Wheat Reproductive Heat Tolerance
Exploiting Pollen Viability, Stay-Green Trait and Spikelet Fertility

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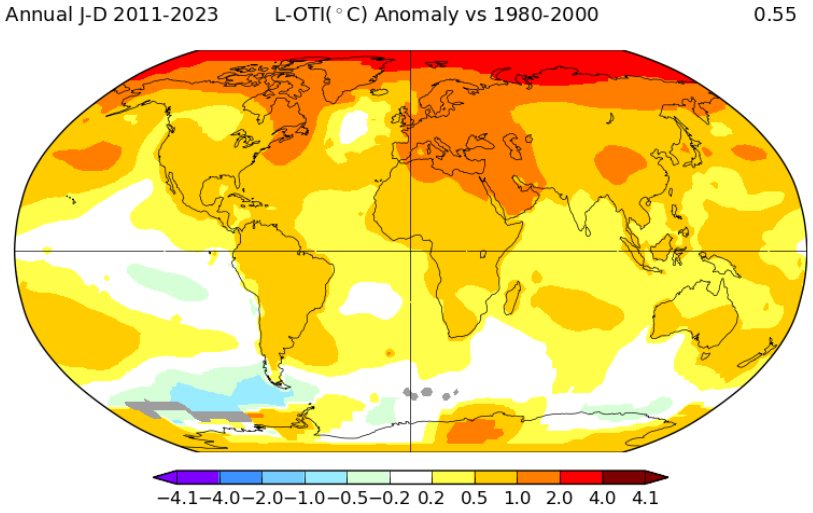
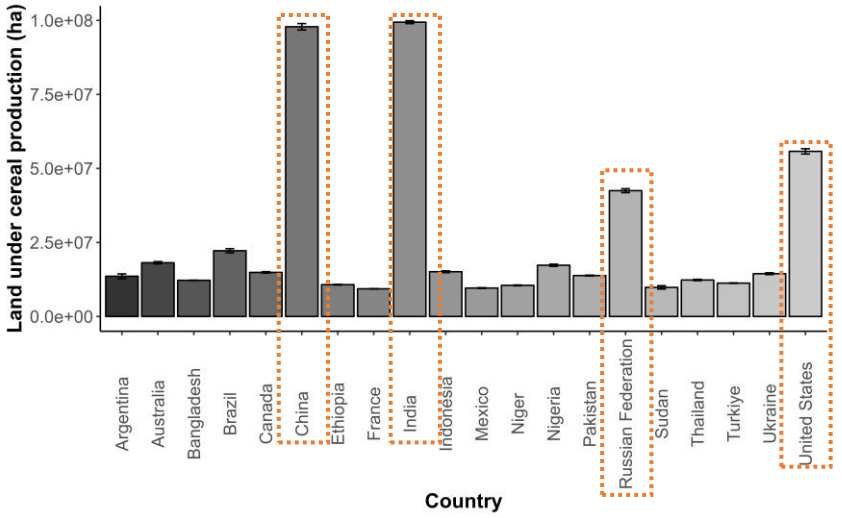
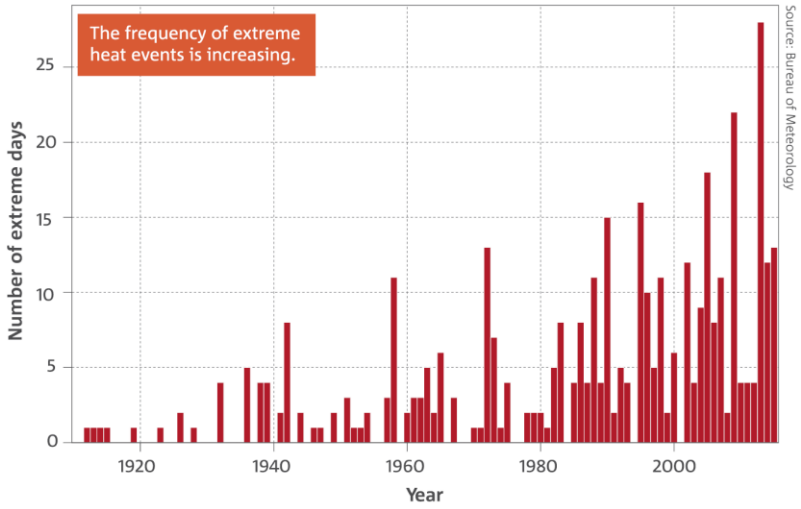
Climate Change and Heat Stress

- Global warming is one of the major outcome of climate change
- Annual temperature in 2011-2023 vs 1980-2000
- Temperature increase in the high wheat producing countries
- Increase of extreme heat waves in Australia



