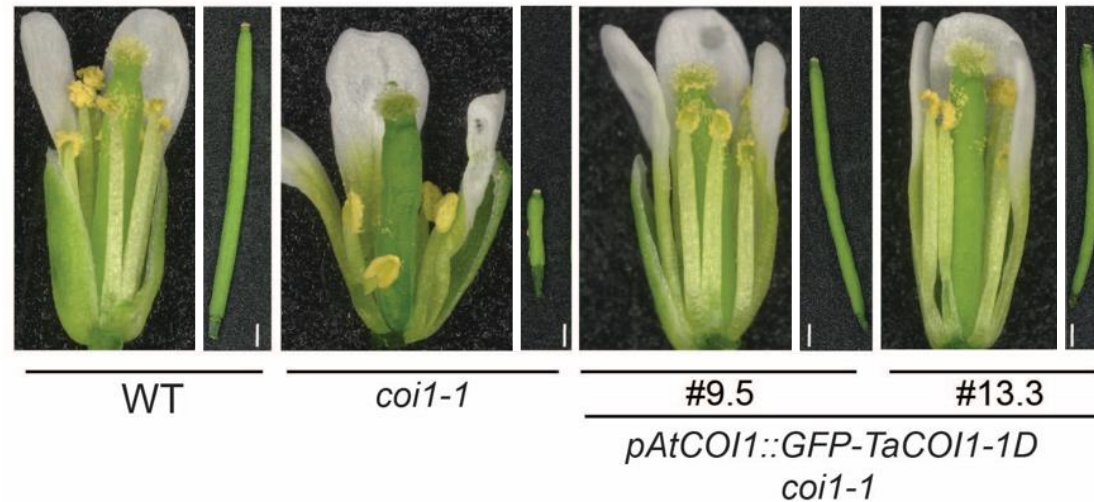
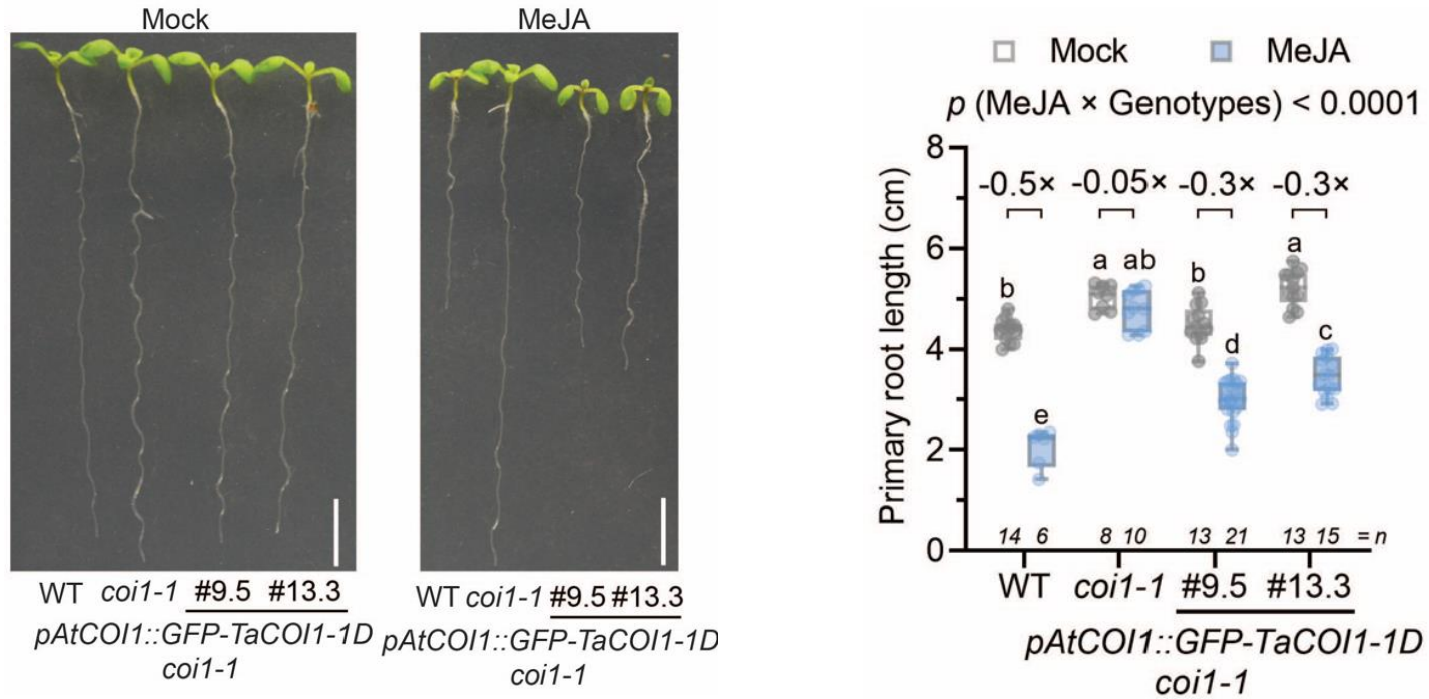
GFP	+	-	-
GFP-TaCOI1-1D	-	+	+
HA-TaASK1-7D	+	+	-
HA	-	-	+



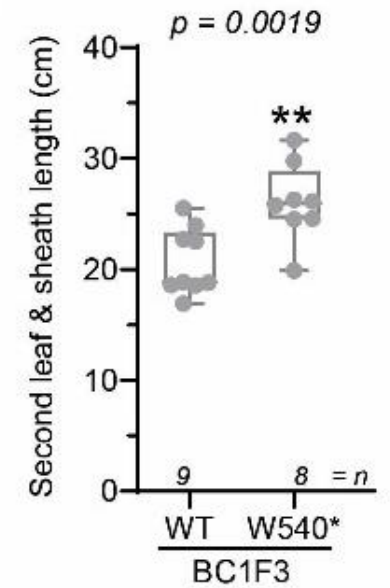
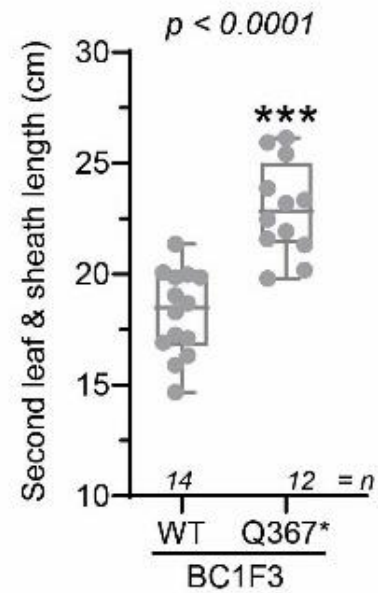
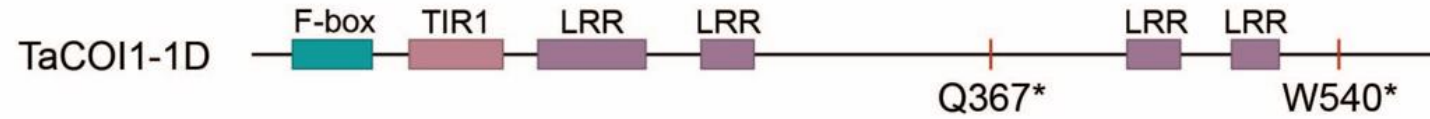
TaCOI1-1D interacts with TaJAZ9-5B in CORONATINE-dependent way



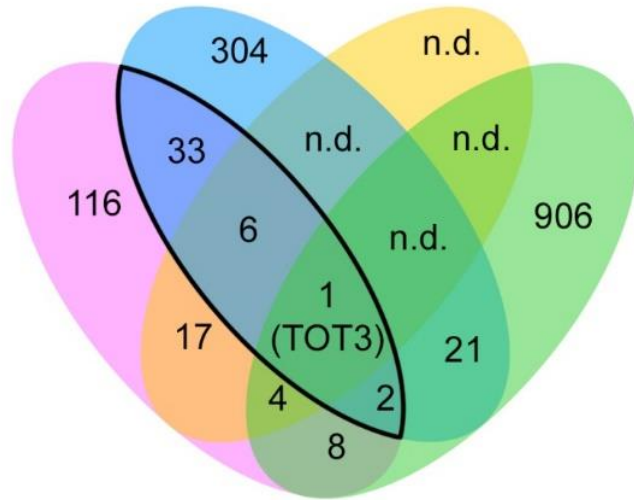
Wheat TaCOI1-1D can rescue Arabidopsis *coi1-1* mutant



