

# Understanding abiotic stress signalling in wheat through (phospho)proteomics

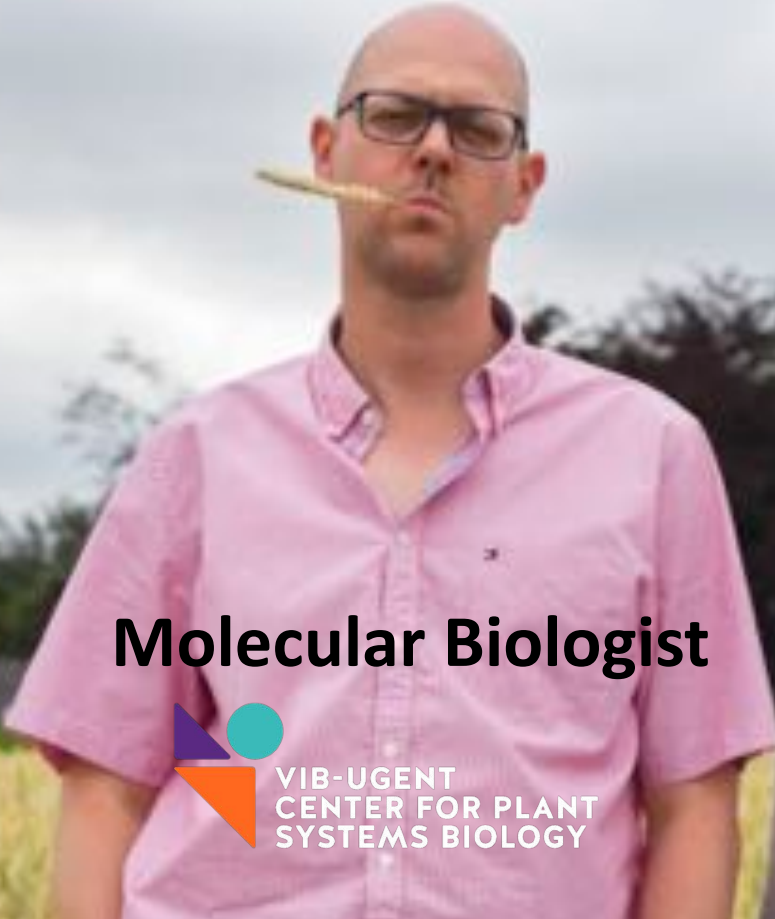
Prof. Dr. Ive De Smet

# INTRODUCTION – #ArtGenetics



The Harvesters - Pieter Bruegel the Elder, 1565 (downloaded from [images.metmuseum.org/CRDImages/ep/original/DP119115.jpg](https://images.metmuseum.org/CRDImages/ep/original/DP119115.jpg); public domain)

# INTRODUCTION – #ArtGenetics



**Molecular Biologist**



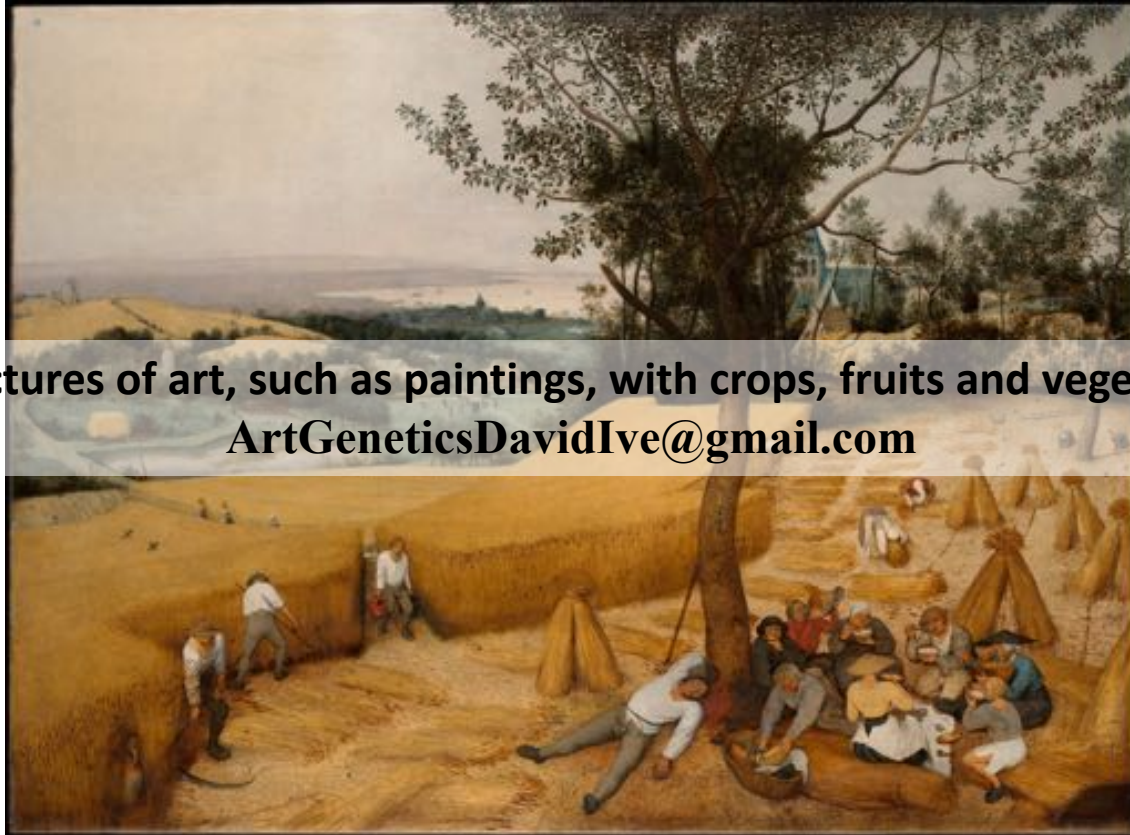
VIB-UGENT  
CENTER FOR PLANT  
SYSTEMS BIOLOGY



**(Art) historian**



# INTRODUCTION – #ArtGenetics



**Send pictures of art, such as paintings, with crops, fruits and vegetables to  
[ArtGeneticsDavidIve@gmail.com](mailto:ArtGeneticsDavidIve@gmail.com)**