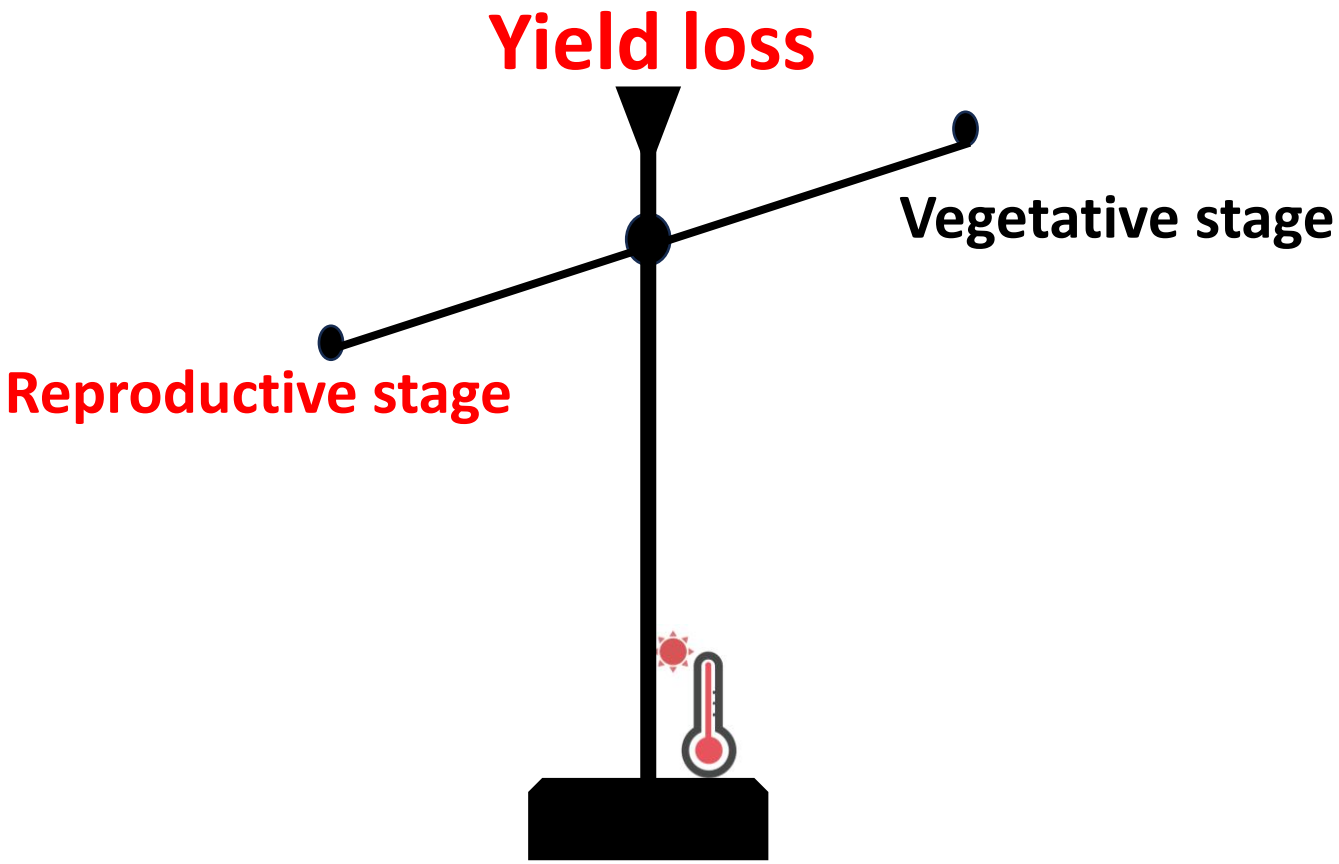


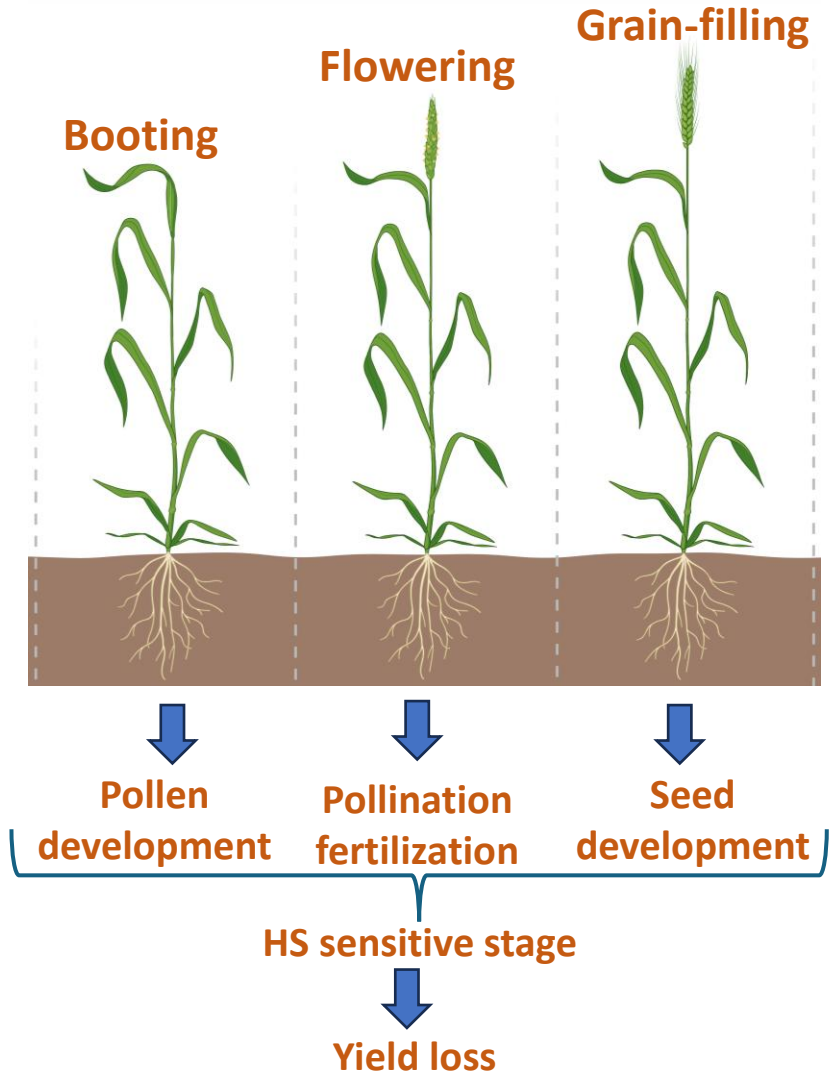


Siddique et al. 2025, Plant Stress

Reproductive Stage Heat Stress

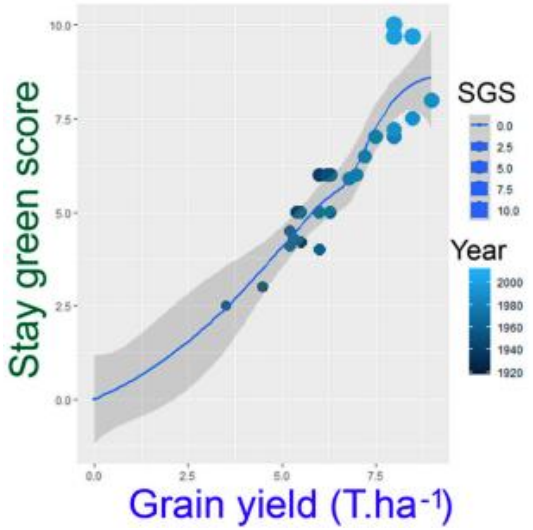
- Heat stress is one of the major limiting factors for wheat production
- HS during reproductive stage is more detrimental



