Project FY24-GD-003: Einkorn genomics to identify FHB-resistance genes in wheat

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1. **What are the major goals and objectives of the research project?**

    1. Extensive phenotyping of sequenced einkorn wheat panel of 221 accessions for FHB resistance in GH
    2. Perform K-mer GWAS matrix on the FHB phenotypic datasets to identify novel genes and alleles
    3. Use biparental RIL population to validate GWAS QTL /gene
    4. Use a bridge cross to transfer useful genes from selected einkorn accession with marker datasets:

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address these three items below.)*

What were the major activities?

Extensive phenotyping of sequenced einkorn wheat panel of 221 accessions for FHB resistance in GH: During the first year of the project, we phenotyped the first set of einkorn association panel. Phenotyping of the einkorn panel was perform using the protocol described in Chhabra et al., 2021. Initial results suggested more than 3 genomic regions involved for the FHB resistance in the panel.

Perform K-mer GWAS matrix on the FHB phenotypic datasets to identify novel genes and alleles: To identify this phenotype-genotype association for the FHB resistance in our einkorn GWAS panel, we have adopted a *k*-mer-based association mapping pipeline developed by our OWWC as well as employing the 'standard' association approaches using SNP variants. Our work on identification of k-mers from 221 sequenced einkorn accessions (with at least 10X coverage) has provided us 800 million k-mers. We have validated the k-mer matrix for einkorn wheat by identifying known genes and alleles for stem rust resistance genes. We are working on performing GWAS analysis and results will be reported in the next cycle.

Use biparental RIL population to validate GWAS QTL /gene: Phenotypic evaluation of parental lines indicated that wild parent TA4342-L95 is highly resistant to the FHB spread and domesticated accession TA4342-L96 is moderately susceptible to FHB. Now we are currently growing the population in two sets for its critical phenotyping. Since we already have genotypic data on the entire population (with a resolution of a marker at every 500 Kb). We are working on independently mapping the QTL or genes directly to the genetic map. We will then compare the results with GWAS results.

Use a bridge cross to transfer useful genes from selected einkorn accession with marker datasets: We have identified 36 einkorn lines showing excellent resistance against FHB disease severity. 20 lines showed significantly lower DON content than wheat and einkorn checks. A total of 4 einkorn wheat accessions were selected to initiate bridge crosses with tetraploid wheat cultivar Svevo and these accessions included UMD95, UMD107, UMD187, and UMD1660. All these four accessions showed excellent FHB response against disease spread and DON content for two straight years under GH testing with point inoculation. We selected Svevo for developing bridge crosses due to availability of reference genome sequence that will allow us to delete einkorn sequences using skim sequencing approaches in the backcrossed germplasm. F1 plants obtained from these crosses and in the next growing season these will be heavily backcrossed with adopted MD cultivar MD315. We have used the pipeline as suggested in figure 2.

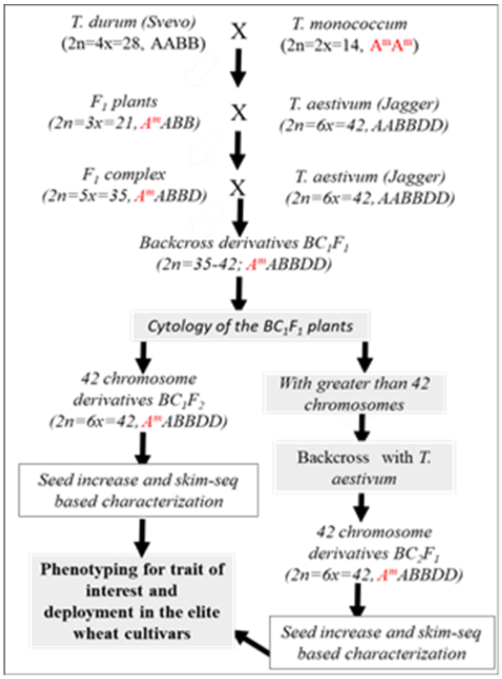

Fig. 2. Schematic presentation of bridge crossing plan to generate einkorn introgressions in tetraploid and hexaploid wheat

What were the significant results?