TaCOI1-1D mediates TaJAZ9-5B degradation



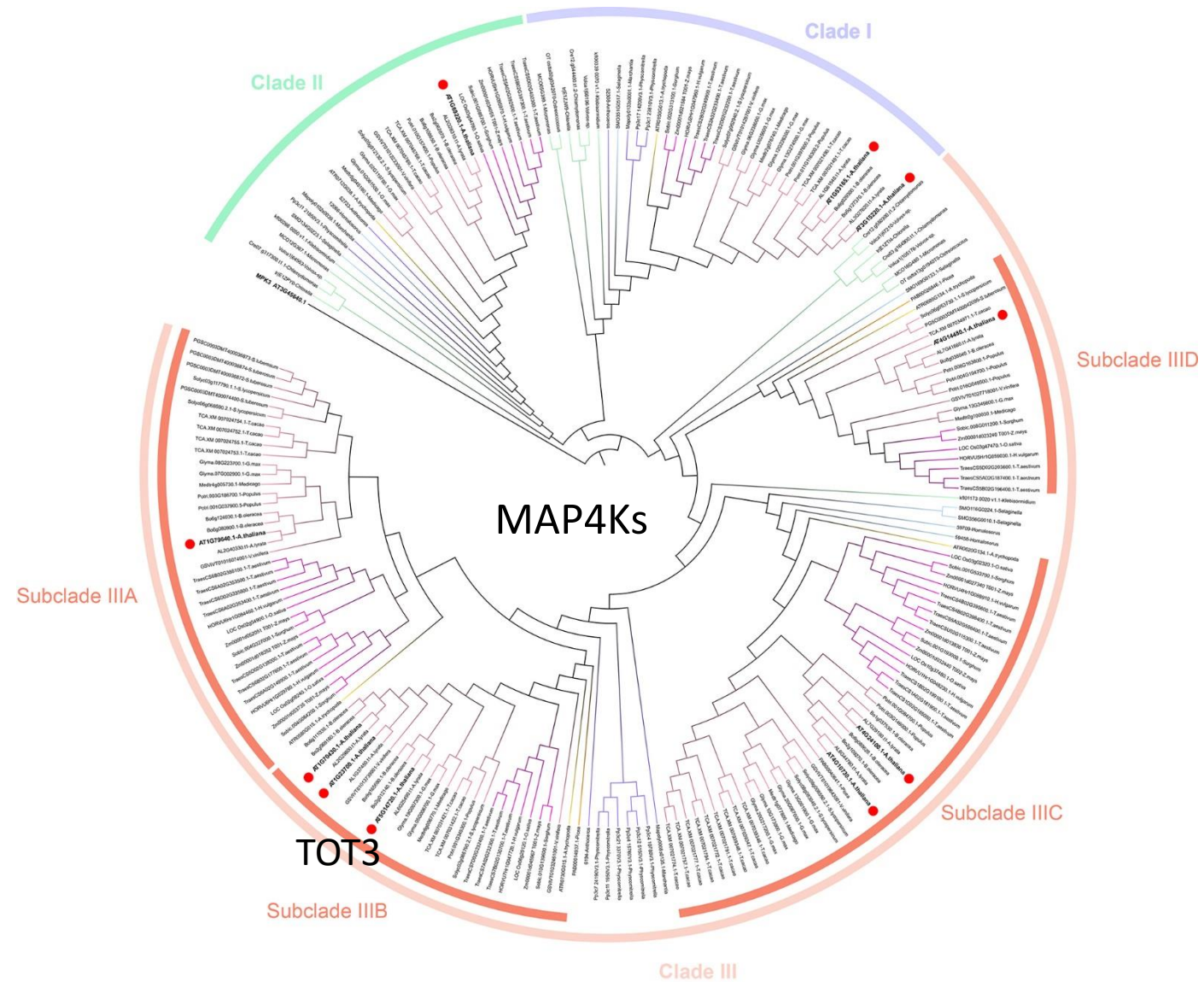
Are there conserved temperature-regulated proteins?



- TARGETs OF TEMPERATURE (TOTs)
- Warm temperature *A. thaliana* phosphoproteome (21 °C vs 27 °C)
- Warm temperature wheat / soybean phosphoproteome
- Predicted membrane localization of *A. thaliana* proteins
- *A. thaliana* kinases



TOT3 is a MAP4K

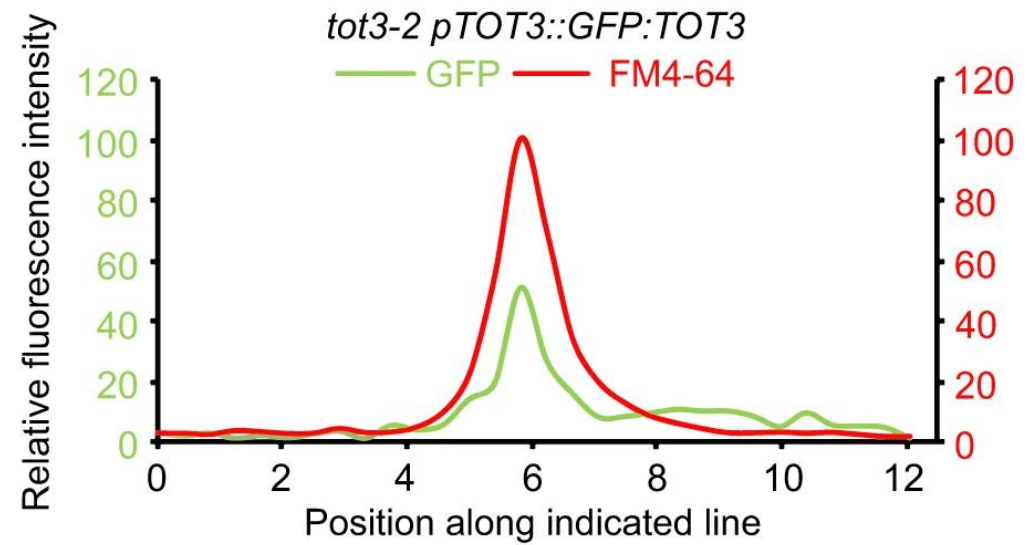
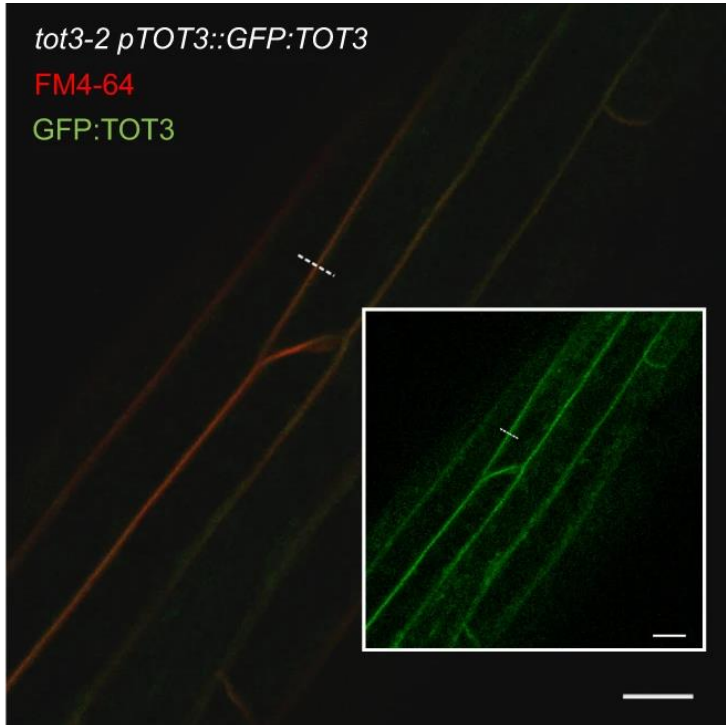


MAP4Ks are hardly explored in plants

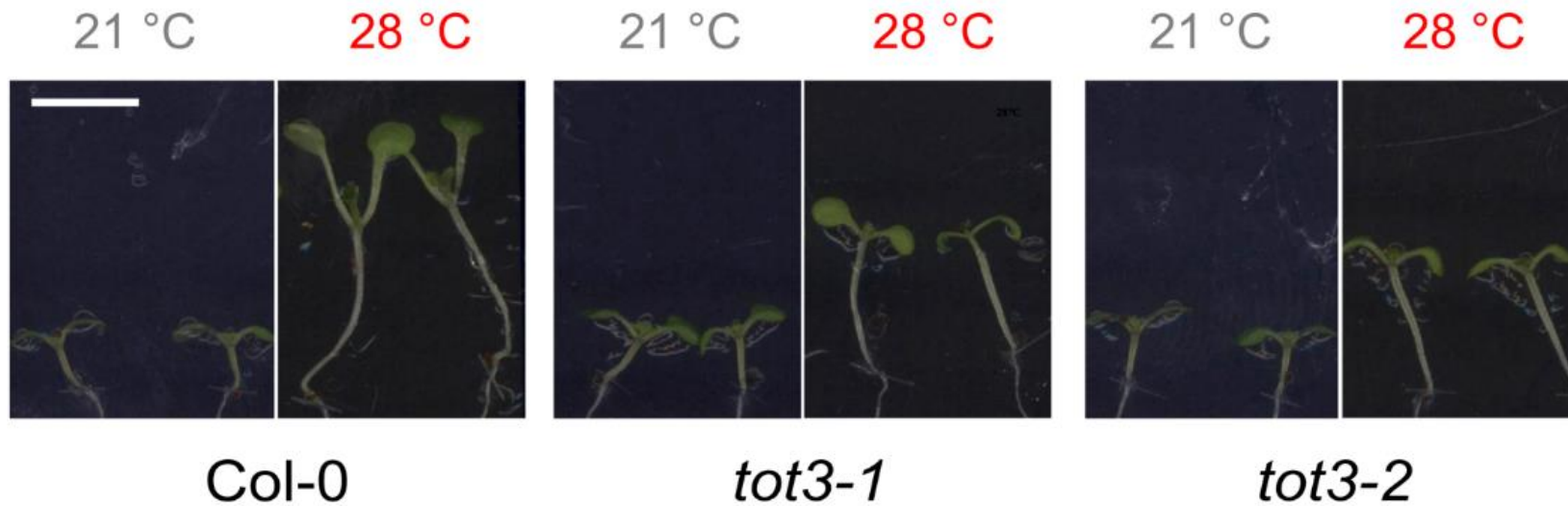
	NON PLANT			PLANT					
	Yeast	Human		Zea mays	Arabidopsis thaliana				
Signal	Mating/Pheromone Signal	Apoptosis Stimuli		?	Pathogen	Blue Light	Growth signal		
Receptor	G protein		TAOK STRIPAK	MARK	?	?	PHOT1/2	?	ER
MAP4K	Ste20	HPK1	MST1/2	MIK	SIK1	MAP4K4	BLUS1	SIK1	
Target Protein			LATS1/2 MOB1	?	BIK1 ROBDH	BIK1 PP2C38	?	MOB1	
MAP3K	Ste11	MEKK1/MLK3		?			?	?	YDA
MAP2K	Ste7	MKK4/MKK7		?			?	?	MKK4/MKK5
MPK	Fus3/Kss1	SAPK/JNK		?			?	?	MPK3/MPK6
Target Protein	Fus1		YAP TAZ	?			PP1 H ⁺ -ATPase	?	SPCH