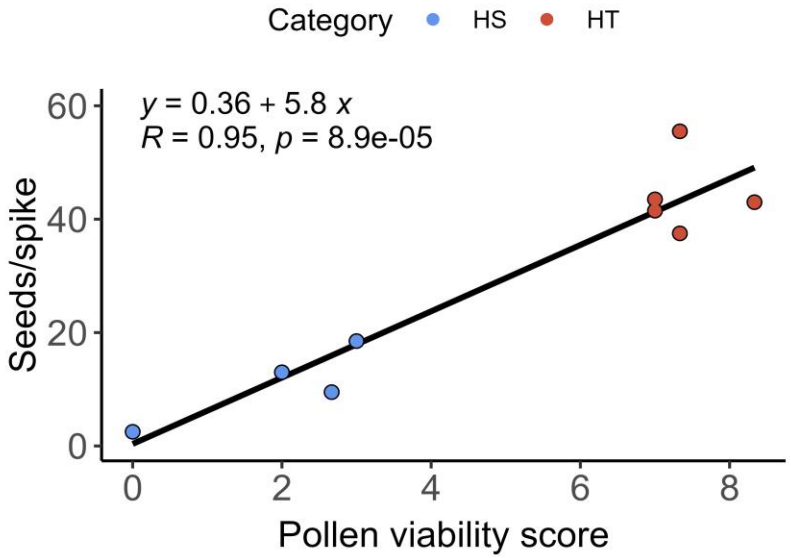


Key heat tolerance traits

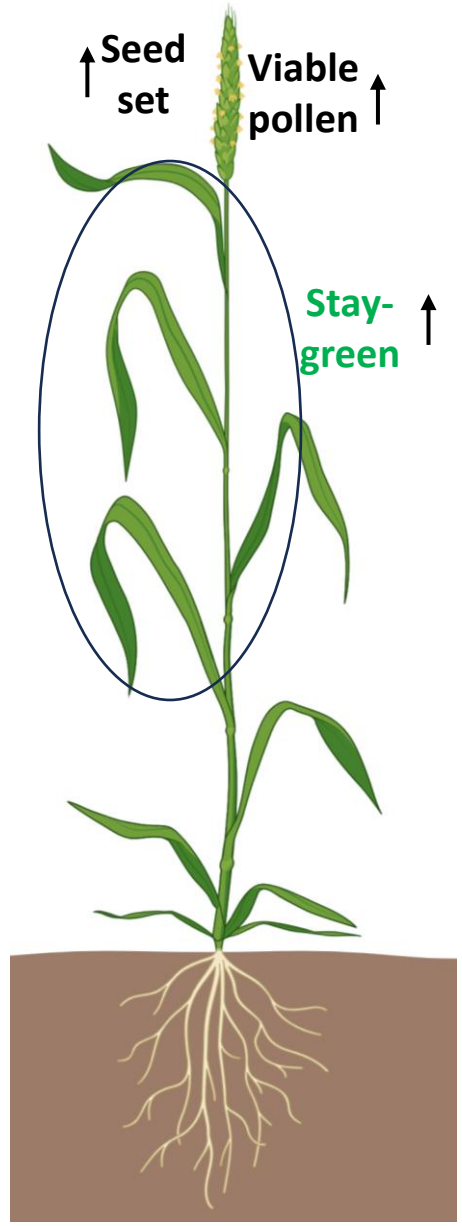
- Stay-green: stronger source strength, leading to energy supply to flower
- Viable pollen: Ensures the fertilization and seed set
- Strong source and viable pollen: higher seed set and yield



(Thomas et al., 2014)