# INTRODUCTION – Wheat is under stress

Seed Borne Diseases

Rusts

Viral Diseases

Crown and Root Rot Diseases

Cold Stress

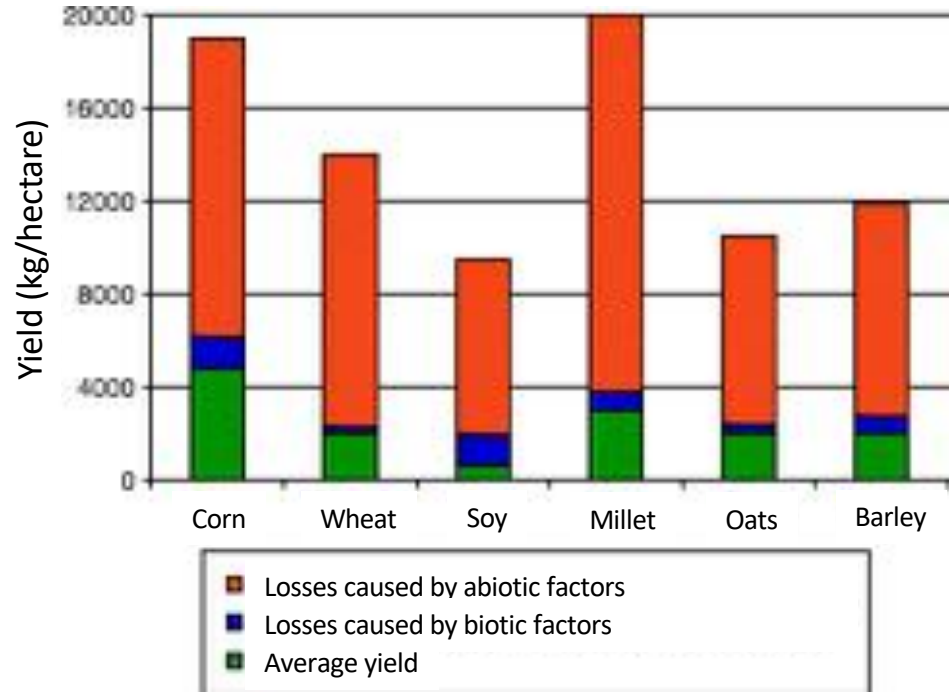
Water Logging Stress

Heat Stress

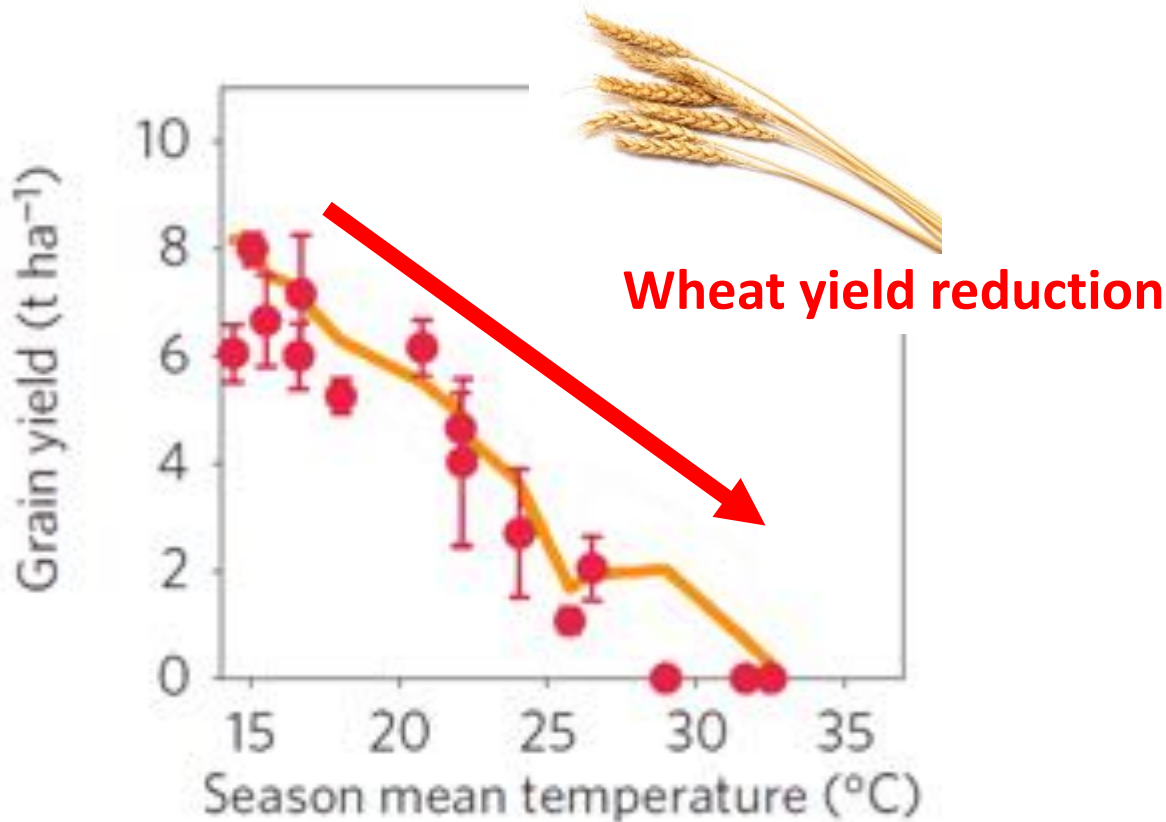
Minerals Stress

Drought Stress

# INTRODUCTION – Abiotic stress impacts wheat yield

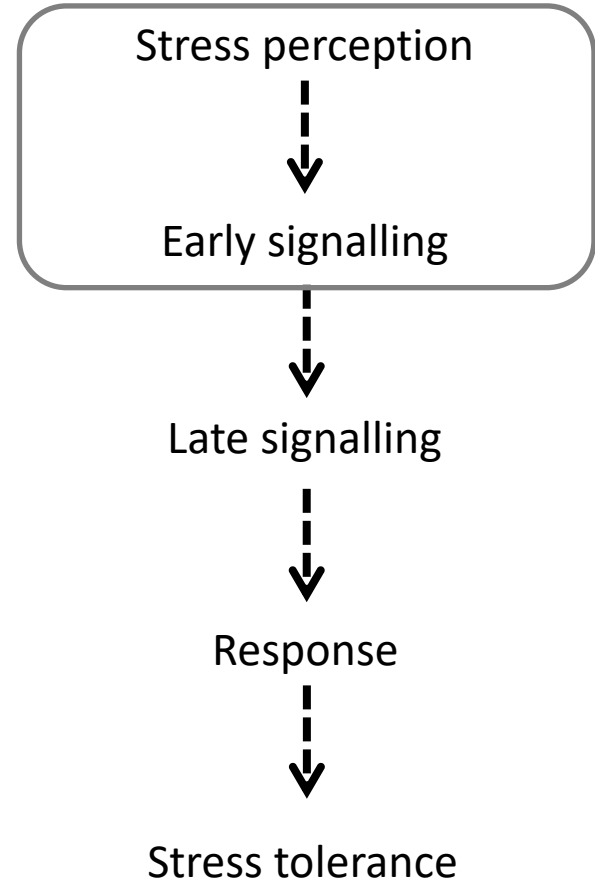
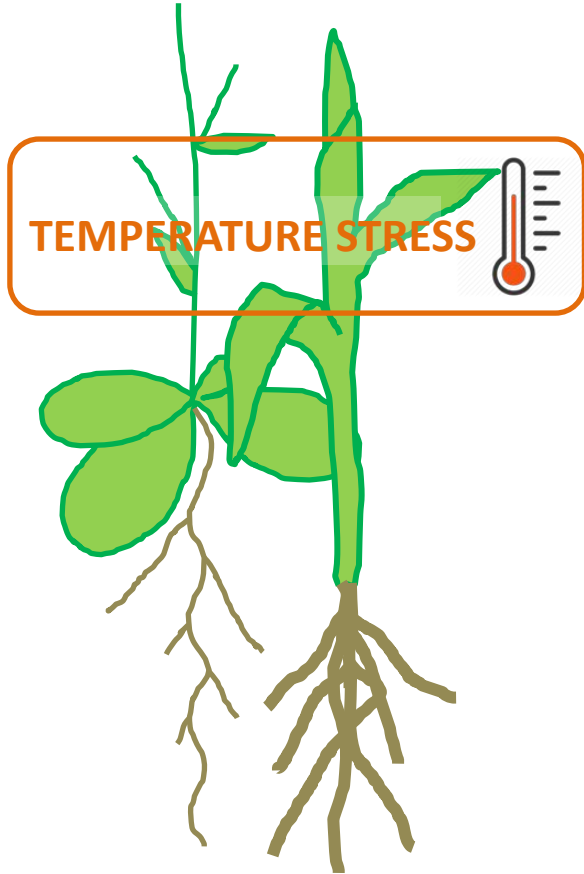


# INTRODUCTION – High temperature reduces crop yield



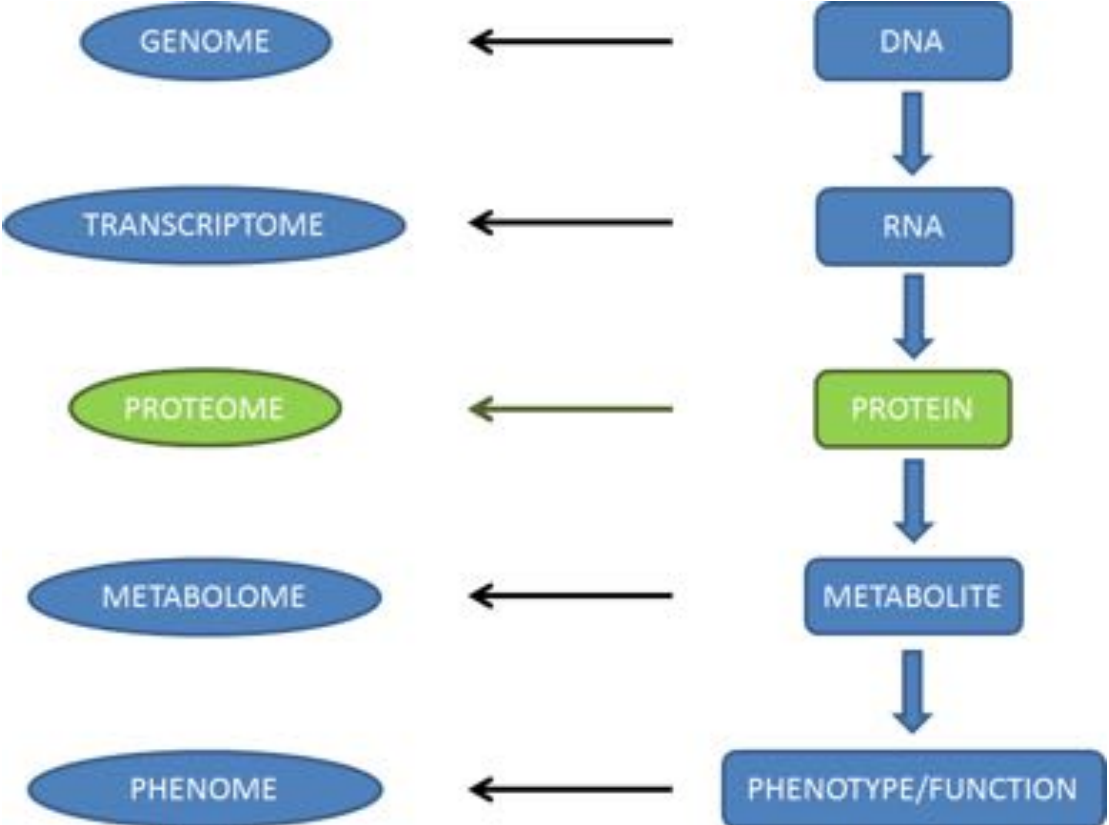
Adapted from Asseng et al (2014) Nature Climate Change 5:143-147

