

## Introduction

### Background:

- Heat stress significantly impacts global wheat productivity.
- The frequency of heat waves occurrence during wheat flowering has alarmingly increased in the last few years.

### Aims:

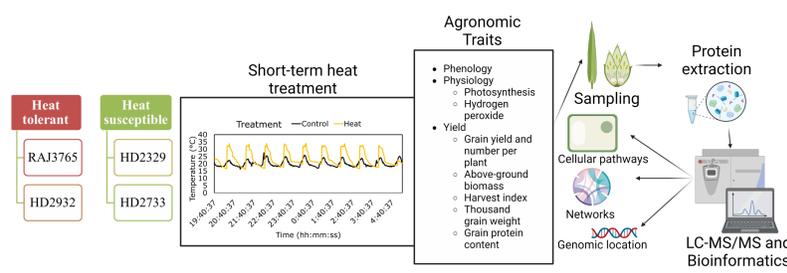
- Investigate the effects of heat stress on physiology, yield and yield components in wheat genotypes with contrasting heat tolerance.
- Identify proteins with differential abundances in flag leaves and spike tissues.

## Methods

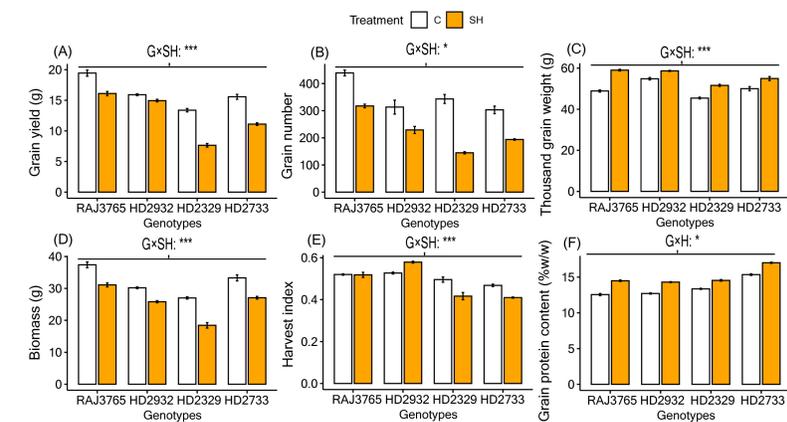
- Wheat genotypes with contrasting heat tolerance (two tolerant [RAJ3765 and HD2932] and two susceptible [HD2329 and HD2733]) were exposed to short-term heat stress at ear peep.
- Physiological traits (leaf gas-exchange and reactive oxygen species accumulation), were measured on days 1, 3 and 5 during the heat-treatment and on day 12 after recovery.
- Flag leaves and spike tissues were collected on days 1, 3 and 5 during the heat-treatment and on day 12 after recovery.
- Protein extraction was performed using a chloroform/methanol extraction method, developed in the Taylor lab.
- LC-MS/MS analysis was done using Thermo Exploris 480 with Dionex Ultimate 3000 series Ultra-High-Performance Liquid Chromatography.
- Bioinformatics was performed on label-free quantification output from MaxQuant using DEP (v. 1.22.0) package.
- Proteins with false discovery rate-corrected p-value  $\leq 0.05$  and  $2 \leq$  fold change  $\leq -2$  were considered as differentially abundant proteins.
- Functional annotation and over-representation analysis were done using MapMan (v.3.6.0RC1) with the PageMan tool. Networks were made using the WGCNA (v.1.72-1) package.
- Yield and yield components were measured post-maturity.

## Results

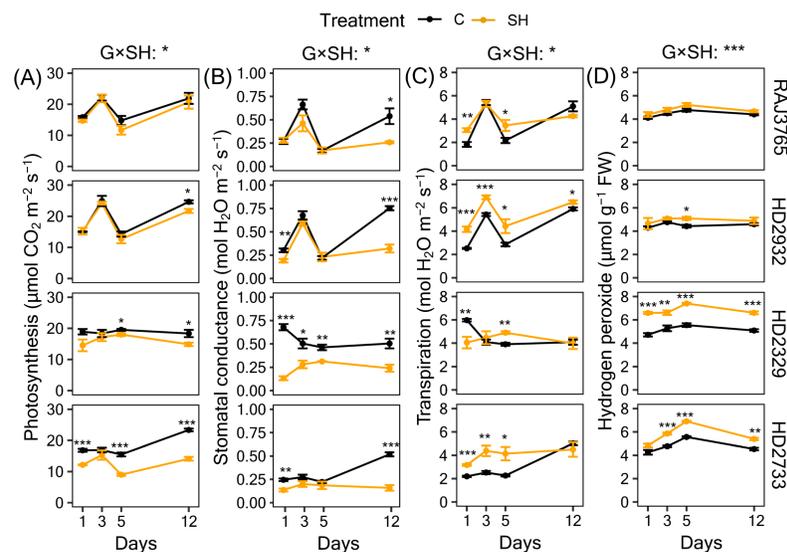
- Under short-term heat stress, heat-tolerant genotypes show less reduction in yield and yield components than heat-susceptible genotypes (Fig. 2).
- Thousand grain weight and grain protein content increased in all genotypes (Fig. 2).
- All physiological measurements show a significant genotype $\times$ treatment interaction, except for intercellular CO<sub>2</sub> (Fig. 3).
- We identified 31 and 60 proteins with significant differential abundance in flag leaves and spike tissues, respectively.
- Functional annotation of the differentially abundant proteins revealed key pathways responsive to heat stress.
- Correlations of co-expression networks of proteins and agronomics traits revealed proteins correlated with important agronomic traits (Fig. 4).
- Over-representation analysis in PageMan revealed the pathways that had proteins with significantly increased or decreased abundance in various comparisons (Fig. 5).