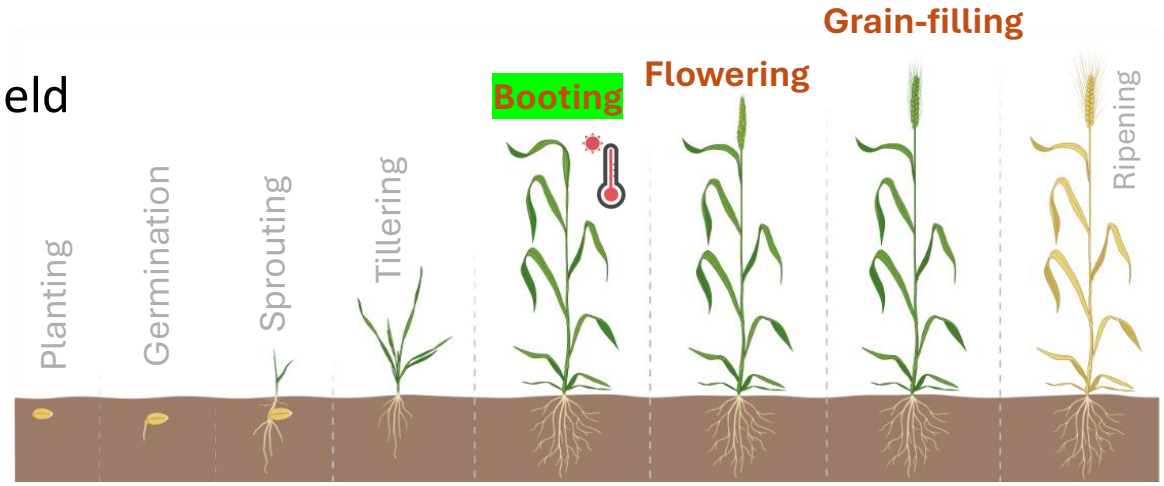


(Zhao, Siddique et al., 2024, aBIOTECH)



Limitations of Field-Based Study

- Large scale pollen and stay-green phenotyping in field condition limited
  - HS appearance at untargeted stage
  - Diversity and variation in natural population



- Lack of GWAS study on pollen viability and stay-green trait

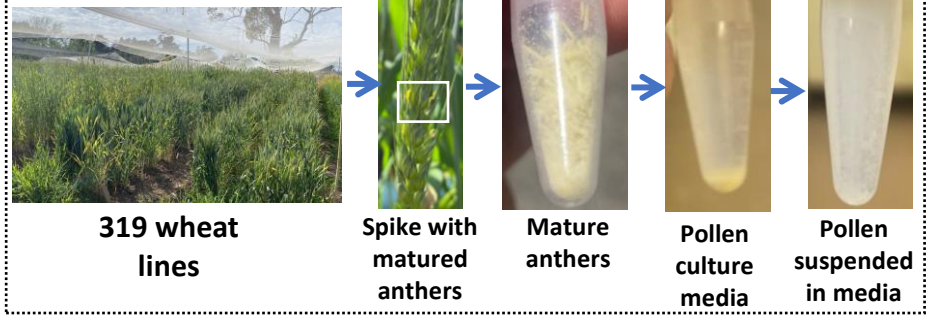
- This study was conducted

1. To establish flexible in-vitro methods for phenotyping PVS using field grown plants
2. To develop faster stay-green traits phenotyping in wheat under controlled heat condition
3. To identify major marker trait associations linked to heat stress tolerance

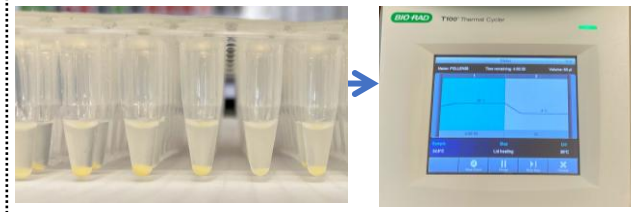
Pollen viability phenotyping

- Pollen viability score was recorded using high-throughput method (Zhao, Siddique et al., 2024)
- A total of 2871 images were scored for 319 lines in 3 replicates