# RESEARCH FOCUS – Stress perception and early signalling

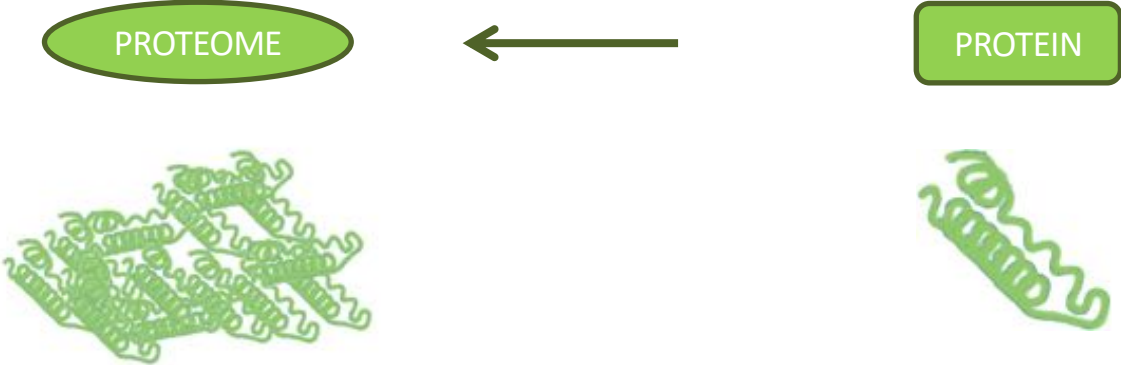




# RESEARCH FOCUS – Stress perception and early signalling



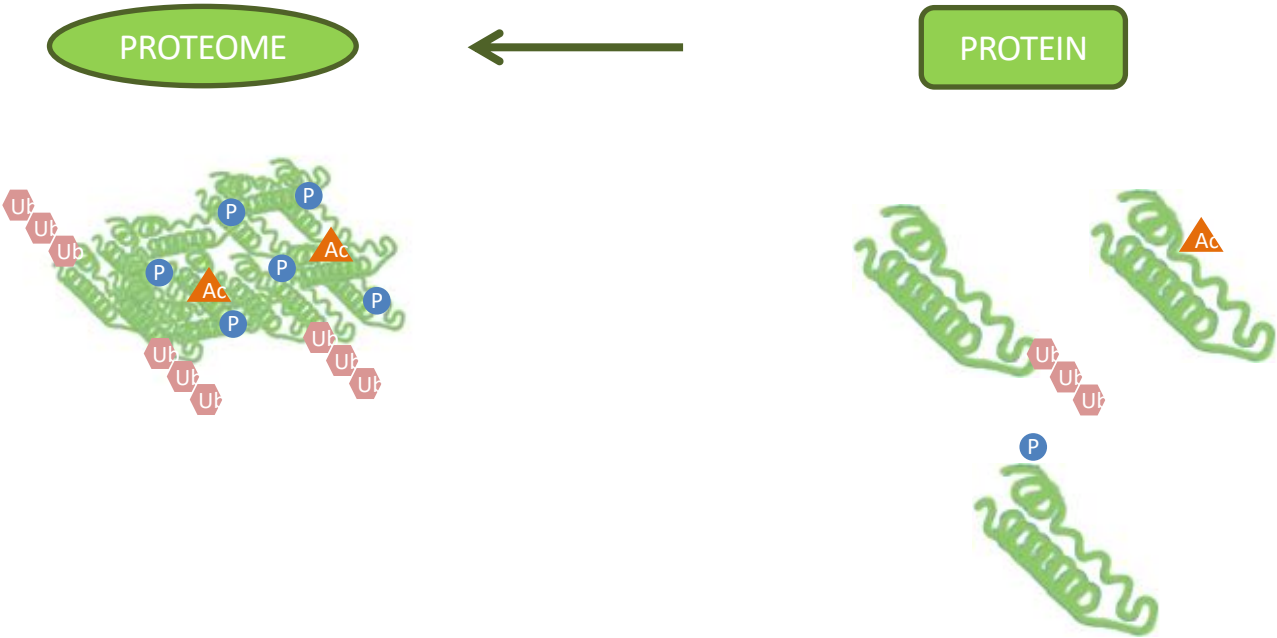
# INTRODUCTION – Protein pool expands through post-translational modifications



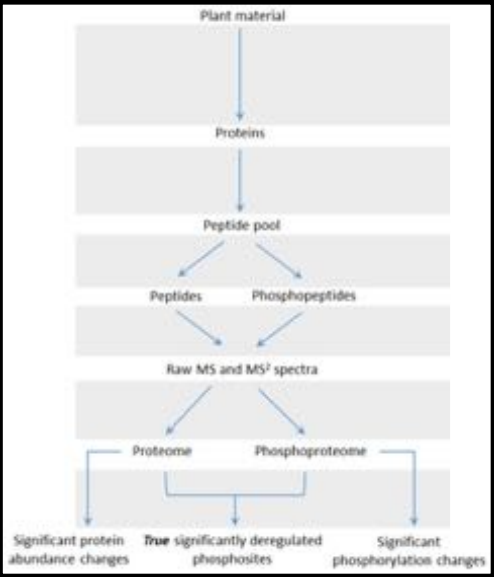
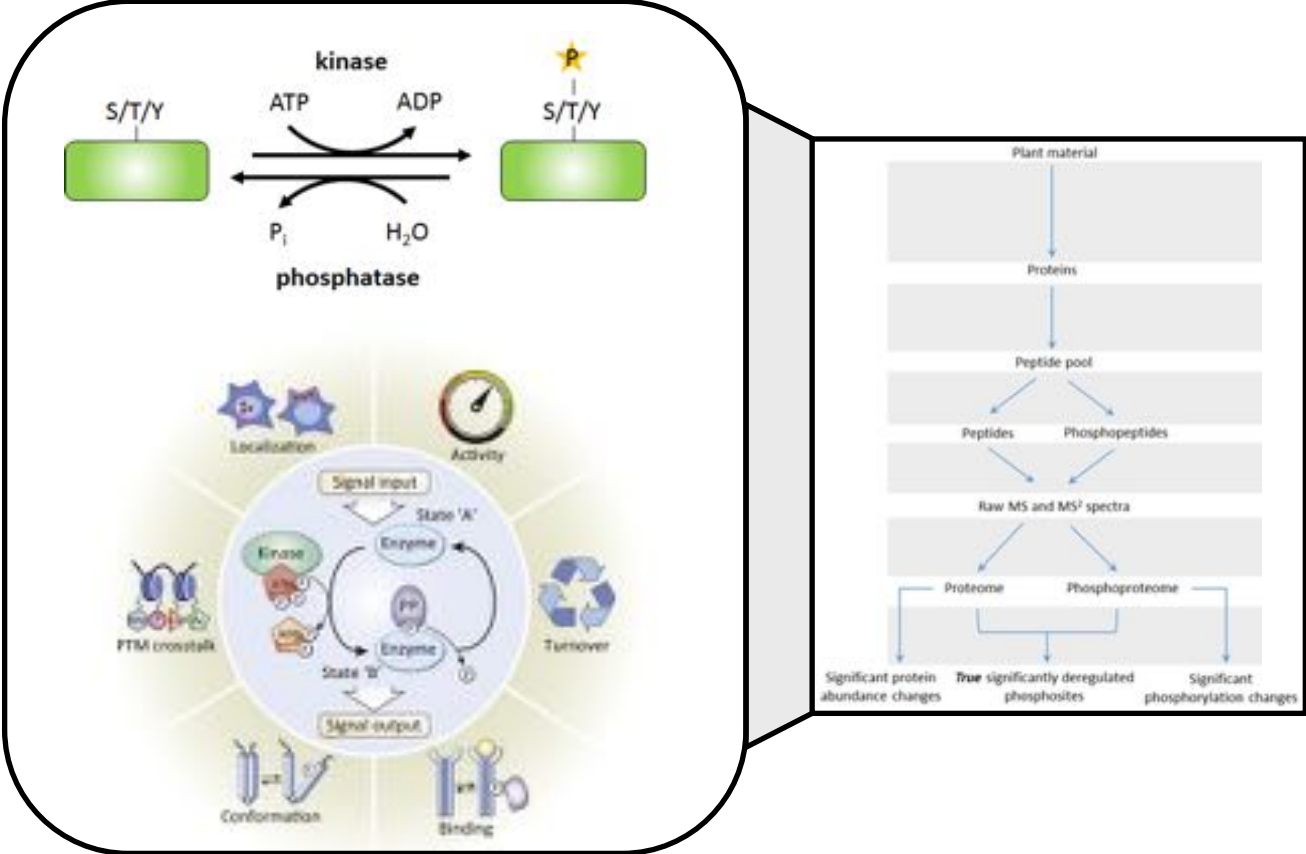
# INTRODUCTION – Protein pool expands through post-translational modifications



# INTRODUCTION – Protein pool expands through post-translational modifications



# APPROACH – Phosphorylation is involved in everything



## APPROACH – Mass spectrometry-based approaches require well-annotated genome

IWGSC PopSeq PGSB/MIPS v2.2 database (100 344 entries) (from wheatproteome.org)