MAP4K pathways in plants

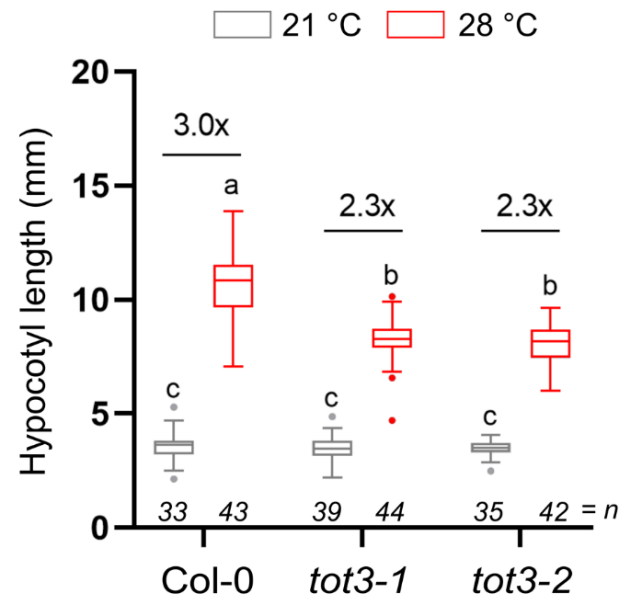
TOT3 localizes to the plasma membrane



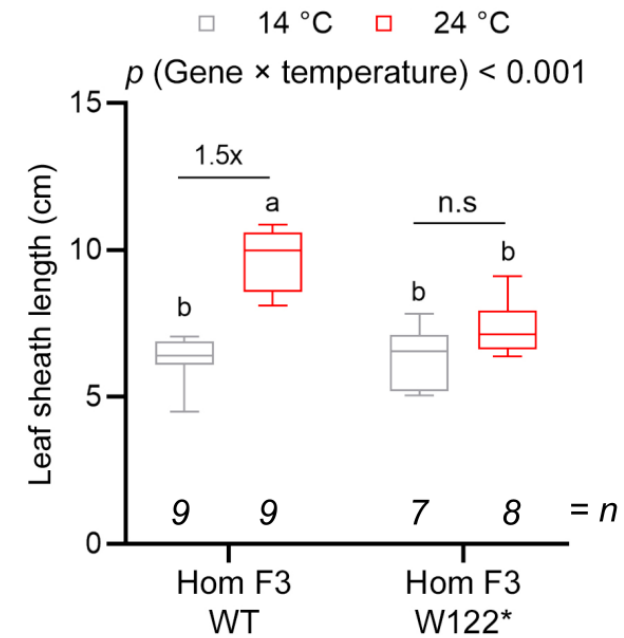
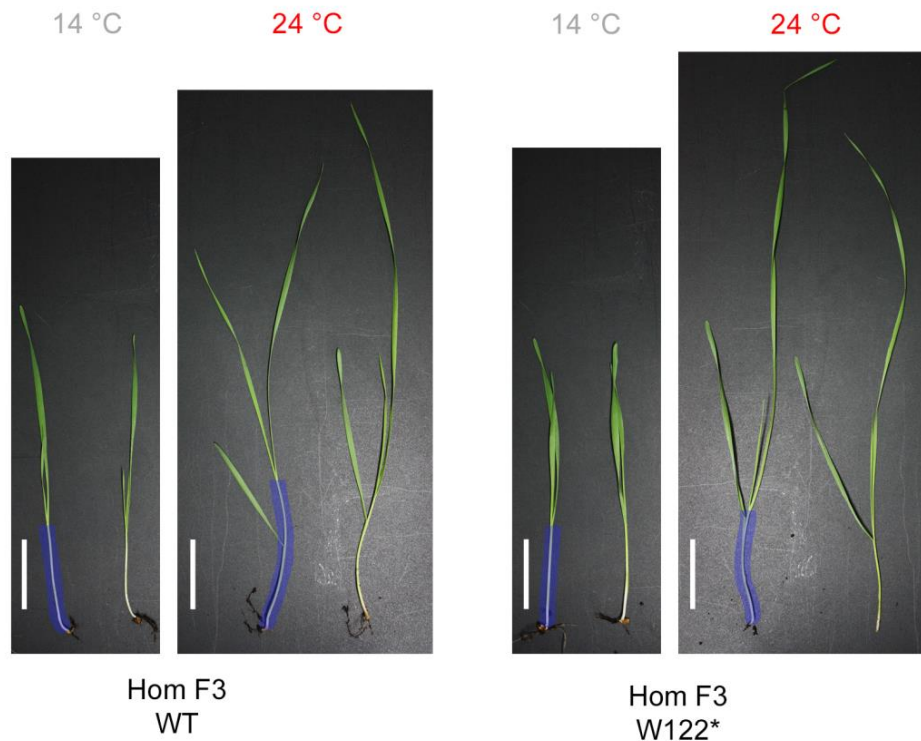
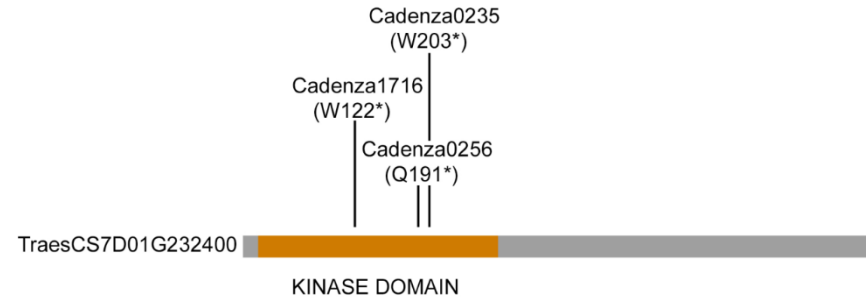
TOT3 is required for warm temperature-mediated growth in *Arabidopsis*



p (Gene \times temperature) < 0.0001



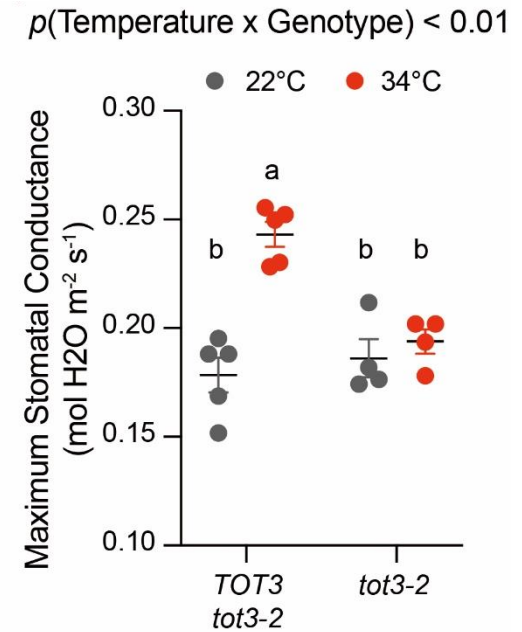
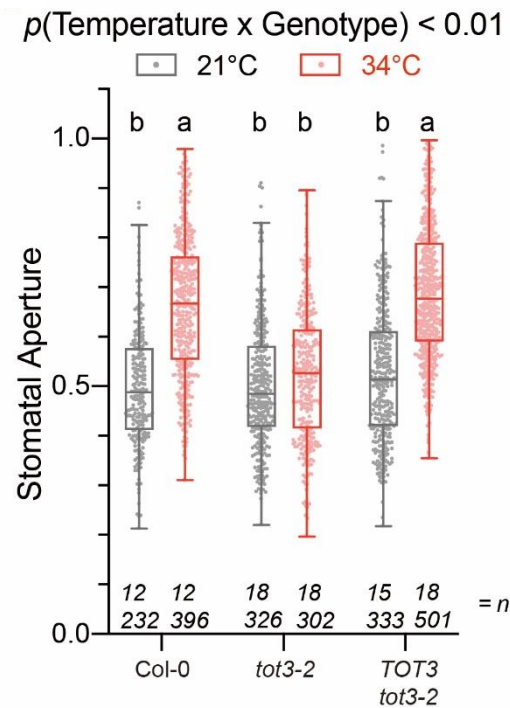
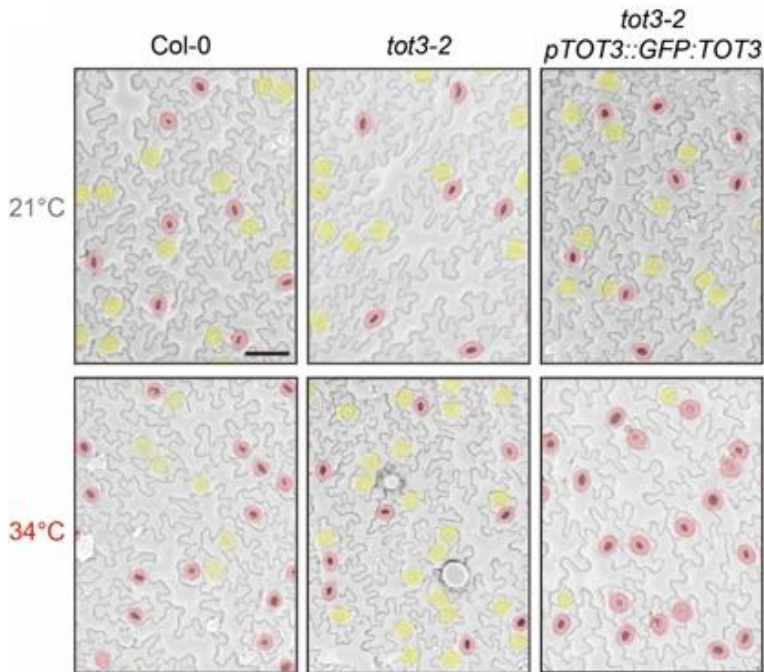
TOT3 is required for warm temperature-mediated growth in *wheat*