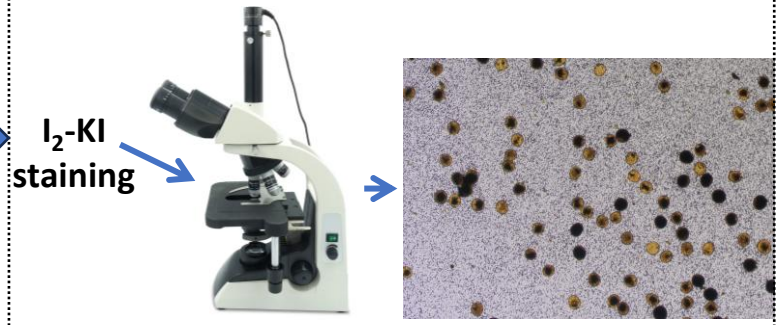
Step 1. Pollen collection



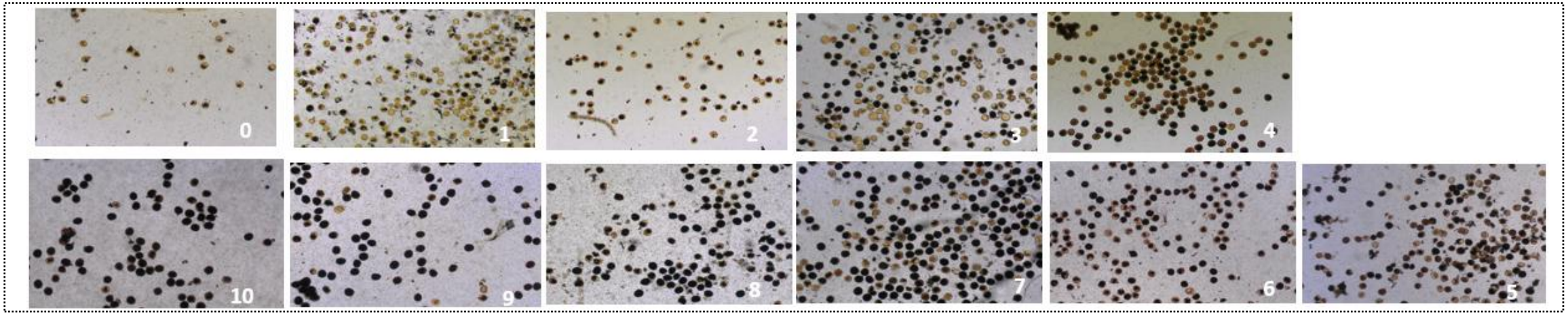
Step 2. Heat incubation



Step 3. Imaging



Step 4. Image processing and score assignment



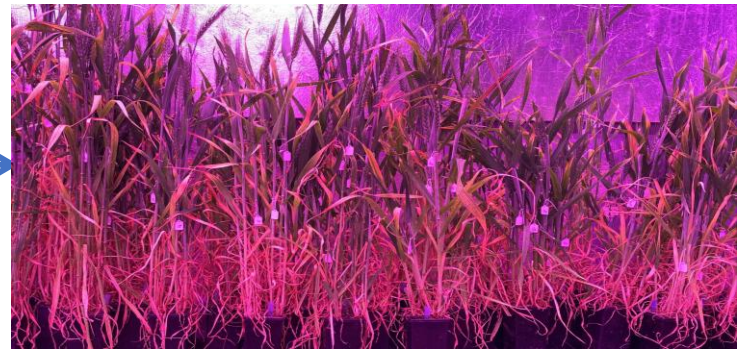
Siddique et al., 2026 (under review)

Phenotyping stay-green and spikelet fertility

- 319 diverse lines used for two years experiment
- Heat treatment in four ear emergence stage
- Stay-green was scored with 0-9
- Individual heads were harvested for spikelet fertility