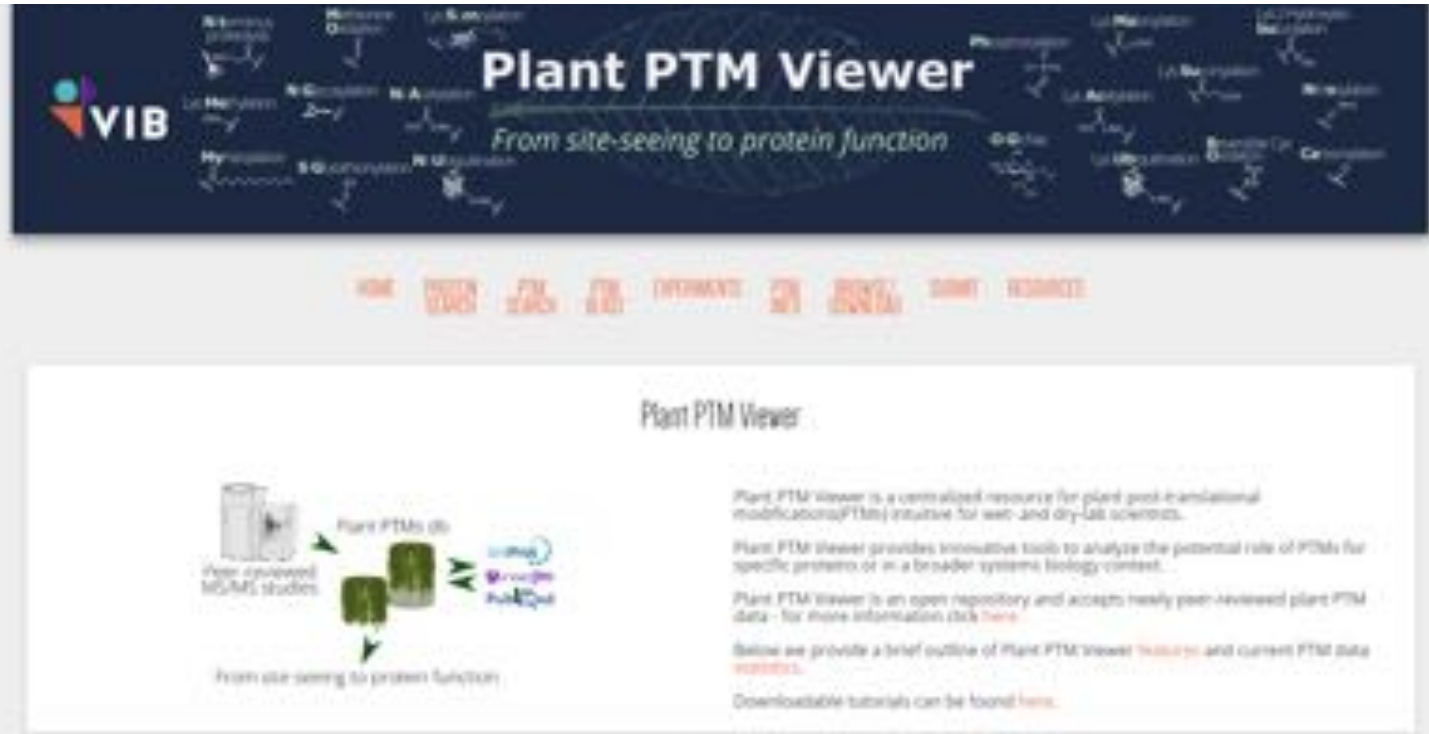
vs.

IWGSC RefSeq v1.0 database for *Triticum aestivum* (137 052 entries) (wheat-urgi.versailles.inra.fr/Seq-Repository/Assemblies)

→ an increase of 30% and 34% of identifications for leaf and spikelet samples, respectively

→ seems to correlate with the increase of 36.5% in the number of entries

# APPROACH – Plant PTM Viewer to explore the data

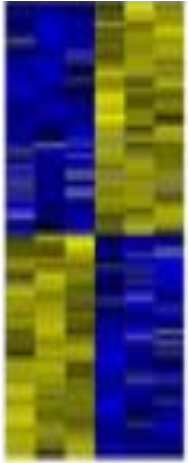


The image shows a screenshot of the Plant PTM Viewer website. At the top, there is a dark blue header with the VIB logo on the left and the text "Plant PTM Viewer" in large white font. Below the title is the tagline "From site-seeing to protein function". The header also features a network diagram of protein interactions. Below the header is a navigation bar with orange icons and labels for "HOME", "ABOUT", "FAQ", "CONTACT", "TUTORIALS", "NEWS", "CONTACT", "DONATE", and "RESOURCES". The main content area has a white background with the title "Plant PTM Viewer" centered. Below the title is a diagram showing a flow from "Peer-reviewed NGS studies" to "Plant PTMs (db)" and then to "Protein structure" and "Protein function". The text below the diagram reads "From site-seeing to protein function". To the right of the diagram is a text block describing the tool: "Plant PTM Viewer is a centralized resource for plant post-translational modifications (PTMs) initiative 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 [sources](#). Downloadable tutorials can be found [here](#)."

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

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

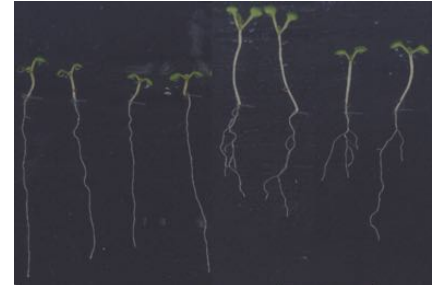
Relevant candidates in wheat



Validation in wheat



Mode-of-action in *Arabidopsis*





## EXAMPLE 1 – Difference in protein level dictates stress tolerance

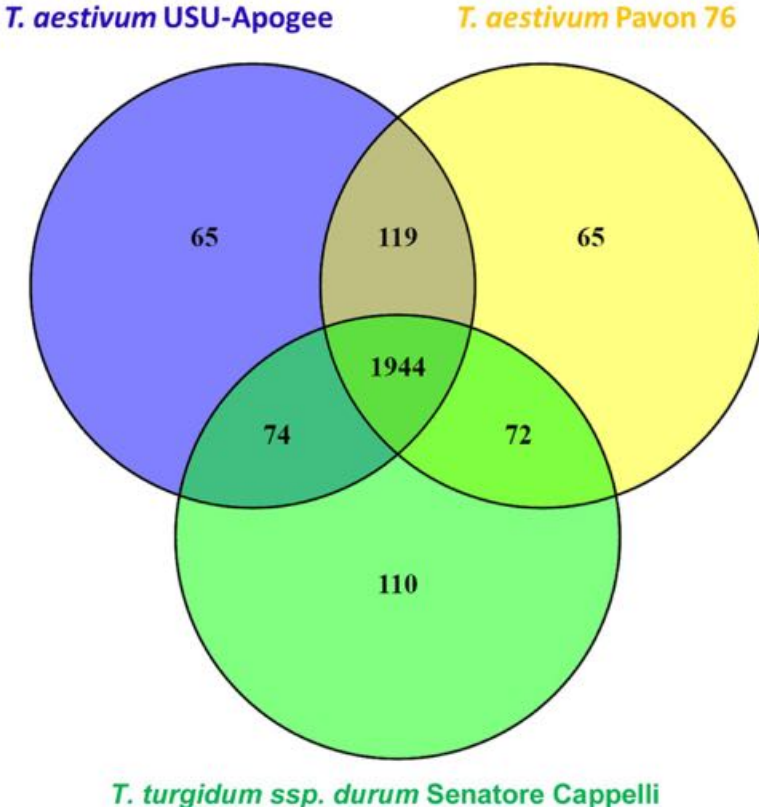


# Comparative wheat proteomics

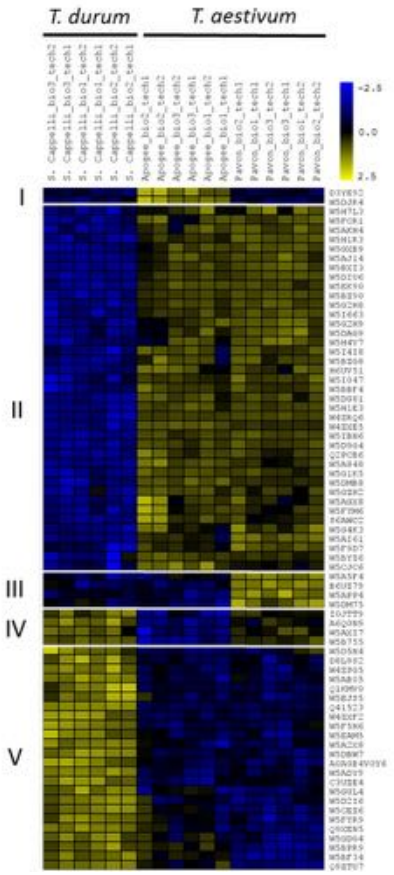


S. Cappelli      Pavon 76      USU-Apogee  
*T. turgidum ssp. durum*      *T. aestivum*

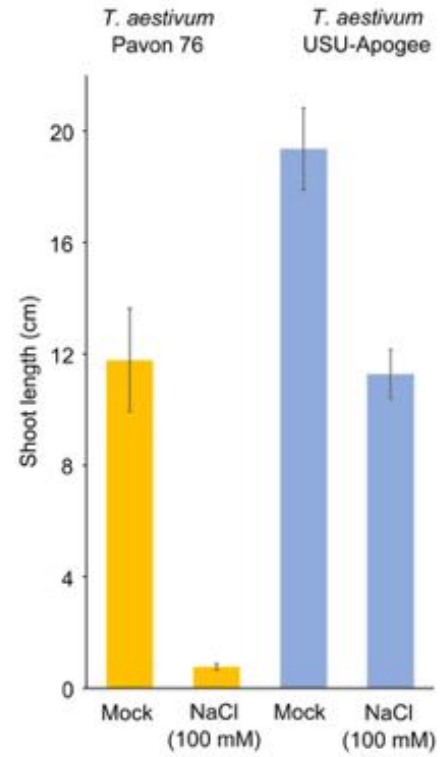
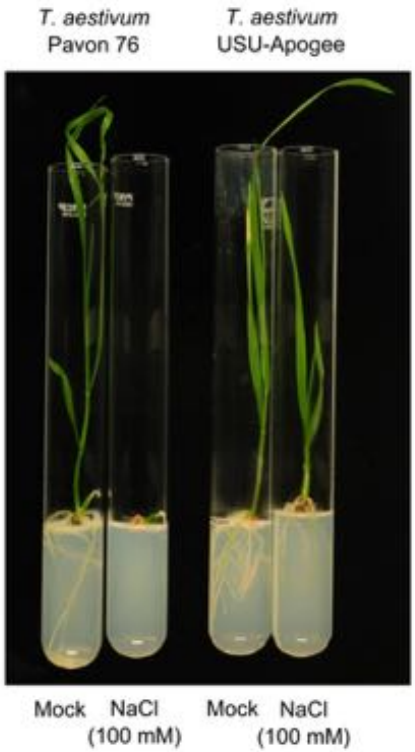
# Comparative wheat proteomics