Replicated phenotyping of the GWAs panel showed that the Einkorn wheat panel has excellent resistance against FHB spread as well as the DON production (Figure 3a. and 3b.) More than 50% of the accessions showed moderate to high resistance against FHB. K-mer based GWAS identified 6 major QTL with two very prominent QTL on chromosome 5A (Figure 4). We are validating the results using a RIL population and data will be presented in the next cycle.

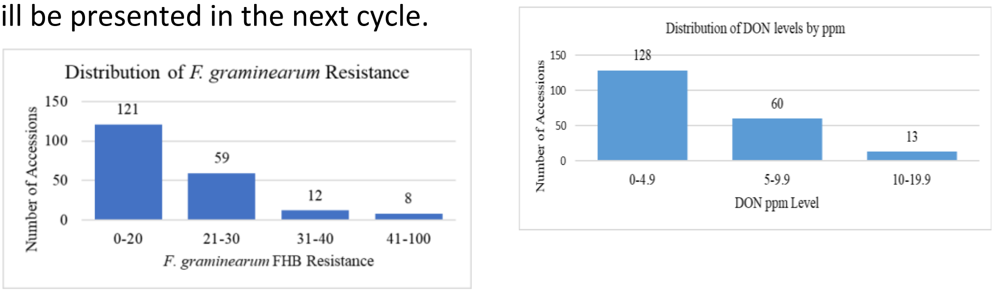

Figure 3. Distribution of FHB spread (3a) and Don content in the einkorn spikes after 21 days post inoculation.

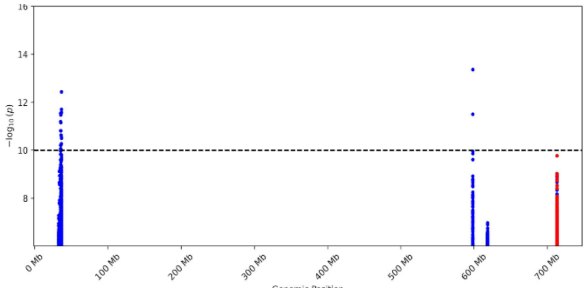

Figure 4. GWAS peak of the FHB resistance on chromosome 5A from einkorn panel of 217 accessions. Two novel regions 5AS (31.06 Mb-31.3Mb) and 5AL (598-3-599.2Mb) are consistent in three replicated studies.

List key outcomes or other achievements.
Einkorn as an important source for FHB resistance
New QTL for FHB resistance and DON content from einkorn panel
Initial results based on back cross derivatives indicate the FHB resistance from einkorn can be transferred to bread wheat.

3. **What opportunities for training and professional development has the project provided?**
   A MS student, Ms. Janelle Lively completed her MS degree in May 2025, and one of her thesis chapters was focused on screening and mapping of Einkorn GWAS panel for the FHB resistance. So, a graduate student and two undergraduate students were directly trained on various aspects of this einkorn trait discovery project.

4. **How have the results been disseminated to communities of interest?**
   Through presentations, and research updates. The PI presented the results in oral presentations and disseminated updates through emails. The graduate students in the team presented the results as posters and handouts to the stakeholders in commodity board meetings. The results were disseminated via emails and the UMD extension system to the broader grower community. We are working on a manuscript to describe novel QTL identified in this study with an expected submission deadline of September 15.

5. **What do you plan to do during the next reporting period to accomplish the goals and objectives?**
   - Summer 2025: Confirm current GWAS results and mapping validation
   - Fall 2025: Test of selected lines and validation of GWAS of QTL using sequence indexed RIL population
   - Fall 2025: Backcrosses of BC1F1 plants with soft red winter wheat cultivars and use the Speed Breeding to get adopted germplasm using recurrent backcrosses.
   - Spring 2026: Selfing and marker as well as skim sequencing-based identification of introgressed gene region from einkorn germplasm to wheat. based
   - Summer 2026: sharing the seeds of back crossed lines with breeders