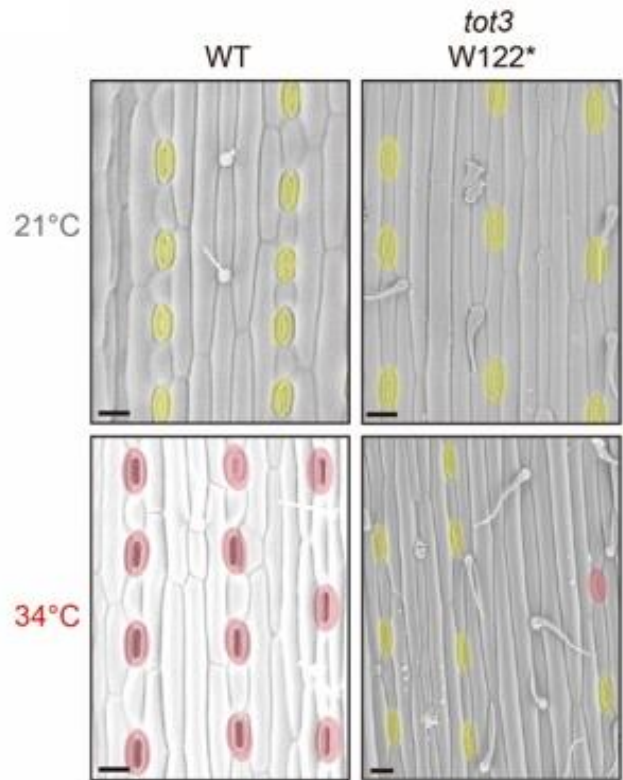
TOT3 is expressed in stomata in Arabidopsis



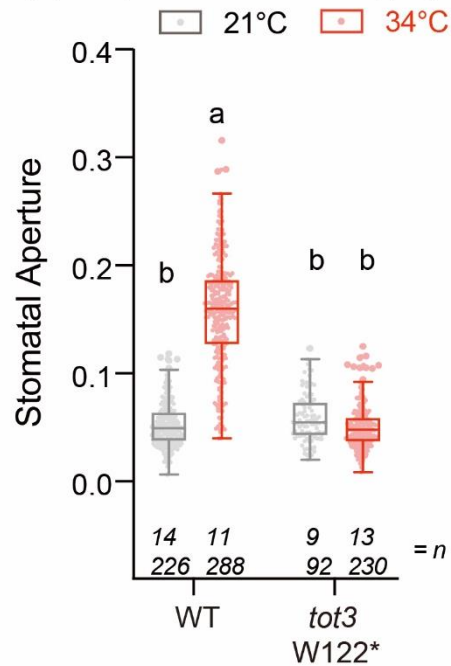
TOT3 is required for stomatal opening at high temperature in *Arabidopsis*



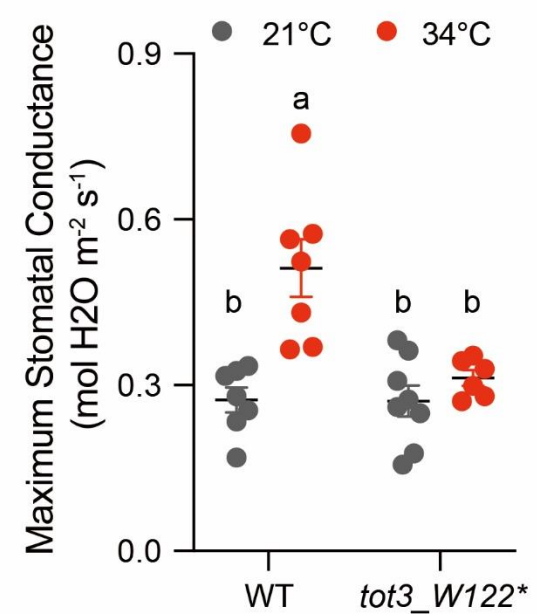
TOT3 is required for stomatal opening at high temperature in *wheat*



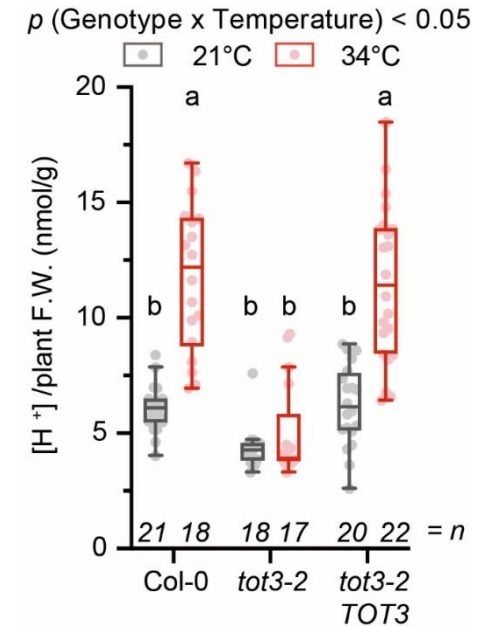
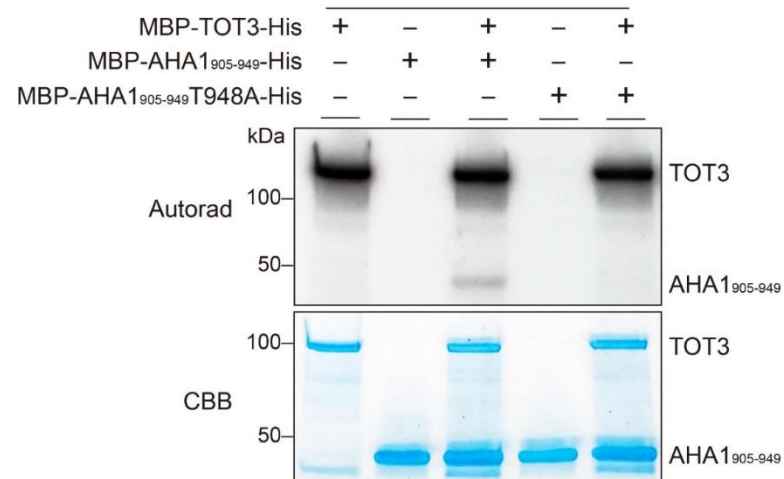
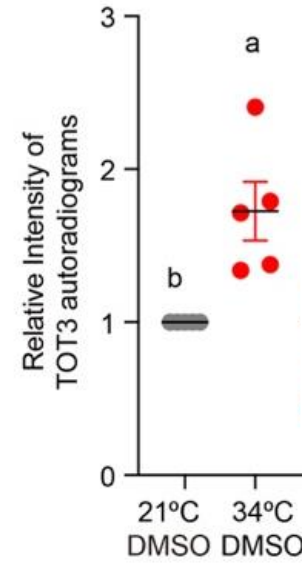
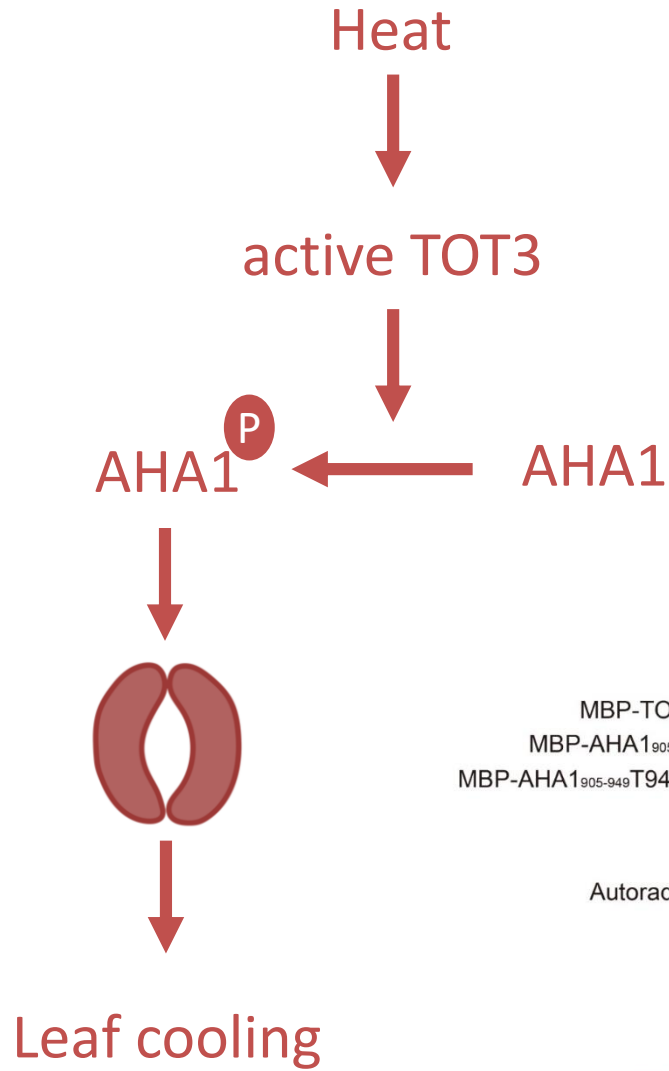
$\rho(\text{Temperature} \times \text{Genotype}) < 0.01$