# Comparative wheat proteomics



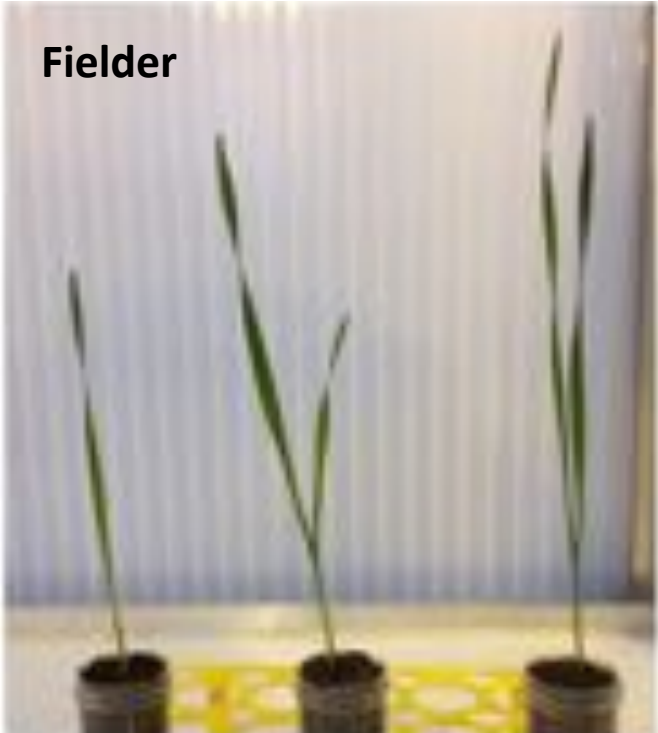
# Comparative wheat proteomics



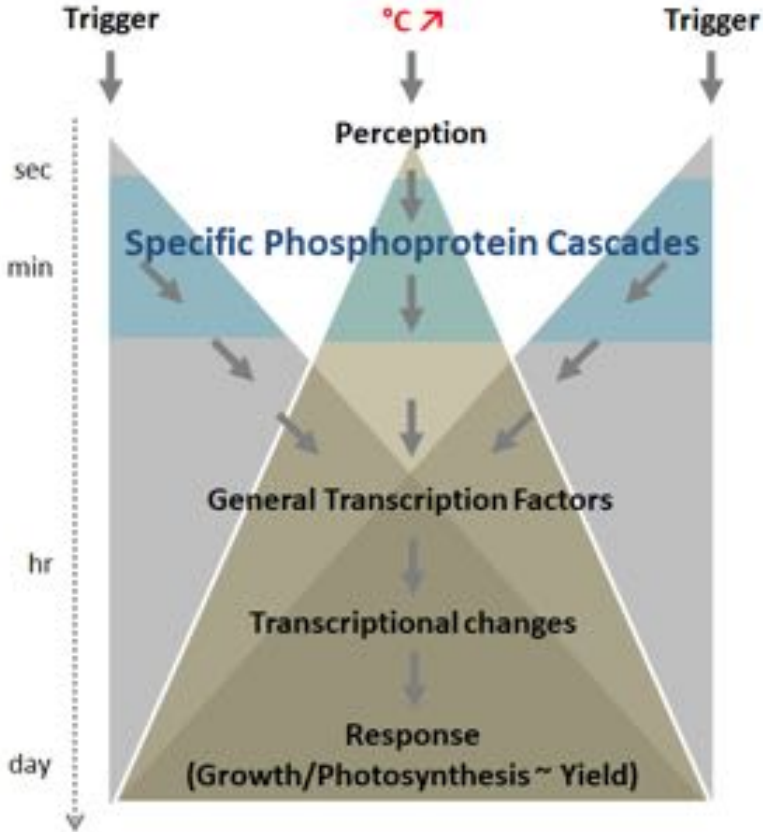
## EXAMPLE 2 – Towards an early temperature-responsive wheat phosphoproteome



# High temperature impacts wheat seedling growth

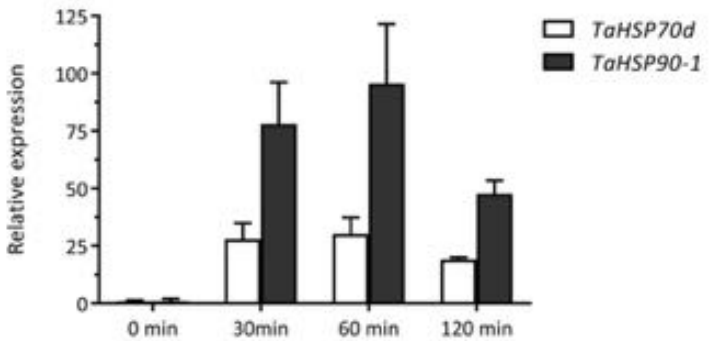
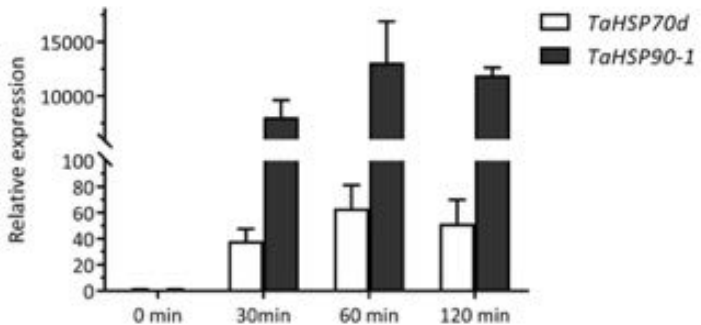


