

Project FY22-BA-011: Development of a Multi-parent Population to Enhance FHB Resistance in Barley

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

The overall long-term goal of this research is to facilitate the development of barley cultivars with resistance to FHB and the accumulation of mycotoxins, such as DON. Our proposal directly addresses the primary mission of the USWBSI “to enhance food safety and supply by reducing the impact of FHB on wheat and barley.” The specific goals for this research within the four-year timeframe are to: 1) develop a Multi-parent Advanced Generation Intercrosses (MAGIC) population using the most resistant barley accessions possessing unique haplotypes at characterized FHB/DON loci; 2) phenotype the MAGIC population for reaction to FHB and DON accumulation in multiple environments; 3) genotype the MAGIC population with SNP markers and perform QTL analyses to identify and map alleles conferring resistance to FHB and DON accumulation; and 4) identify progeny with enhanced resistance and distribute them to barley breeders. Within the Barley Coordinated Project, our proposal aligns closely with Objective 3 (Evaluate and implement modern breeding technologies to further enhance short-term and long-term improvement of FHB resistance in barley, and to efficiently introgress effective resistance genes into barley germplasm) under the Research Area of Variety Development and Host Resistance (VDHR), but is also an important step in advancing Objective 2 (Increase efficiency of coordinated barley breeding programs to develop and release FHB resistant varieties) within VDHR.

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

Objective 1) Develop a Multi-parent Advanced Generation Intercrosses (MAGIC) population using the most resistant barley accessions possessing unique haplotypes at characterized FHB/DON loci.

Major Activities. Preliminary data collected for this project involved the evaluation of over 25,000 *Hordeum* accessions for FHB resistance in the field over the course of our long-term USWBSI-funded research. From this extensive field screening effort, we identified about 230 two-rowed accessions (the type now preferred by the malting and brewing industries) that exhibited a moderate to moderately high level of resistance. To fully validate the resistance of these selections and rigorously assay DON accumulation, we evaluated the lines together in head-to-head trials in up to 11 environments (i.e. unique location-year combinations) over the past six years at Crookston and St. Paul, Minnesota. Twenty-one of the best performing accessions from this group were then selected as candidate parents (Page 2023). The criteria used for selecting these 21 lines was as follows in order of priority: i) lowest DON level; ii) lowest FHB severity; iii) heading date within a few

days of standard barley cultivars; iv) height within about 10 cm of standard barley cultivars; v) genetic diversity as revealed through principal component analysis on genotype data; and vi) improvement status (i.e. breeding lines over unadapted landraces). All 21 candidates exhibited DON levels that were >3.0 ppm lower than the moderately resistant six-rowed malting cultivar 'Quest' released by the University of Minnesota. Within this group of candidates, four are from North America, seven are from Europe, two are from Africa, and the final eight are progeny selections derived from crosses between various FHB-resistant landrace or wild barley (*Hordeum vulgare* ssp. *spontaneum*) accessions and malting barley cultivars. One additional candidate included is an advanced two-rowed malting barley from the Minnesota breeding program.

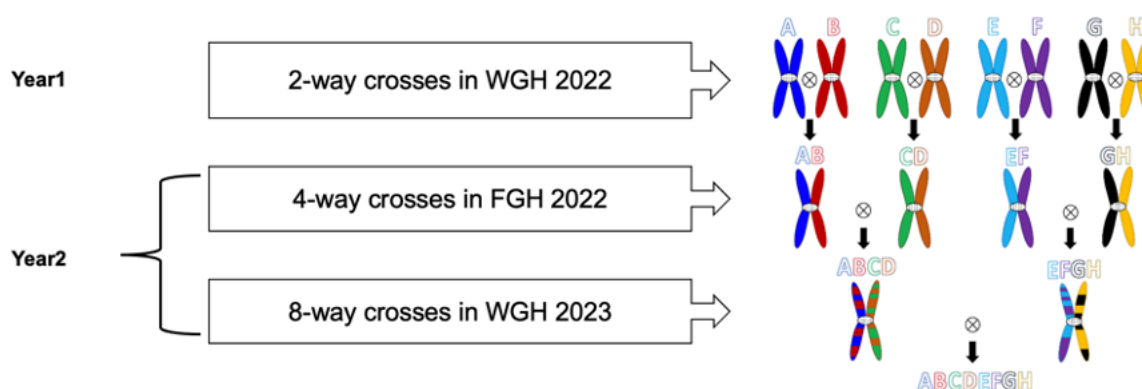
We recently completed an extensive meta-analysis of the genetics of FHB resistance and DON accumulation in barley based on 11 mapping populations and two germplasm panels. Haplotypes at each identified QTL from this meta-analysis were characterized in the 21 founder candidates to aid in the selection of the final 8 parents for MAGIC population development (Page 2023). The final eight parents selected for this population and their origins are given in Table 1. Two (S2M184, an advanced Minnesota breeding line and AC Minoa, a cultivar released by Agriculture and Agri-Food Canada) of the eight founder parents selected for the population are adapted cultivars and will therefore increase the chances of identifying agronomically advanced progeny with low FHB severity and DON levels.