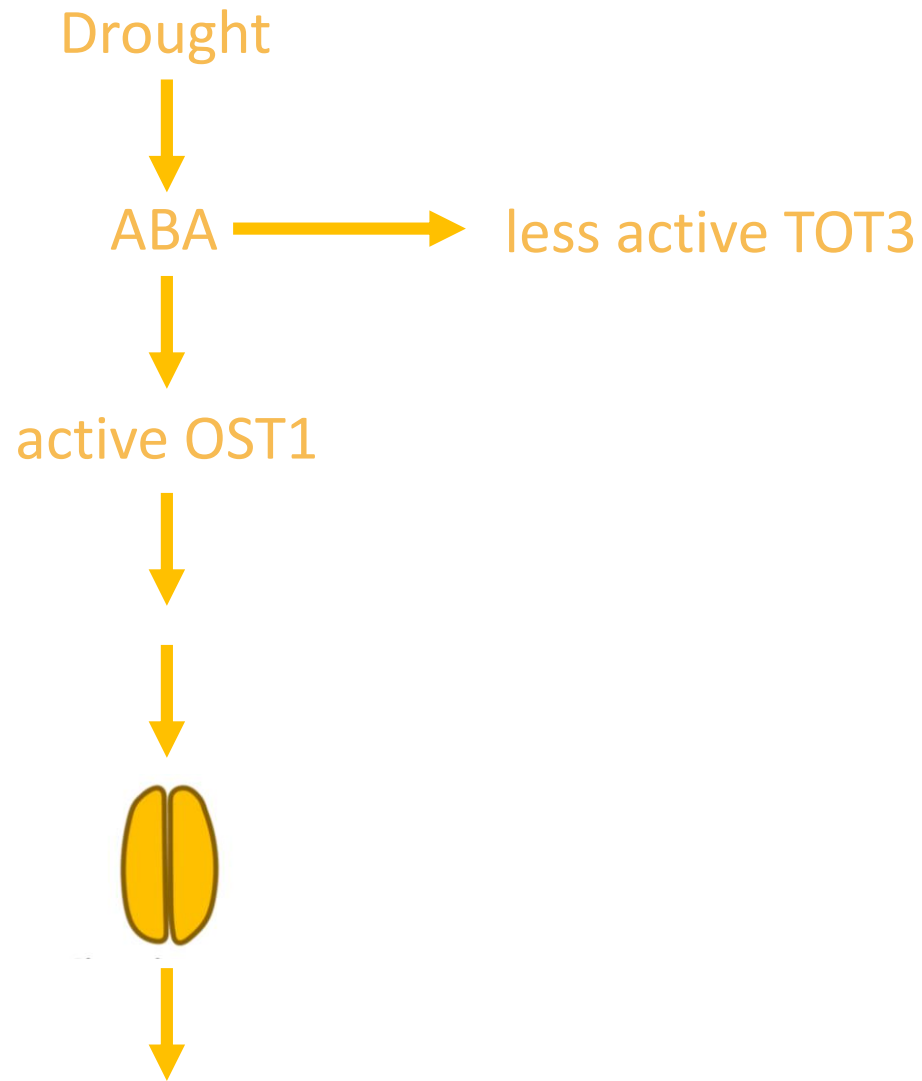
$\rho(\text{Temperature} \times \text{Genotype}) < 0.01$



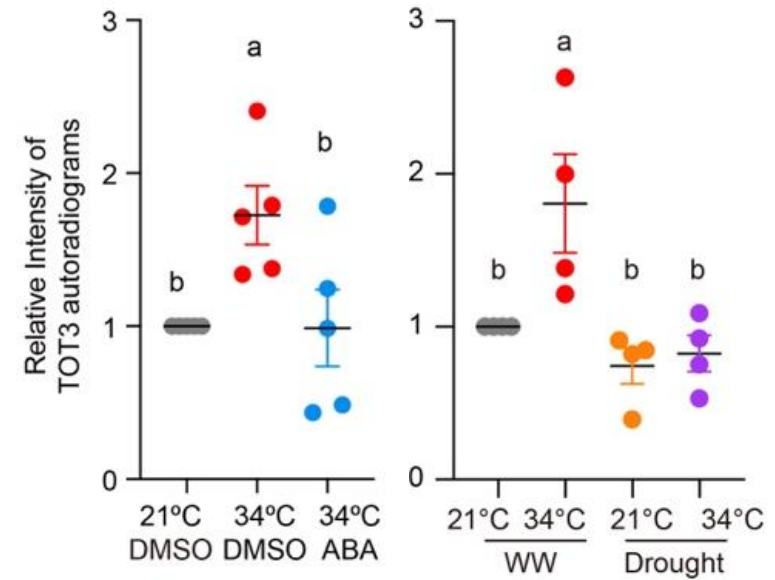
TOT3 controls AHA1 activity to open stomata by phosphorylating T948 upon heat



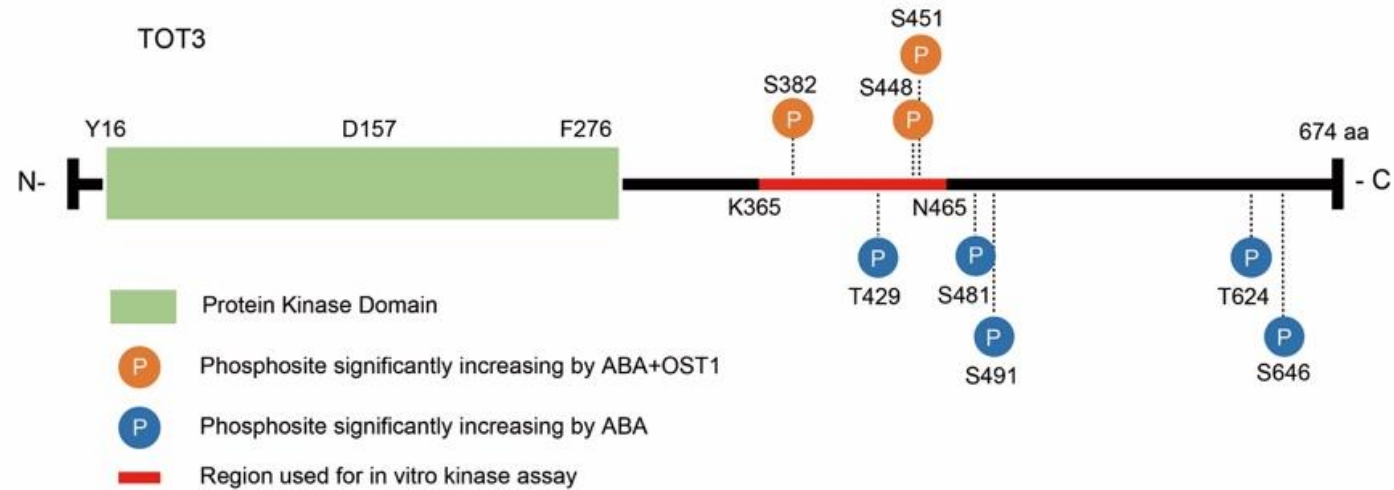
What happens under drought?