319 lines, 4 seeds/pot

Tagging S1, S2, S3, and S4 stage



2 weeks heat treatment, 37°C

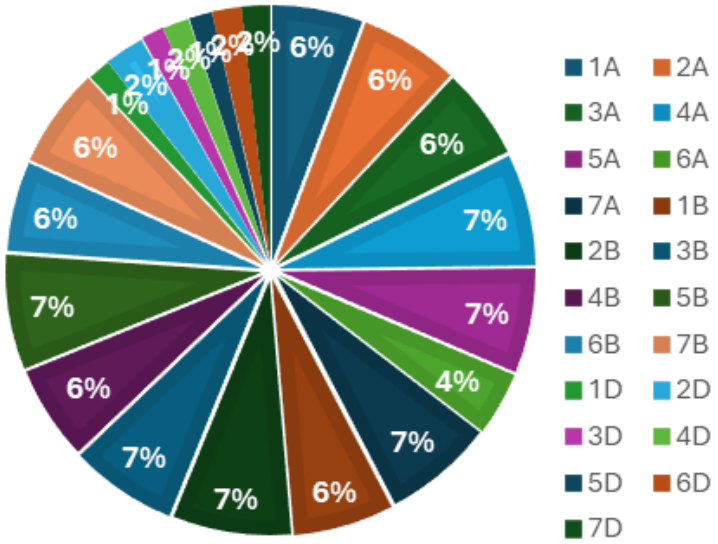
Recording seeds/spike

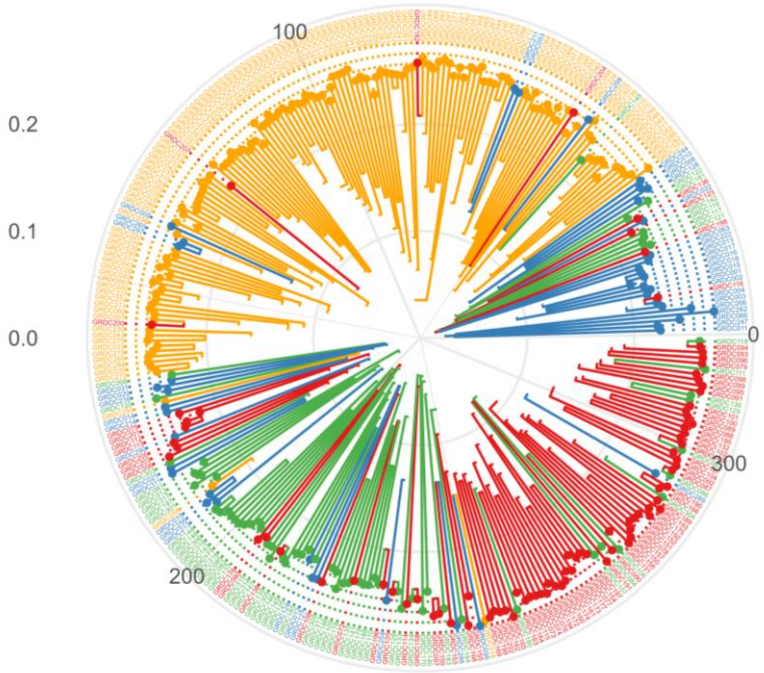
Scoring stay-green phenotype

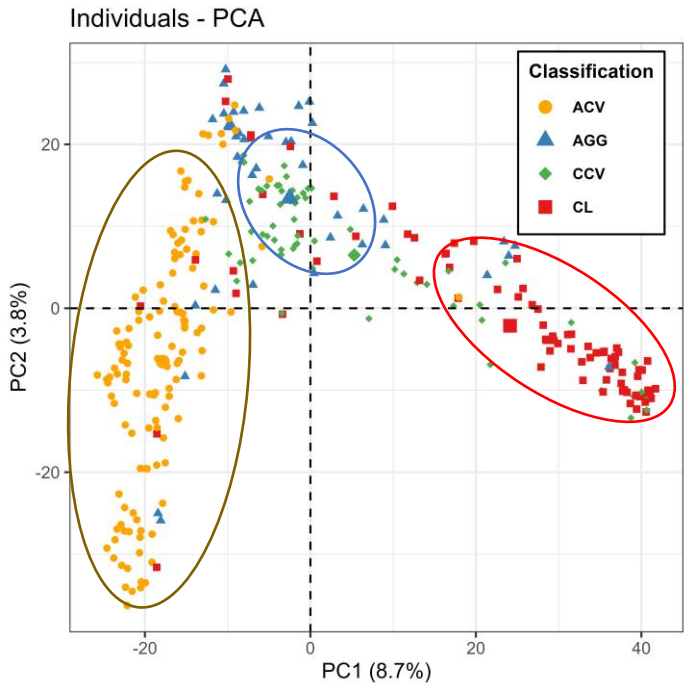
Siddique et al., 2026 (under review)

Population Structure

- 24,955 SNPs generated by Illumina iSelect 90K SNP bead chip assay
- 5,171 high quality SNPs were selected (minor allele frequency < 0.05) and missing values > 10%),
- Distinct separation based on their origin



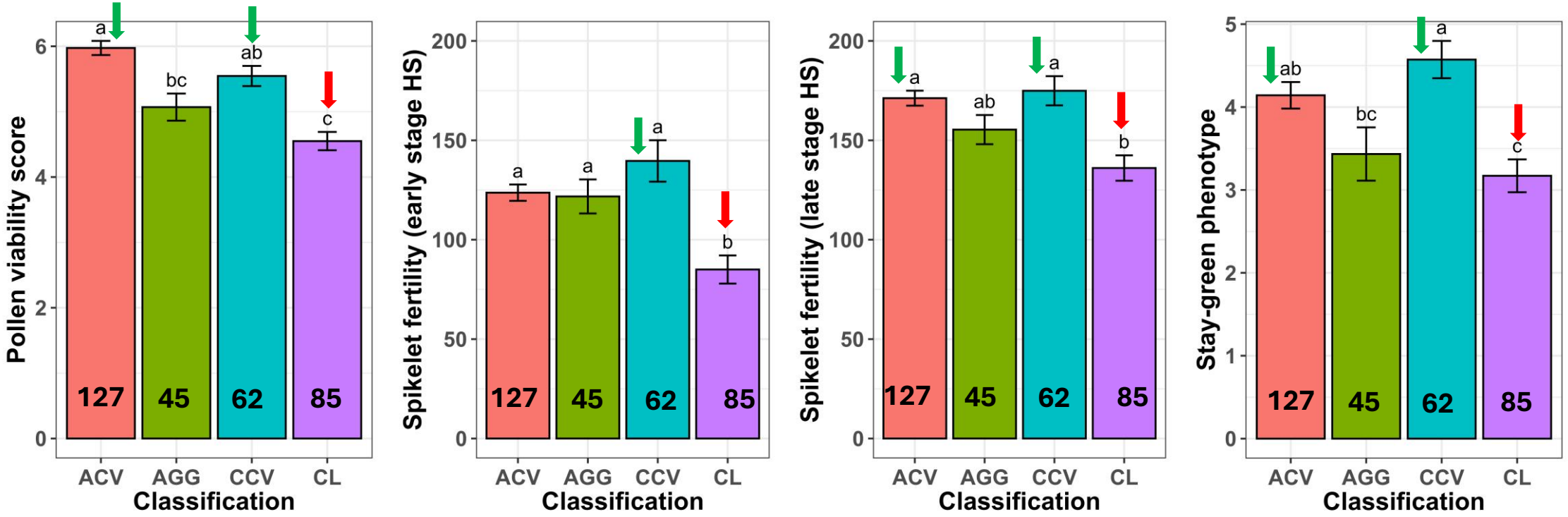




Siddique et al., 2026 (under review)

Phenotypic variation

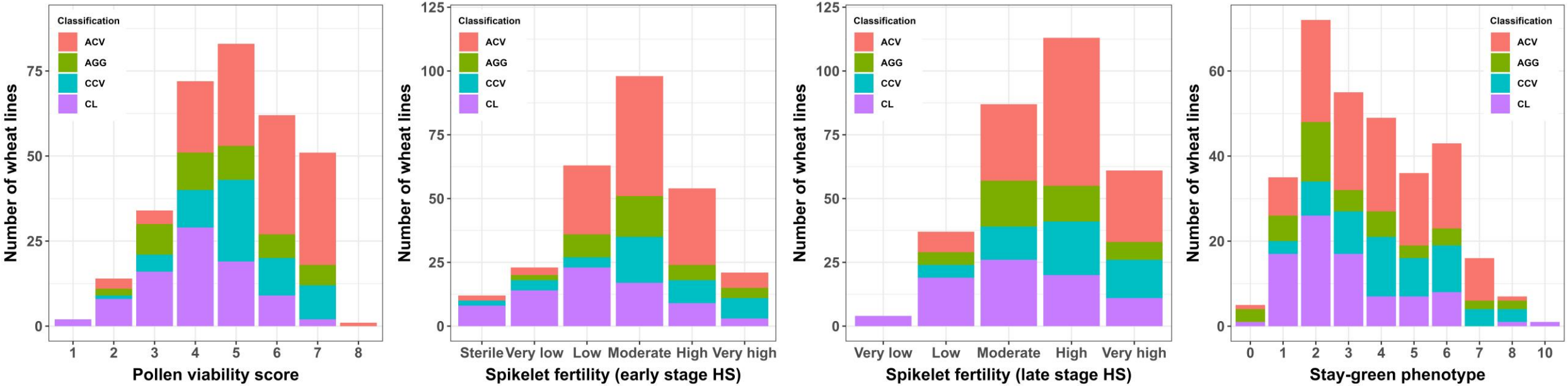
- Chinese landraces showed significant lower PVS, spikelet fertility, and stay-green score under HS
- Modern cultivars (CCV and ACV) exhibited higher mean value for all traits
- Significant breeding efforts to improve these traits