Table 1. Final eight founder parents selected for development of the barley MAGIC population.

Accession	Origin
S42IL_170	Backcross line from Scarlett/ISR42-8 (wild barley from Israel)
AC_MINOA	Cultivar from Agriculture and Agri-Food Canada
KTYQST_53_4	Backcross line from Quest/Kutahya (Netherlands)
PI_356118	Landrace from Ethiopia
HSR_31C	Backcross line from Rasmusson/PI466423 (wild barley from Israel)
VIR_18426	Landrace from Romania
2ND29827	Breeding line from North Dakota State University
S2M184	Breeding line from University of Minnesota

Significant Results: To develop the MAGIC population, F₁s were generated from inter-crosses with eight selected founder parents (i.e. A, B, C, D, E, F, G, and H) during the 2022 winter greenhouse season (Figure 1). Next, F₁'s from these initial crosses (A×B; C×D; E×F; and G×H) were used in four-way inter-crosses (AB×CD and EF×GH) during the 2022 fall greenhouse season. The eight-way inter-crossing of parent pairs (ABCD×EFGH) was done during the 2023 winter greenhouse season, generating 1,075 F₁ seeds. Due to the late spring harvest of crossed seed from the eight-way intercrosses, we were not able to increase F₁ seeds from the eight-way inter-cross in the spring. Instead, F₁ seeds were selfed during the 2023 Fall greenhouse season to produce F₂ seed. The F₂ was advanced to the F₃ generation in the 2024 Winter greenhouse season. From this increase, 888 F₃ lines were harvested from the greenhouse and sown at Crookston in May 2024. Agronomic observations will be made on the MAGIC population in July 2024. Lines that are excessively late or taller than standard breeding material or have poor plant growth were discarded. We advanced a population of ~860 F₄ lines in the 2024 Fall greenhouse season. In the 2025 Winter greenhouse, the F₅ lines will be sown in large pots to produce enough F₆ seed for planting two FHB screening nurseries: one at St. Paul and one at Crookston. DNA will be extracted from F₆ plants in the greenhouse and genotyped with the 50K genotyping array.

Figure 1. The scheme and timeline for development of a MAGIC population to enhance resistance to FHB and DON accumulation in barley. Displayed is only one of seven barley chromosomes.



Key Outcomes or Other Achievements: We successfully developed a MAGIC population by intercrossing eight founder accessions possessing unique alleles conferring the highest level of resistance to FHB and DON accumulation. We successfully advanced 888 lines to the F_3 generation and planted them in the field for the first observation of agronomic performance in Crookston in May 2024. After discarding progeny having outlier traits, we expect to advance about 800 progeny for genotyping and phenotyping in 2025 and 2026. This large population should generate progeny with different combinations of alleles that contribute to enhanced resistance to FHB and DON accumulation.

Objective 2) Phenotype the MAGIC population for reaction to FHB and DON accumulation in multiple environments.

Major Activities. Controls and the 860 F_6 lines of the MAGIC population were sown in the field at St. Paul and Crookston to assess their level of resistance to FHB and DON accumulation. The nurseries were sown in late April and mid-May 2025, respectively. Three inoculations were performed at St. Paul: i) the first inoculation was made using grain spawn, generating ascospores as the inoculum source, whereas the second and third inoculations were made using foliar sprays of conidia two to three days apart. At Crookston, a single grain spawn inoculation was made in late May.