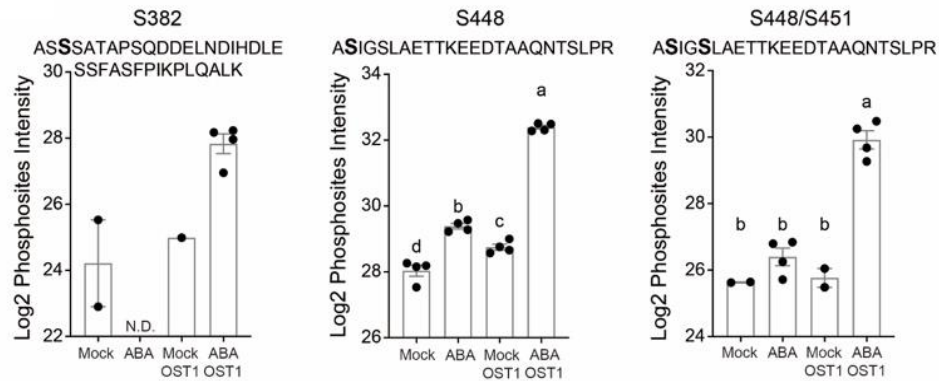
Water retainment



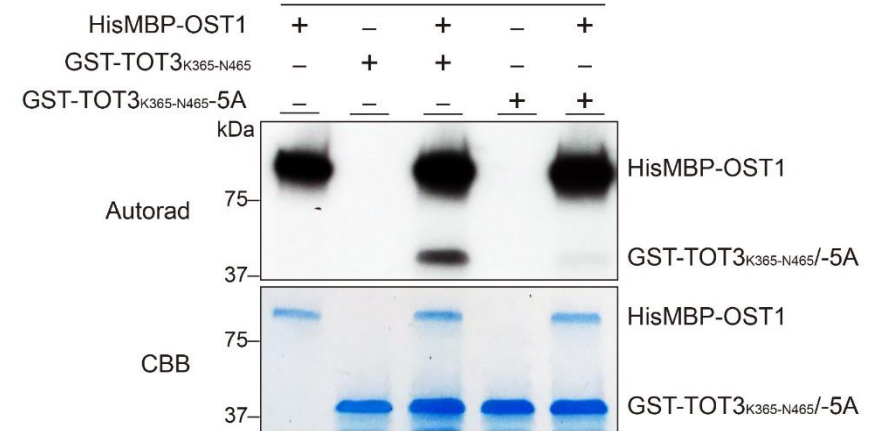
OST1 directly phosphorylates TOT3



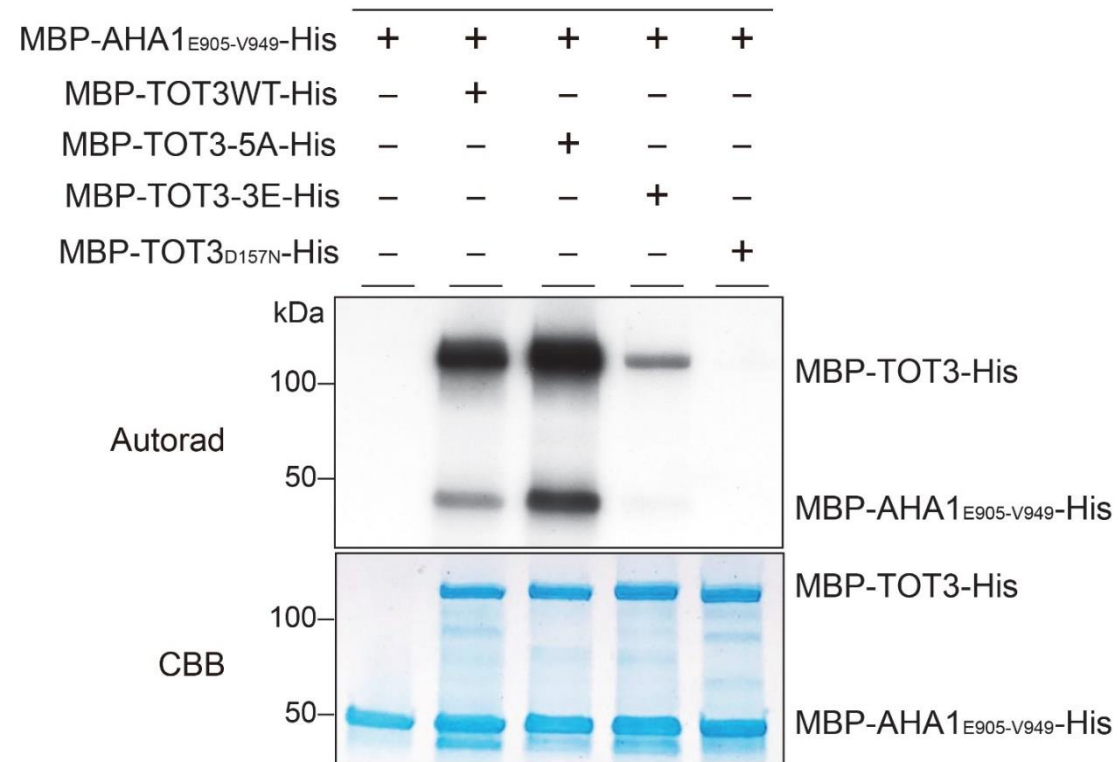
Transient tobacco assay



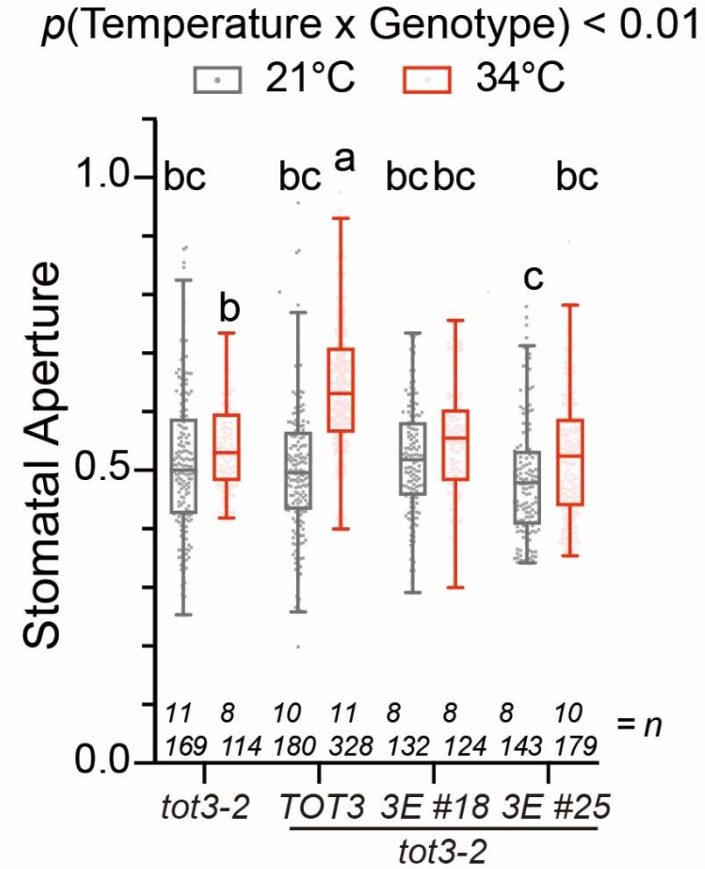
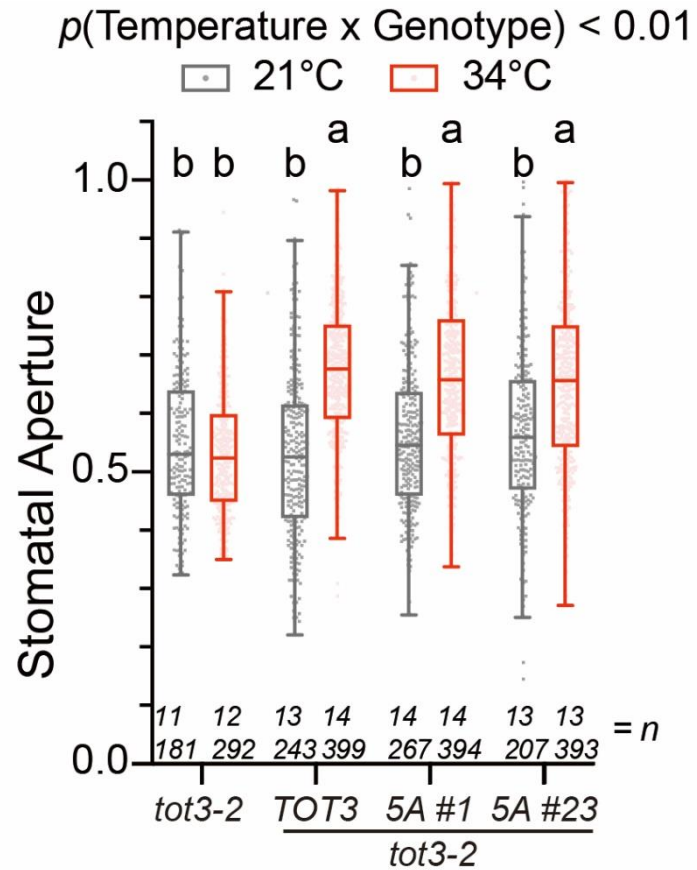
In vitro kinase assay



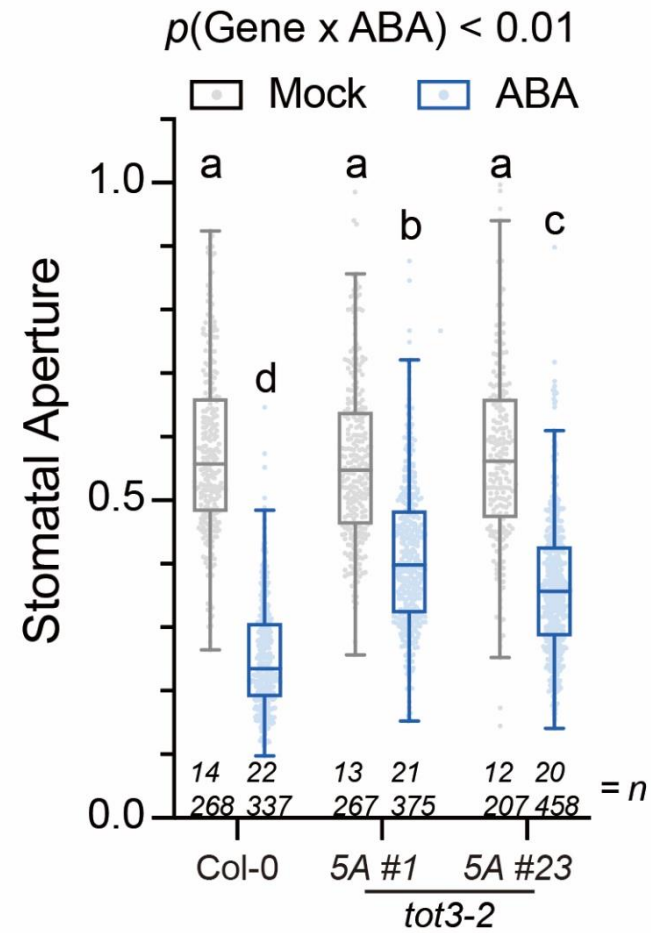
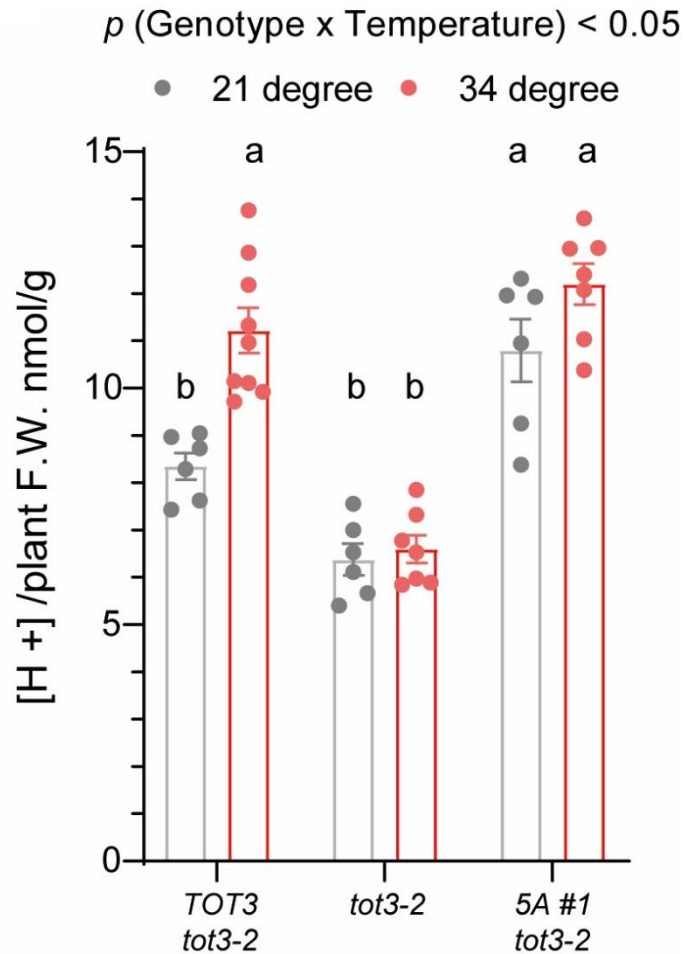
OST1-mediated TOT3 phosphorylation leads to loss of kinase activity