Siddique et al., 2026 (under review)

Phenotypic variation

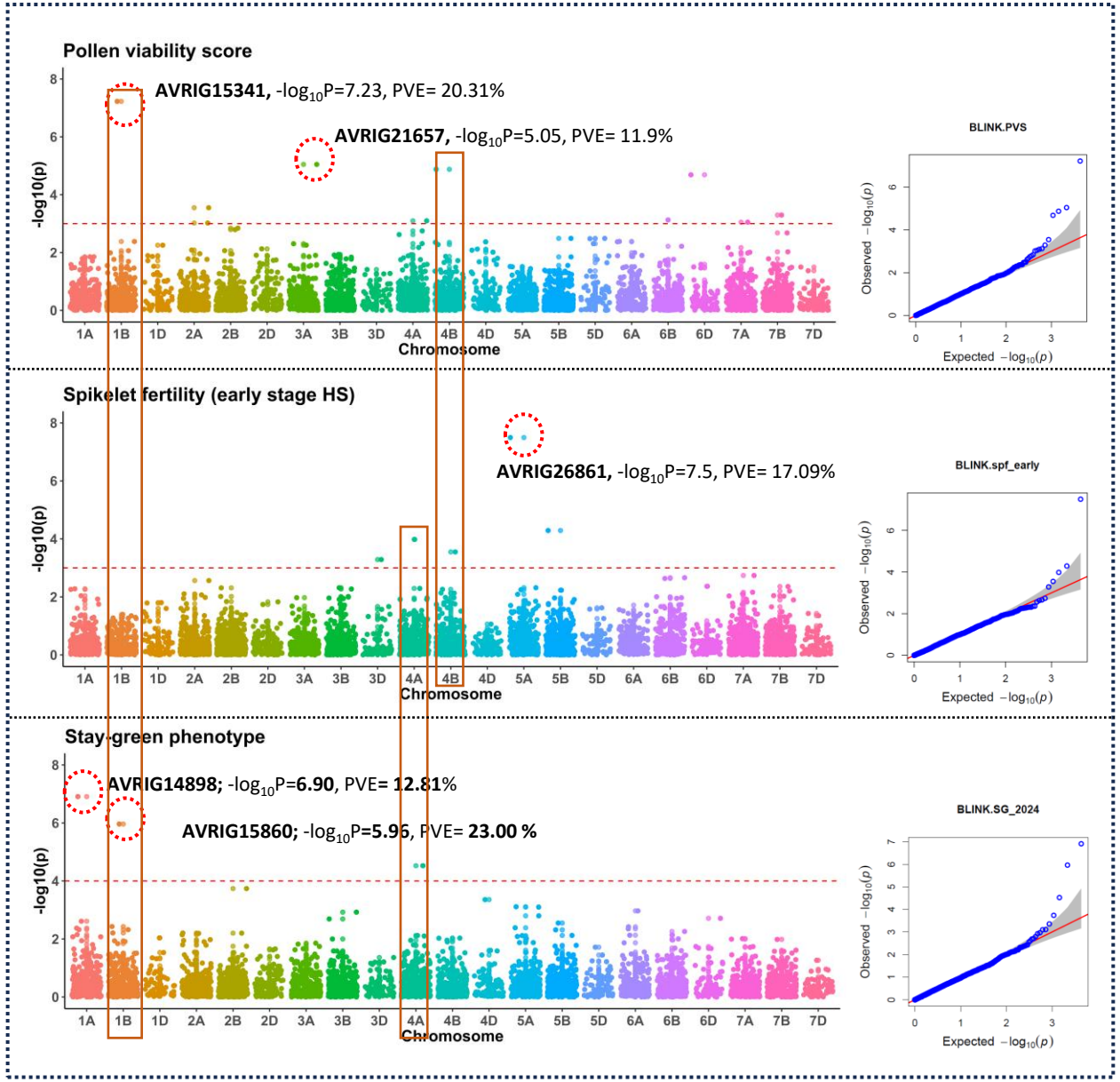
- Significant variations for PVS, spikelet fertility, and stay-green score
- Wheat lines from same origin showed variation, indicating presence of region-specific variation
- The newly developed screening method potentially capture the variation



Siddique et al., 2026 (under review)

