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

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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: 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 10 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.

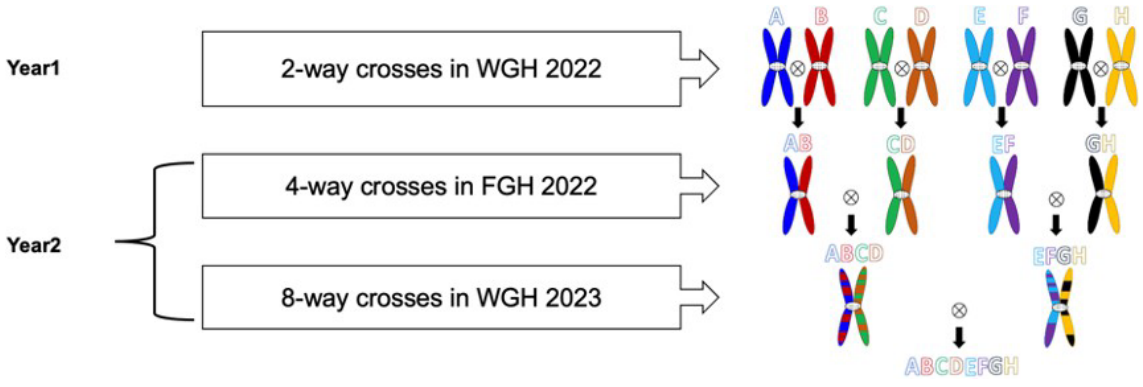
We recently completed an extensive meta-analysis of the genetics of FHB resistance and DON accumulation in barley based on 11 mapping populations and two germplasm panels. Haplotypes at each identified QTL from this meta-analysis were characterized in the 21 founder candidates to aid in the selection 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

Objective 1: Significant Results: To develop the MAGIC population, F_1s 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, F_1'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 F_1 seeds. Due to the late spring harvest of crossed

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.



seed from the eight-way intercrosses, we were not able to increase F_1 seeds from the eight-way inter-cross in the spring. Instead, F_1 seeds were selfed during the 2023 Fall greenhouse season to produce F_2 seed. The F_2 was advanced to the F_3 generation in the 2024 Winter greenhouse season. From this increase, 888 F_3 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 will be discarded. We anticipate advancing a population of about 800 F_4 lines in the 2024 Fall greenhouse season. In the 2025 Winter greenhouse, F_5 lines will be sown in large pots to produce enough F_6 seed for planting two FHB screening nurseries: one at St. Paul and one at Crookston. DNA will be extracted from F_6 plants in the greenhouse and genotyped with the 50K genotyping array.

Objective 1: 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 di^erent combinations of alleles that contribute to enhanced resistance to FHB and DON accumulation.

Research on Objective 2 (phenotype the MAGIC population for reaction to FHB and DON accumulation in multiple environments); **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 **Objective 4** (identify progeny with enhanced resistance and distribute them to barley breeders) will commence once the MAGIC population is selfed to the F_6 generation and the first disease and mycotoxin phenotypes are collected.

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. student Yoonjung Lee; and M.S. student Connor Slawin. Undergraduate students assisting on this project in various capacities include Emma Le^, Annie Russell-Pribnow, Arno Swart, Ava Pederson, and Hazel Fritz. 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. 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 advance about 800 F_4 lines to the F_5 generation in the 2024 Fall greenhouse season and resulting F_5 lines to the F_6 generation in the 2025 Winter greenhouse. DNA will be extracted from the leaves of F_6 lines and genotyped with the 50K genotyping array. The first phenotyping assays for FHB resistance and subsequently DON accumulation will be conducted in St. Paul and Crookston in 2025.