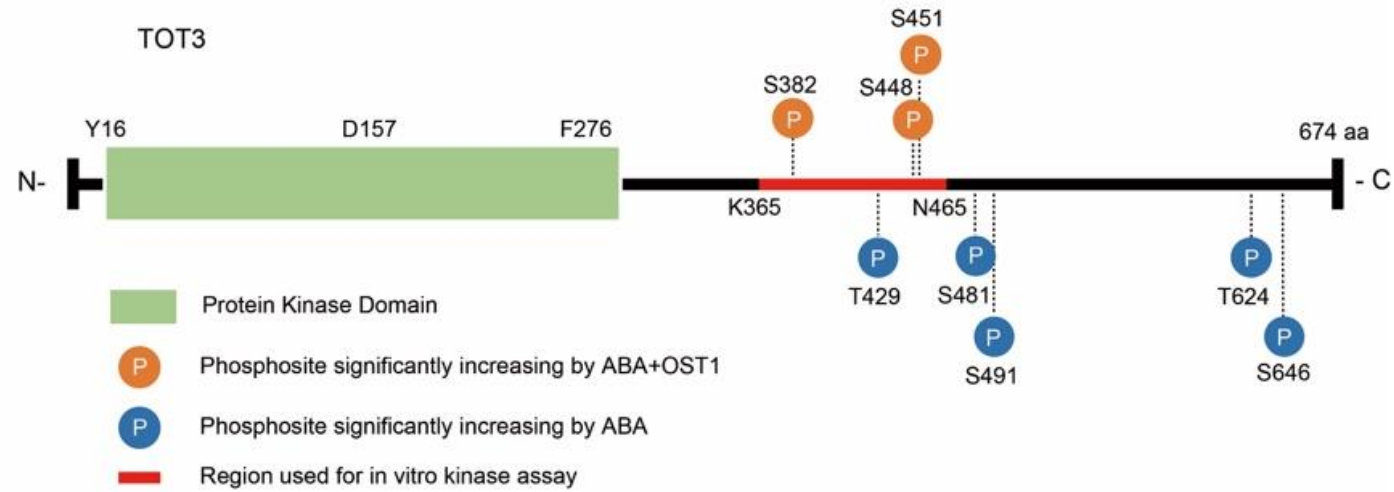
TOT3-5A = active / TOT3-3E = inactive



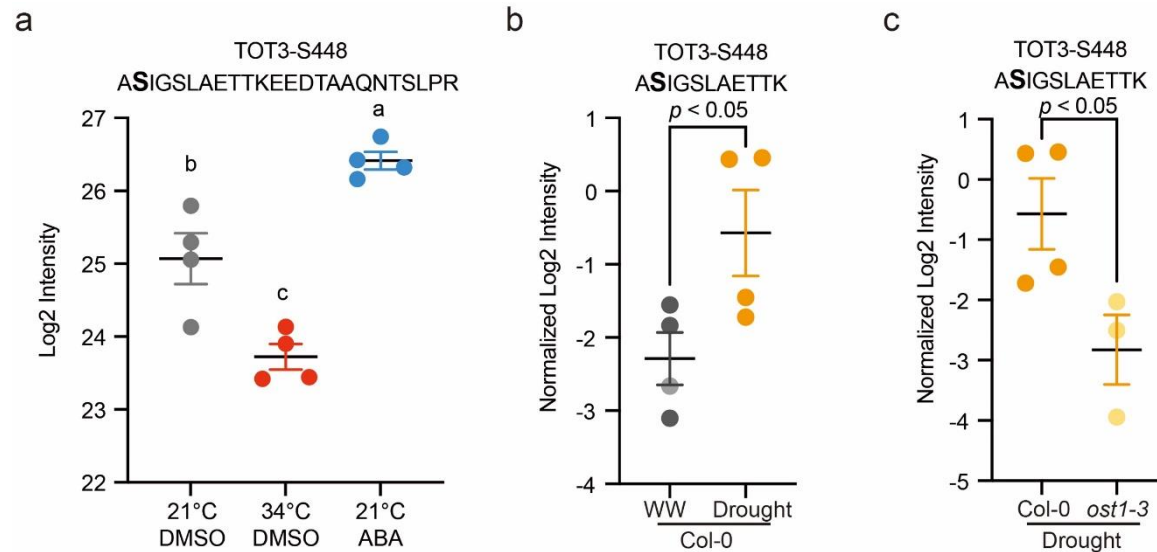
Impaired OST1-mediated TOT3 phosphorylation leads to more proton pump activity and reduced ABA-activated stomatal closure



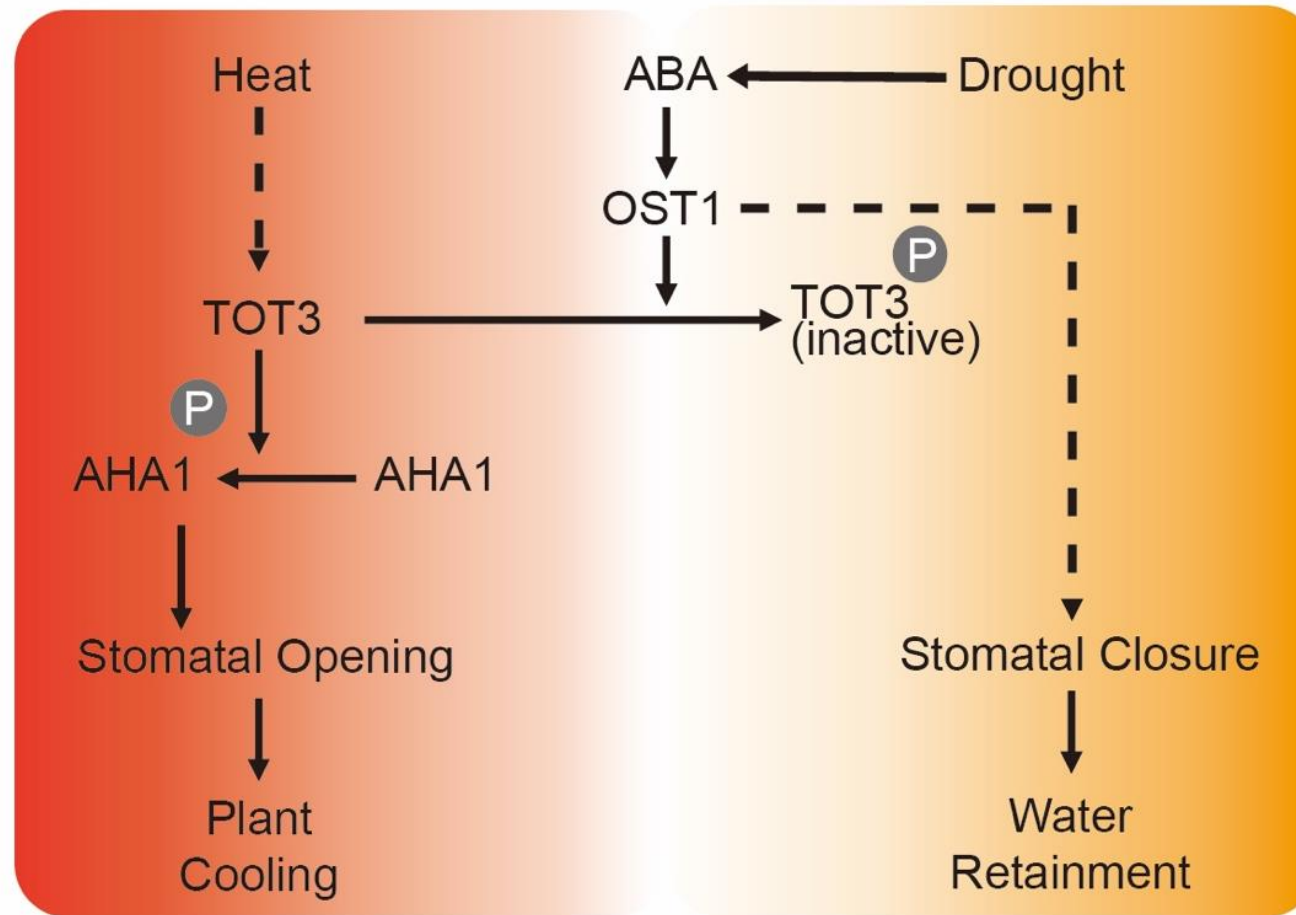
OST1 phosphorylates TOT3 upon ABA or drought



Arabidopsis



OST1 and TOT3 interplay balances water loss / cooling under drought / heat



Take Home Messages

- Capturing protein phosphorylation allows the identification of novel signaling pathways, including components that control temperature signaling and plasticity
- Phosphorylated proteins can be valuable breeding markers
- TOT3 function is conserved in wheat and Arabidopsis
- The TOT3-AHA1-OST1 signaling module integrates environmental signaling
- TaCOI1-1D encodes a JA receptor that is involved in regulating wheat growth

WHO DID THE WORK?