Significant Results: At St. Paul, disease severity assessments for FHB were made based on a 0-100% rating scale on July 11, 2025. Marked differences for disease severity were observed among the progeny lines and ranged from about 2 to 60%. FHB assessments were also made using a smartphone to capture images of the infection levels on spikes across the row. The images captured using a smartphone will be analyzed for their FHB severity using the model developed by Cory Hirsch's group, who is also funded by the USWBSI. In addition to the FHB assessments, data were also be taken on various agro-morphological traits that may influence the level of infection. Disease assessments for the MAGIC population at Crookston will be made in late July or early August.

Key Outcomes or Other Achievements: We successfully developed a MAGIC population of about 860 F_6 lines and are evaluating them for their reaction to FHB and various agro-

morphological traits. When the lines are fully mature, they will be harvested and assayed for their level of DON.

Objective 3) Genotype the MAGIC population with SNP markers and perform QTL analyses to identify and map alleles conferring resistance to FHB and DON accumulation; and

Major Activities. In the 2025 Winter greenhouse, leaf tissue from F₅ lines was collected and sent to the USDA-ARS Red River Valley Agricultural Research Center for DNA extraction and genotyping.

Significant Results: Genotyping was completed with the 50k genotyping platform. The filtering of markers is in progress.

Key Outcomes or Other Achievements: Once the phenotyping datasets are available, we will conduct the QTL analyses.

Objective 4) Identify progeny with enhanced resistance and distribute them to barley breeders.

Major Activities. In the 2025 FHB nurseries at St. Paul and Crookston, we will take careful notes to identify progeny with a high level of FHB resistance. The same will be done in the 2026 FHB nurseries. We will also carefully analyze subsequent DON data and select those lines with the lowest accumulation for use in the breeding program.

Significant Results: This aspect of Objective 4 will be completed once a second season of FHB and DON phenotyping is completed.

Key Outcomes or Other Achievements: This aspect of Objective 4 will be completed once a second season of FHB and DON phenotyping is completed.

3. What opportunities for training and professional development has the project provided?

This USWBSI-funded research has provided an excellent training opportunity for many scientists over the past year. The basis for the current research project was part of the Ph.D. thesis of my graduate student Rae Page who submitted her dissertation entitled “Genetics of Fusarium head blight resistance in barley and of rust resistance in the wild wheat relative *Aegilops longissima*” in May 2023. Other participants in this research included post-doctoral research associates Ahmad Sallam, Eric Nazareno, Oadi Matny, and Rebecca Spanner; Researcher 2 scientist Alexis Feist; Researcher 3 scientist James Nesbit; Ph.D. students Reecha Acharya, Yoonjung Lee, and Connor Slawin. Undergraduate students assisting on this project in various capacities include Emma Leff, Annie Russell-Pribnow, Arno Swart, Ava Pederson, Hazel Fritz, Ellie Nadeau, Carmen Florentz, and Milica Ceperkovic. All these individuals were trained in the methodology for working with FHB, including production and storage of inoculum; inoculation techniques; disease severity scoring; and DON analyses. Moreover, several members of my senior research team gained valuable experience in SNP genotyping, molecular map construction and QTL analysis.

4. How have the results been disseminated to communities of interest?

A portion of this research project is part of Rae Page’s Ph.D. thesis. She presented her research at several venues listed below, including the USWBSI Forum. She also completed her dissertation in

May 2023. Two of Rae's thesis chapters have been accepted for publication in Crop Science and will appear in an upcoming issue in the autumn. Another key research publication was on meta-

analysis of the genetics of resistance to Fusarium head blight in barley and considerations for breeding. This was a summary of more than 18 years of genetic studies and formed the basis for the haplotype analyses that ultimately contributed to the selection of the eight final parents of the MAGIC population.

5. What do you plan to do during the next reporting period to accomplish the goals and objectives?

For the next reporting period, we will summarize data for FHB, DON and agro-morphological traits on the 860 F₆ lines of the MAGIC population. We will also complete the filtering of the genotypic data and begin a preliminary QTL analysis. The MAGIC population will again be evaluated in the field at St. Paul and Crookston.