# Focus on early and temperature-specific signalling

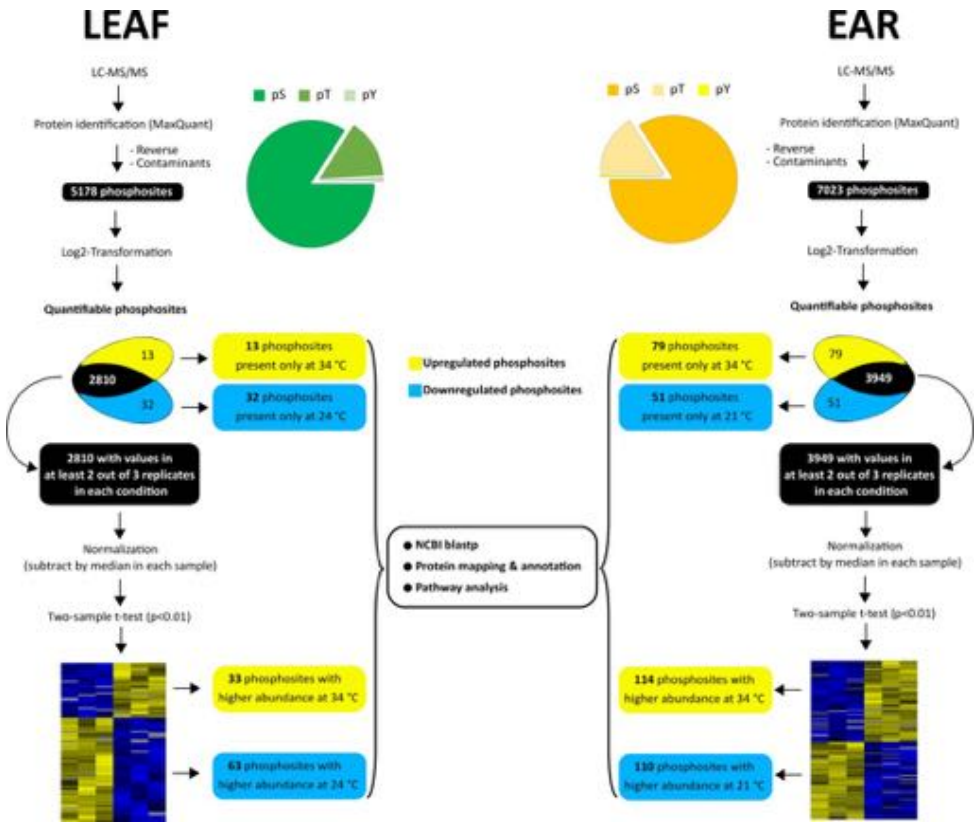




# HEAT SHOCK PROTEINs are transcriptionally rapidly up-regulated

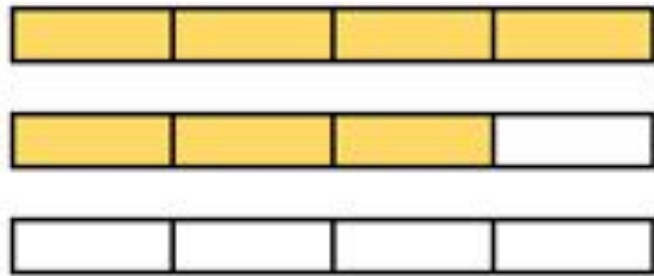


# Towards an early temperature-responsive wheat phosphoproteome

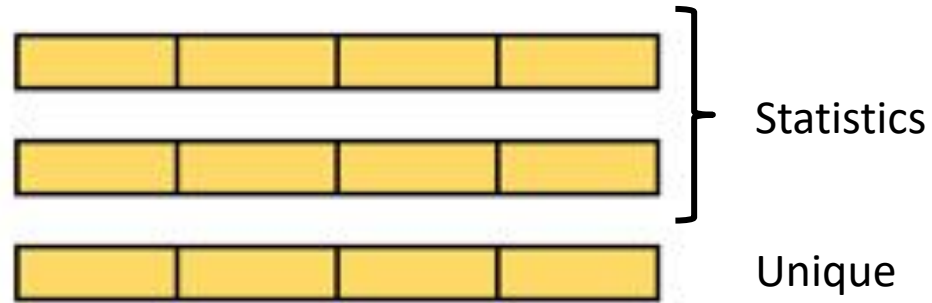


# Towards an early temperature-responsive wheat phosphoproteome

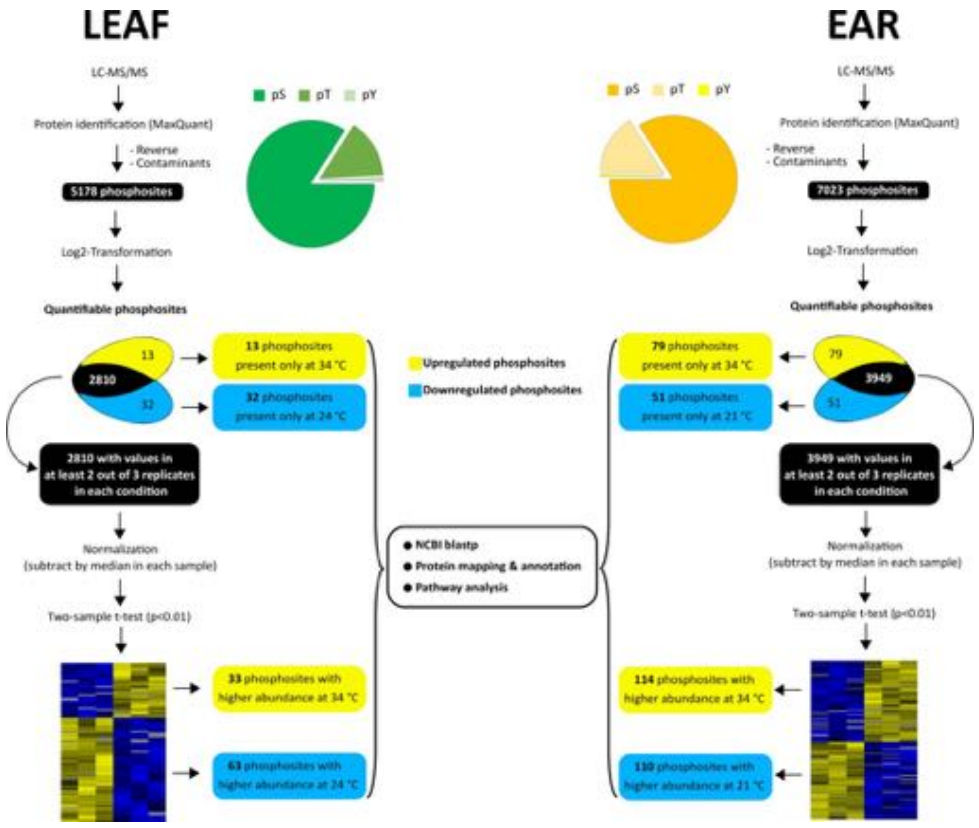
CONTROL



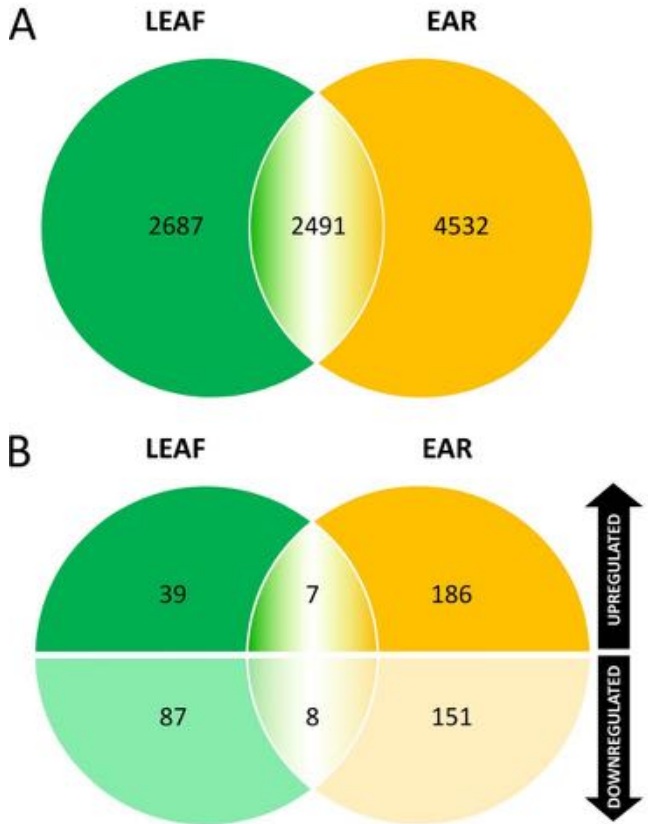
TREATMENT



# Towards an early temperature-responsive wheat phosphoproteome



# Towards an early temperature-responsive wheat phosphoproteome

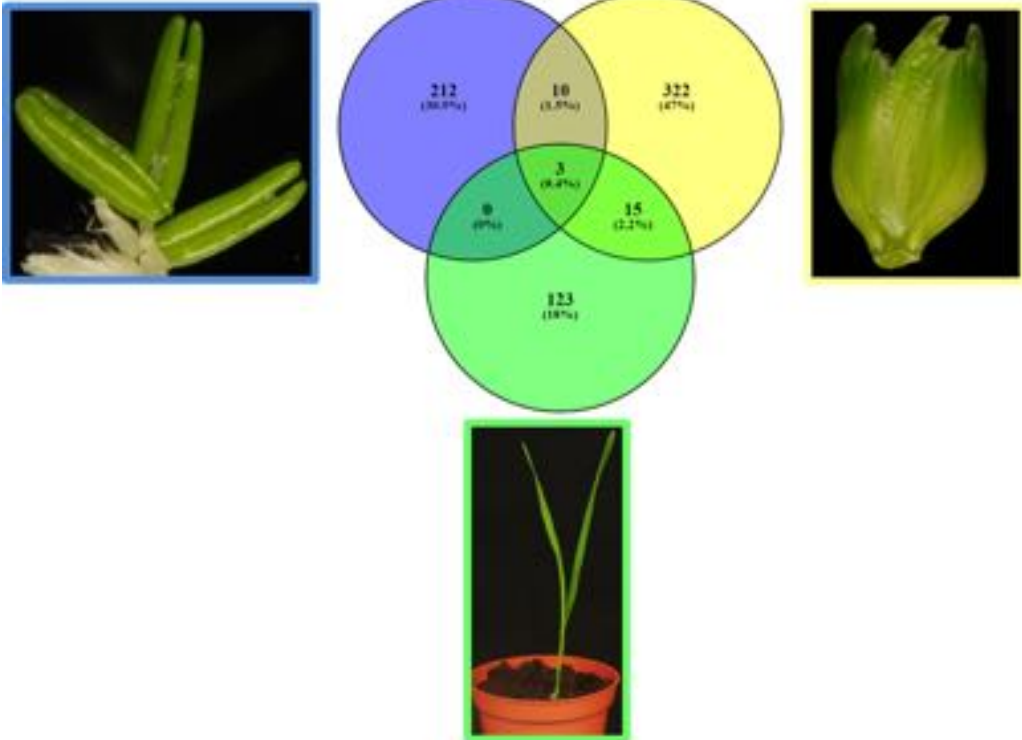


**EXAMPLE 3 – Capturing the early temperature-responsive wheat phosphoproteome**

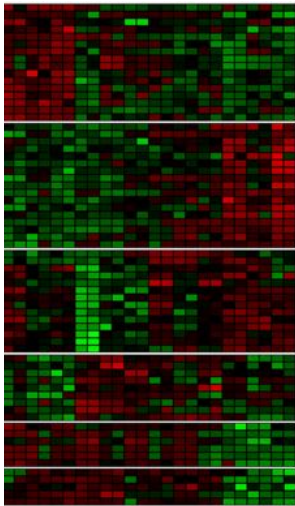
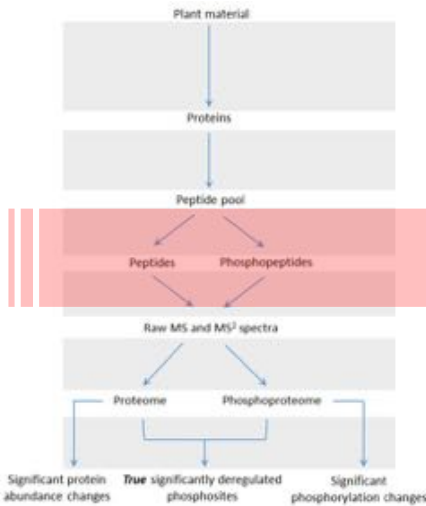
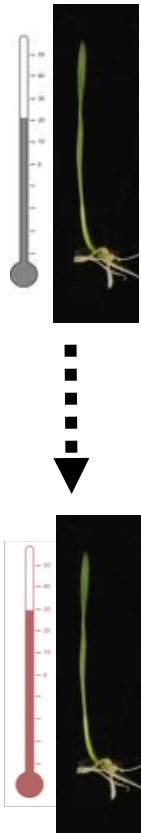