GWAS outcome

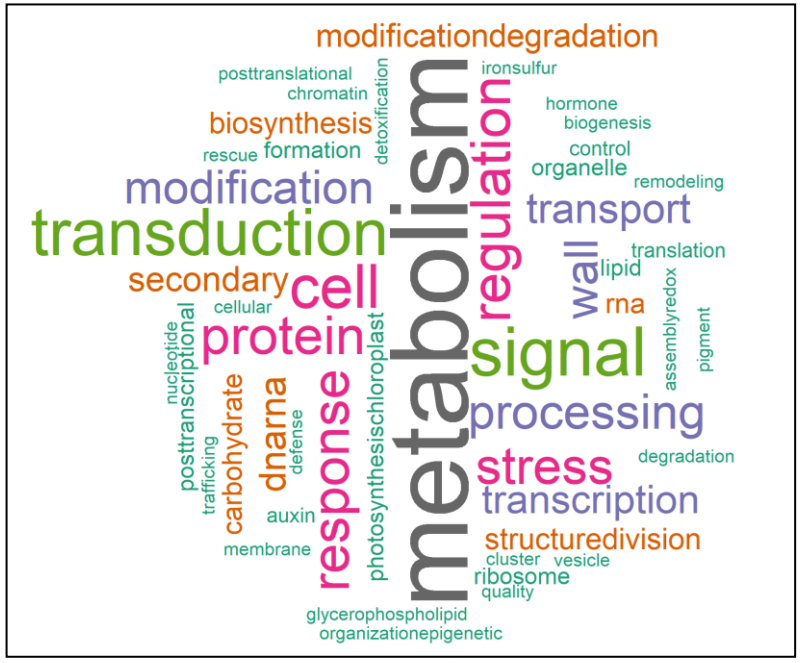
- PVS: Major SNPs AVRIG15341 and AVRIG21657on Chr 1B and 3A
- SPF: Major SNP AVRIG26861 on Chr 5A
- Stay-green: Major SNP AVRIG14898 and AVRIG15860 on Chr 1A and 1B
- Common regions associated with PVS and SPF; and Stay-green and SPF
- These indicate higher reliability of the methods to dissect the genetic control of these key traits

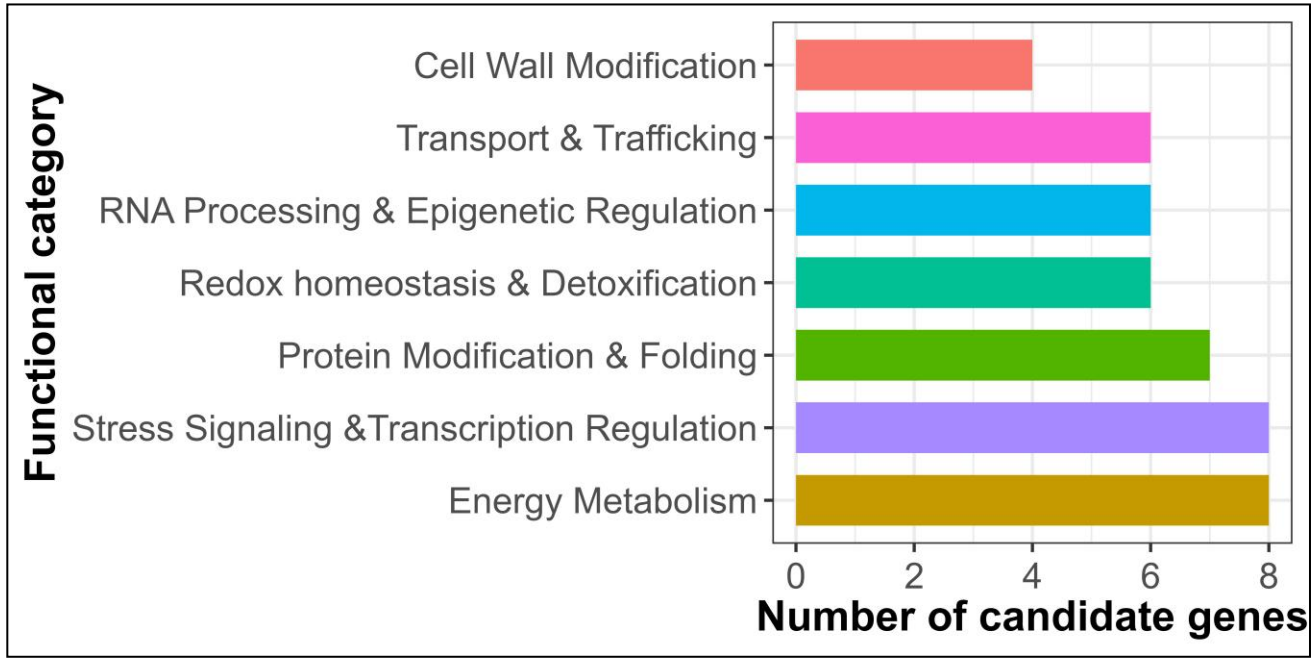


Siddique et al., 2026 (under review)

Candidate genes

- A ±2 Mb window around the highly significant SNPs were chosen for candidate gene identification
- 44 candidate genes were found around the 7 highly significant SNPs
- Candidate genes show diverse functions
- Two candidate genes from **Formin like-protein** and **Nudix hydrolase** group





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Conclusion and Future Prospective

- Established method is rapid, precise, and highly efficient in capturing variation and screening for pollen thermotolerance and stay-green phenotype
- GWAS identified major marker-trait associations linked to key reproductive stage heat tolerance
- Validation of identified MTAs in F_2 and BC lines and their introgression in superior wheat variety
- Integration of AI and machine learning based training model to score the PVS and stay-green trait

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THANK YOU

Question and Suggestions?

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