Group alumni

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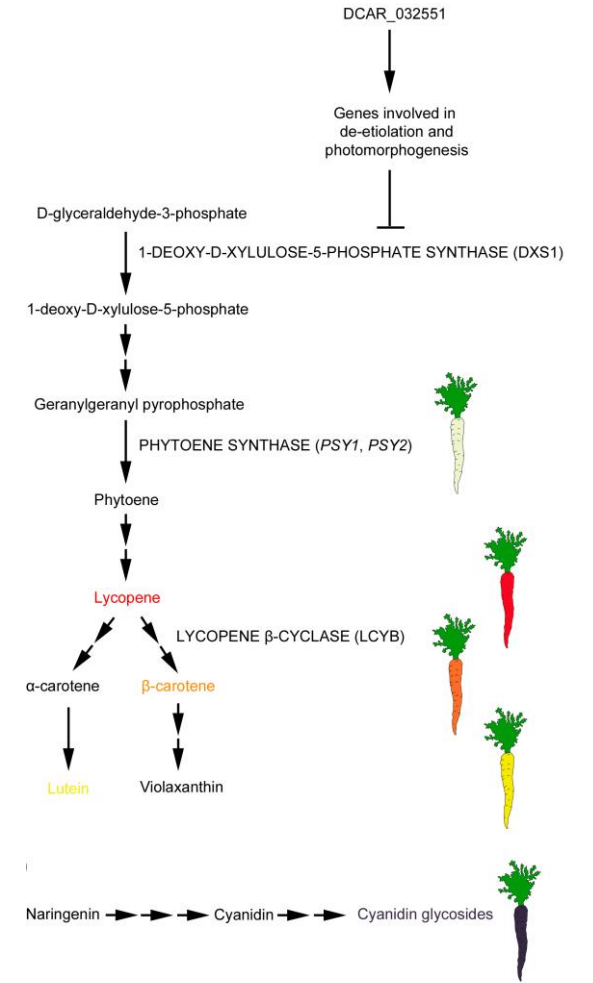
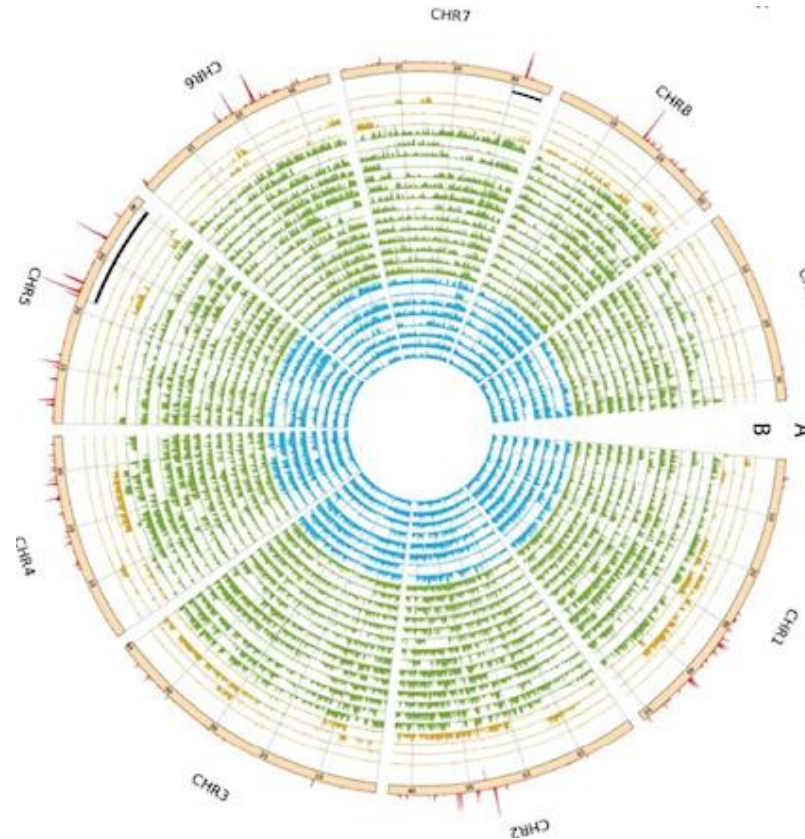


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#artgenetics - Belgian researchers

@david.vergauwen (art historian) and @desmetive

(VIB-UGent) reveal plant diversity over time 🥕 🥝

🥕 🥕 ... meer

Trends in Plant Science

CellPress

Science & Society Down the Rabbit Hole—Carrots, Genetics and Art

David Vergauwen¹ and
Ive De Smet^{2,3,*}

The recent carrot genome assembly provides insight into carotenoid accumulation in carrots, and allows—together with other genetic information—to provide a molecular explanation for color differences observed in carrots painted throughout the centuries.

Carrots and Art

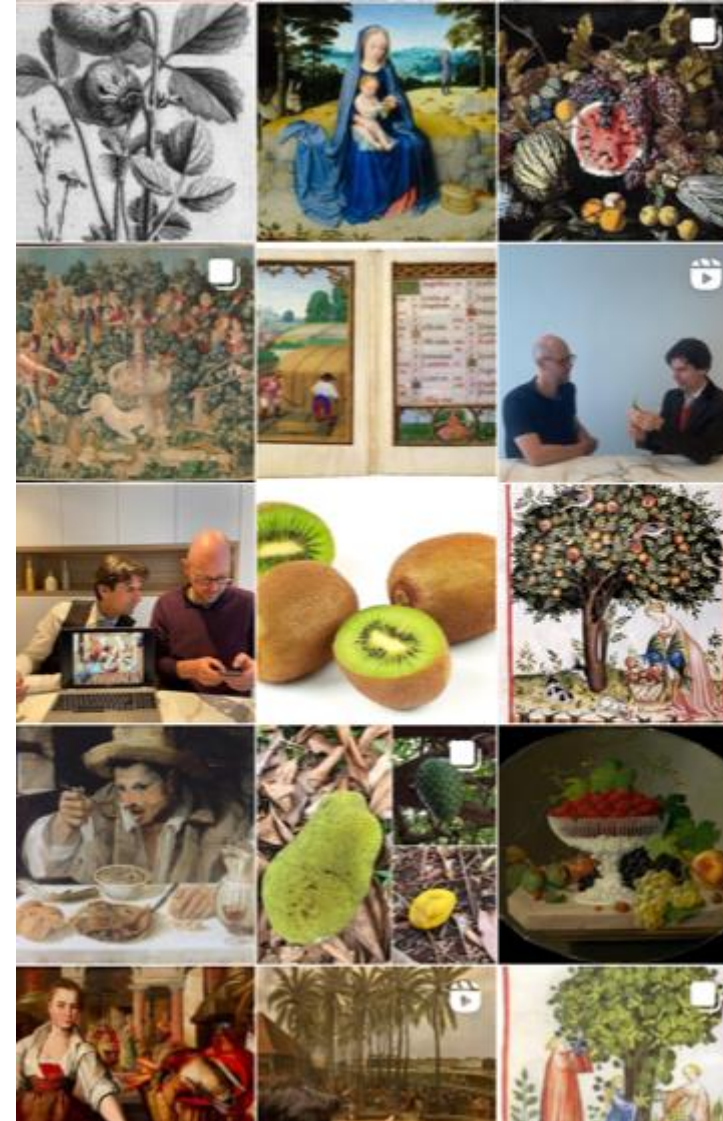
The large, unbranched cultivated carrot

some of the molecular mechanisms and underlying genetic network responsible for the observed colour differences.

On the Origin of the Carrot

The origin of carrot is complex, but recent studies shed some light on the geographical distribution and domestication origin of wild and cultivated carrot [3]. Eastern and Western type carrots can be recognized, with the origin of the former in western to central Asia (purple, red or orange-yellow roots) and the latter derived from the Eastern type (yellow, orange, or sometimes-white roots) [3,4]. The carrot was probably first domesticated somewhere in the area between the north east of Kabul (Afghanistan) and north Kashmir (India) around AD 900 [5]. Later, in the 10th century, a Bagdad-based cook, Ibn Sayyar al-Warraq, mentioned in his 'Kitāb al-Tabī', a book with about 600

artist to truthfully represent the plants and their color. Assumptions on the exactness of the image can be made according to when and where it was made. A fine painting by a seventeenth century Dutch painter can on historical and stylistic grounds be said to be more 'realistic' than an Egyptian mural and will therefore be a more accurate historical source. In addition, color tones or intensity can be affected over time depending on the preservation of the art. A fresco in a Roman bar ('Casaggiato del Termopolo') dated between 98 and 138 depicts a vegetable that is conic shaped and vaguely yellow, which might be one of the first representations of a cultivated carrot, although it is more likely to be a parsnip. In 512, we see next to a pale yellow wild one—an orange, branched carrot depicted in the *Juliana Anicia Codex*. From the 11th century drawings of (yellow) carrots started to



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