# Towards an early temperature-responsive wheat phosphoproteome

Differential phosphosites after 60 min exposure to high temperature



# Wheat phosphoproteome pinpoints TARGETS OF TEMPERATURE

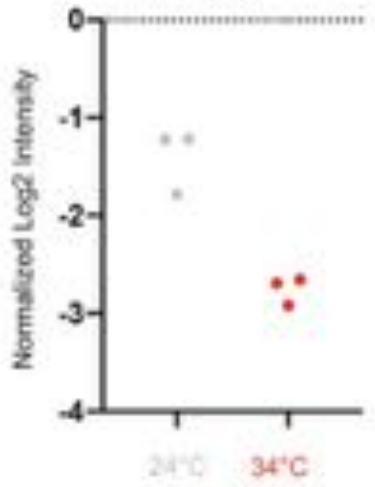


**TARGET  
of  
TEMPERATURE  
(TOT)**

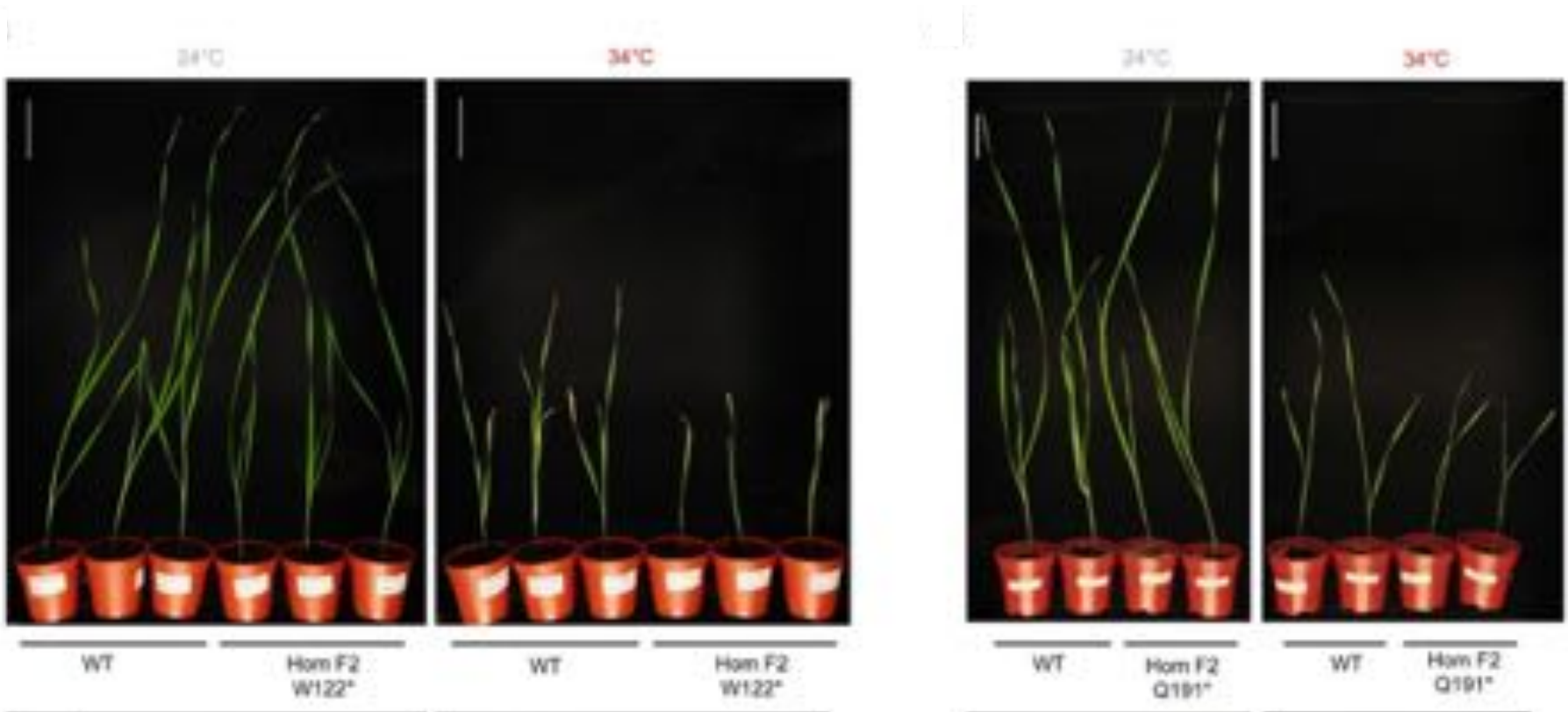


# TOT3 phosphostatus is regulated in wheat at high temperature

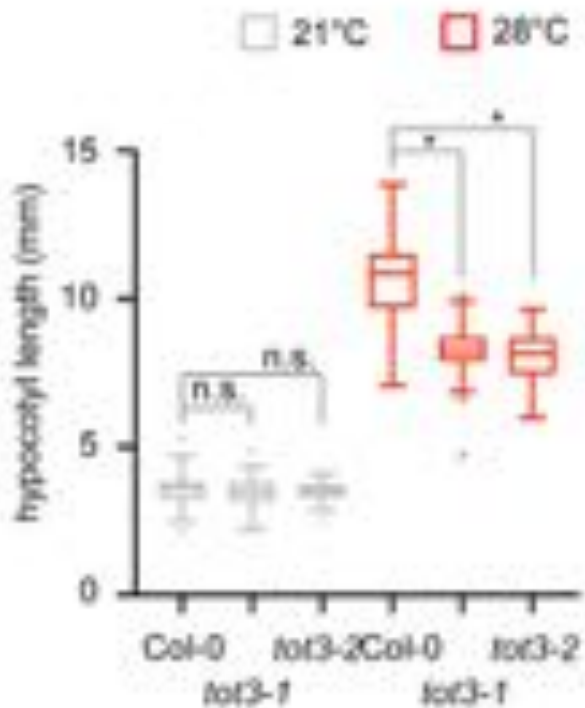
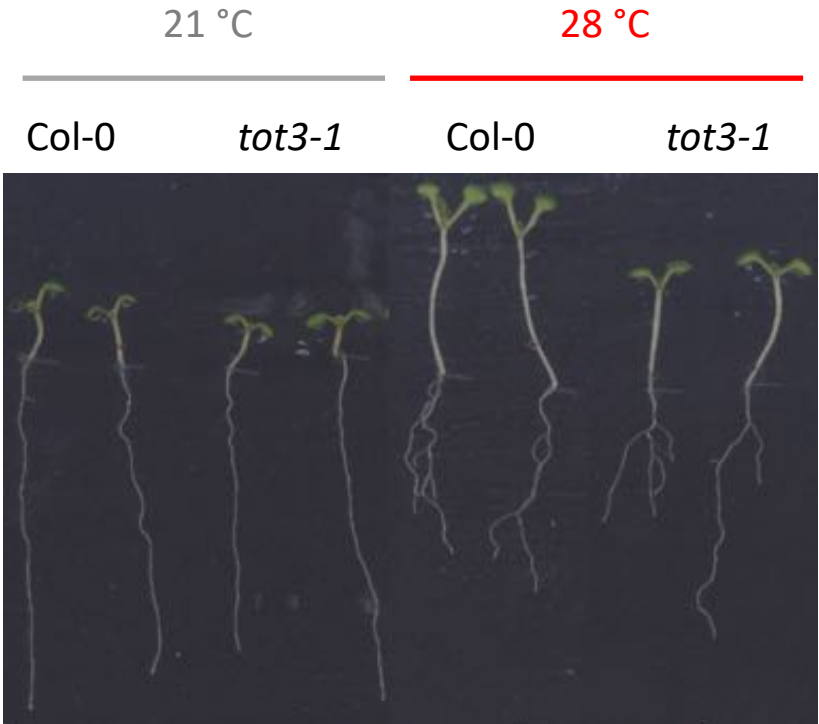
TOT3 – S394