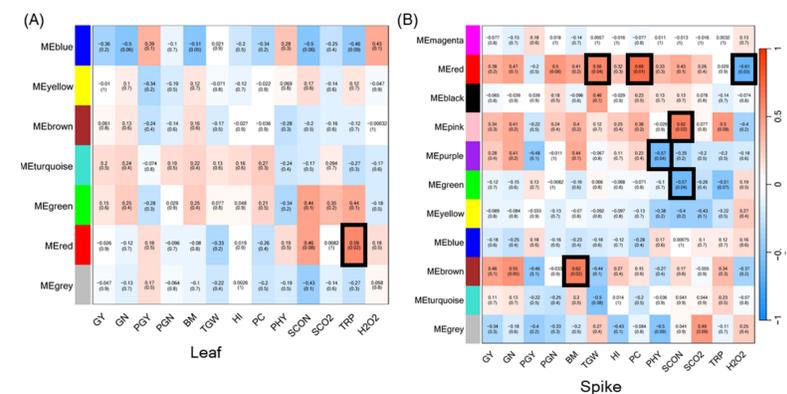
**Fig. 1** Graphical abstract of methodology.



**Fig. 2** Yield and yield components for control (C) and short-term heat treatment (SH), (A) Grain yield per plant (g), (B) grain number per plant, (C) thousand-grain weight (g), (D) above ground biomass per plant (g), (E) harvest index (HI), and (F) grain protein content (% w/w), for four wheat genotypes. Average  $\pm$  standard error (n=4), genotype $\times$ short-term heat treatment interactions (G $\times$ SH) and significance: \* p $\leq$ 0.05, \*\* p $\leq$ 0.01, \*\*\* p $\leq$ 0.001.

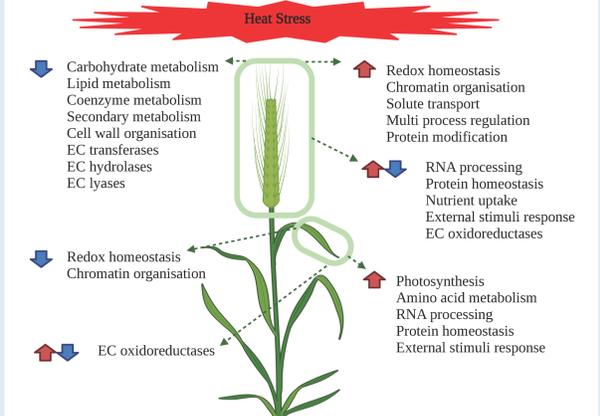


**Fig. 3** (A) Photosynthesis, (B) stomatal conductance, (C) intercellular CO<sub>2</sub>, (D) transpiration, and (E) hydrogen peroxide under control (C) and short-term heat treatment (SH) during the treatment period (Days 1, 3 and 5) and recovery (Day 12) for four wheat genotypes. Average  $\pm$  standard error (n=4), genotype $\times$ short-term heat treatment (G $\times$ SH), significance: \* p $\leq$ 0.05, \*\* p $\leq$ 0.01, \*\*\* p $\leq$ 0.001.



**Fig. 4** Co-expression protein modules and trait correlations for control (C)

**Fig. 4 (contd.)** and short-term heat treatment (SH) of (A) flag leaves and (B) spike tissues during the treatment period (days 1, 3, and 5) and recovery (day 12), for four wheat genotypes. Module to trait correlations (R<sup>2</sup> value and highlighted p-values $\leq$ 0.05).



**Fig. 5** Summary of biological processes associated with differentially abundant proteins for control (C) and short-term heat treatment (SH) of flag leaves and spike tissues during the treatment period (days 1, 3, and 5) and recovery (day 12), for four wheat genotypes. Red arrow indicates decreased abundance, blue arrow indicates increased abundance and combination of red and blue arrows indicate increase and decrease in different comparisons.

## Conclusions

### Key findings

- There were clear differences in the effects of heat treatment on the physiology, yield, and yield components of genotypes with contrasting heat tolerance.
- We identified novel proteins with differential abundances crucial for wheat heat tolerance.
- Correlations between co-expression networks of proteins and agronomic traits offer an alternate understanding of wheat heat tolerance mechanisms.

### Takeaway message

*"The proteins identified in this study provide crucial insights into the mechanisms of wheat heat tolerance."*



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Interested in plant biology, stress physiology, proteomics, mass spectrometry...



## Acknowledgements

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