# TOT3 plays a role in temperature-responsive seedling growth



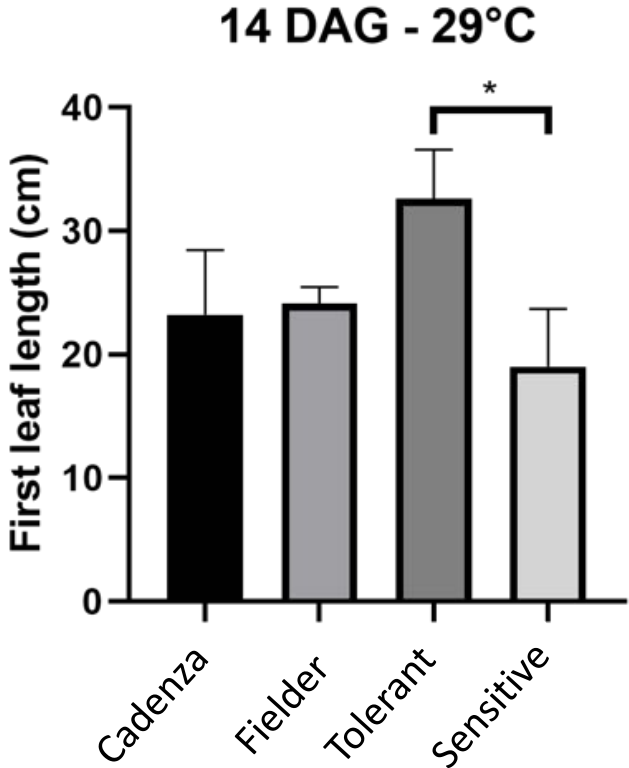
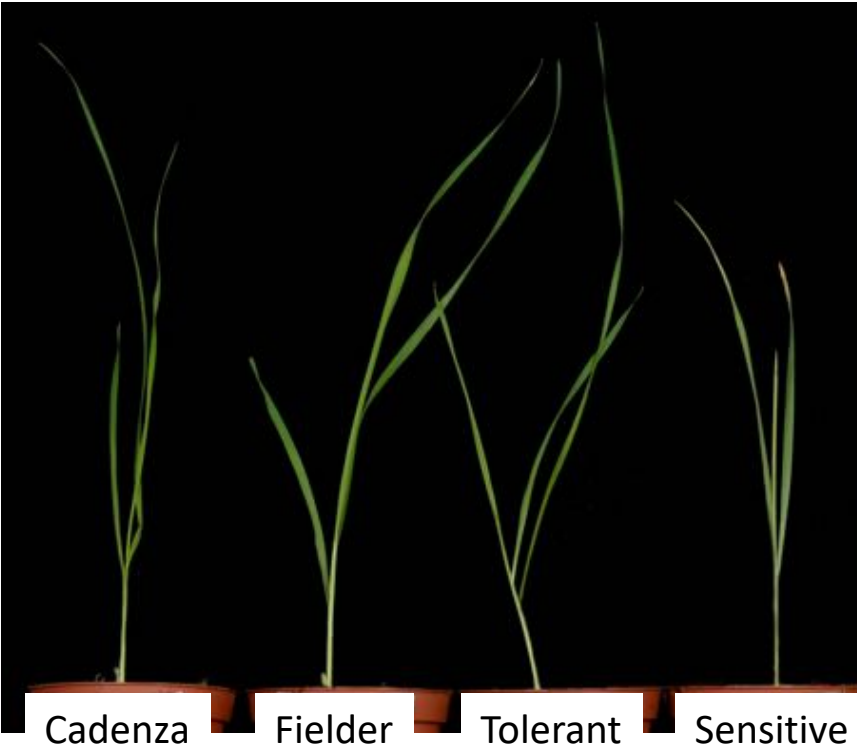
# TOT3 is a conserved growth regulator at high temperature



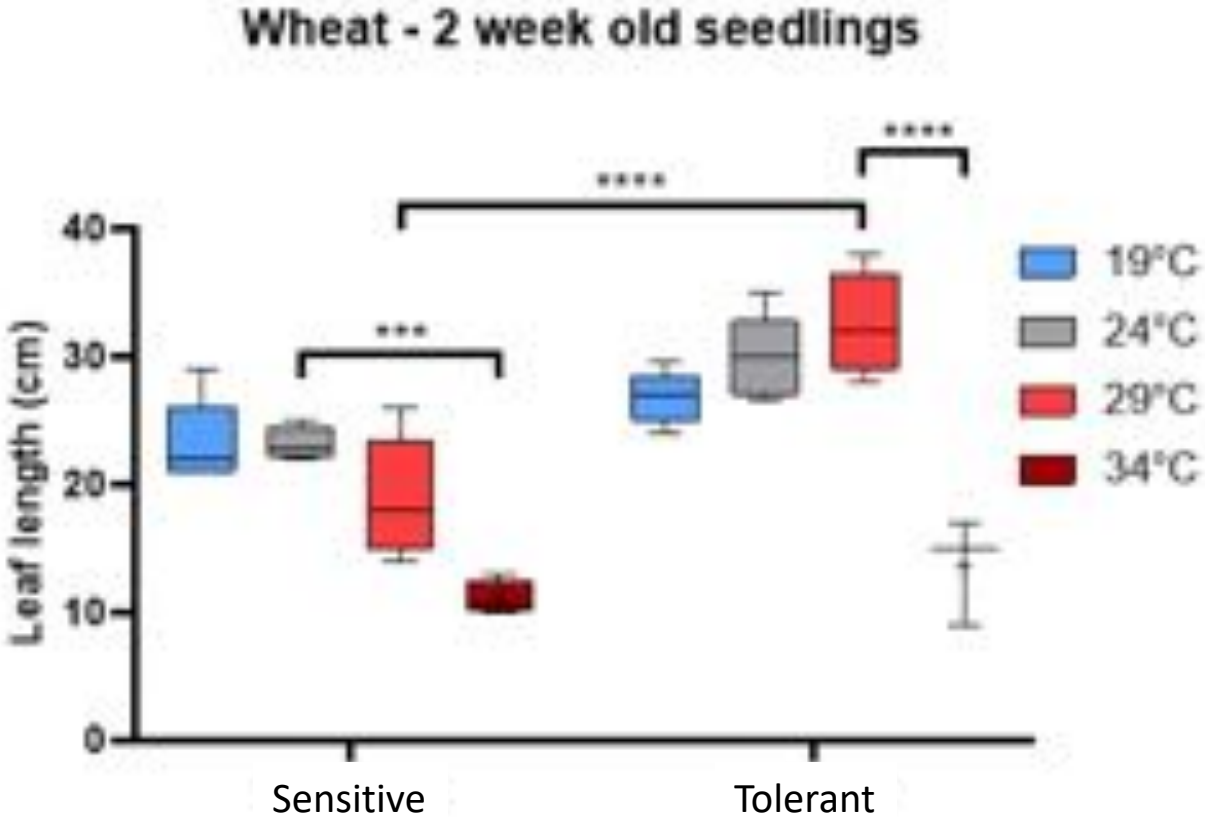
**EXAMPLE 4 – Capturing markers for temperature tolerance and sensitivity**



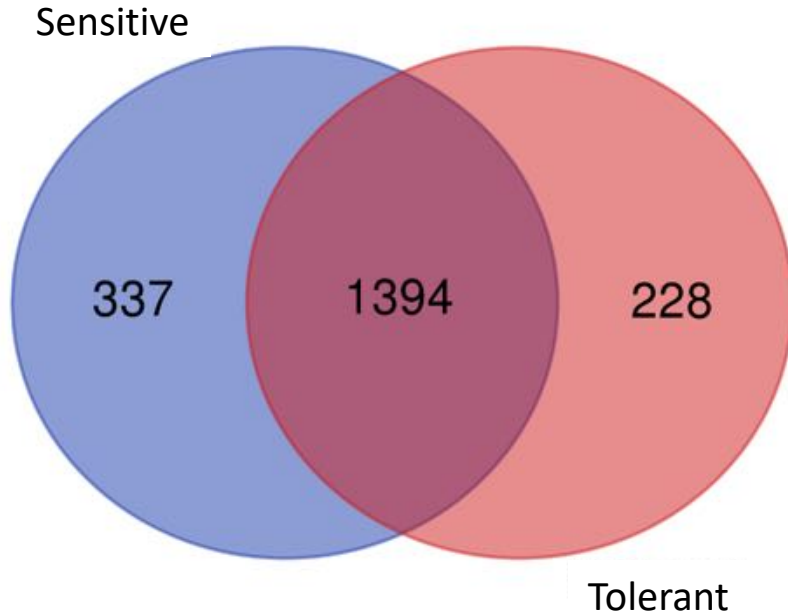
# High temperature impacts wheat seedling growth



# High temperature impacts wheat seedling growth



# High temperature impacts wheat seedling phosphoproteome



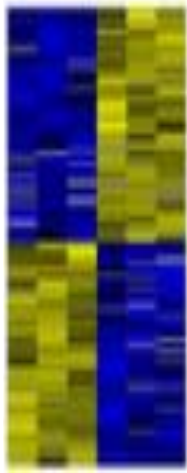
	Tolerant (Log2FC)	Sensitive (Log2FC)
TaTOT100	-2,53	4,83
TaTOT101	-1,94	2,62
TaTOT102	-1,49	6,50
TaTOT103	1,82	-4,30

# CONCLUSION





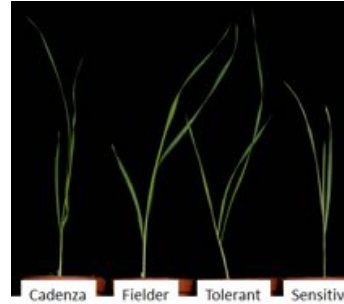
## WHEAT (PHOSPHO)PROTEOME



(Temperature) signalling: e.g. TOT3

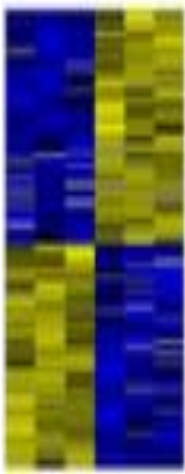


Novel breeding markers



# CONCLUSION

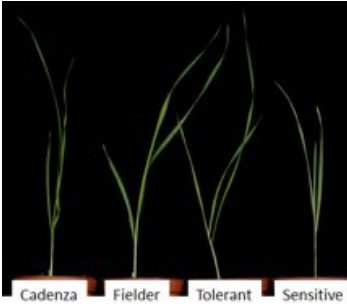
## WHEAT (PHOSPHO)PROTEOME



(Temperature) signalling: e.g. TOT3



Novel breeding markers



*“Farming looks mighty easy when your plow is a pencil and you're a thousand miles from the ~~corn~~ field.”  
wheat*

Dwight D. Eisenhower

# WHO DID THE WORK?




## Group alumni

L. Joos  
I. Verstraeten  
N. Nikonorova

## Collaborators

K. Gevaert  
C. Uauy  
IWGSC  
N. Collins  
M. Xin  
T. Savchenko

 [www.psb.ugent.be/functional-phosphoproteomics](http://www.psb.ugent.be/functional-phosphoproteomics) and [ive.desmet@psb.vib-ugent.be](mailto:ive.desmet@psb.vib-ugent.be)

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PROVEN  
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27% success rate in 2018 call for MSCA  
Compared to overall rate of 12%