USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Performance Report
Due date: July 24, 2020

Cover Page

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Fiscal Year:	2019
USDA-ARS Agreement ID:	59-0206-6-006
USDA-ARS Agreement Title:	Mapping Loci Conferring Resistance to FHB and DON Accumulation in Barley
FY19 USDA-ARS Award Amount:	$ 61,501
Recipient Organization:	Regents of the University of Minnesota Suite 450 Sponsored FIN RPT-P100100001 Minneapolis, MN 55455-2003
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Project/Grant Reporting Period:	5/2/19 - 5/1/20
Reporting Period End Date:	5/1/2020

USWBSI Individual Project(s)

USWBSI Research Category[*]	**Project Title**	**ARS Award Amount**
BAR-CP	Evaluation and Genetic Characterization of Barley Germplasm for FHB Resistance	$ 61,501
	FY19 Total ARS Award Amount	**$ 61,501**

Brian J. Steffenson

July 28, 2020

Principal Investigator Date

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[*] MGMT – FHB Management
FST – Food Safety & Toxicology
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
    SPR – Spring Wheat Region
    NWW – Northern Soft Winter Wheat Region
    SWW – Southern Soft Red Winter Wheat Region

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Project 1: *Evaluation and Genetic Characterization of Barley Germplasm for FHB Resistance*

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

Our major goal is to reduce the economic losses caused by Fusarium head blight (FHB) in barley, including quality discounts due to deoxynivalenol (DON) contamination. This can be best achieved by developing barley cultivars with the highest level of resistance possible, in conjunction with various cultural and chemical control methods. Through extensive germplasm evaluations funded by the USWBSI over the past 16 years, we have identified 78 accessions that possess a level of resistance comparable to the six-rowed and two-rowed controls of Chevron and CIho 4196, respectively. One of the most resistant accessions identified in this group is PI 350725, a two-rowed accession from the Tirol in Austria. Six-rowed barleys have been the preferred type for malting in the Midwest region for more than 80 years. A resistant two-rowed barley was selected for this investigation because this is now the industry-preferred row type for Midwestern malting barley cultivars. Our specific objectives for this proposal are to: 1) determine the number, effect, and chromosomal position of FHB resistance loci in barley accession PI 350725 using the advanced backcross QTL method; 2) conduct rigorous FHB evaluations of the ~200 most resistant *Hordeum* accessions in order to select the very best ones for breeding and genetic analysis; 3) evaluate previously untested barley landraces for FHB reaction, and 4) provide adapted FHB-resistant parental materials to barley improvement programs. This research addresses Barley-CP VDHR objective #2 (Mapping novel QTL for resistance to FHB in barley), but is also an important step in advancing objective #4 (Develop new barley varieties with enhanced resistance to FHB and lower DON). The outputs from this work will be new accessions with novel genes for FHB resistance. Use of this germplasm in breeding will help fulfill the USWBSI primary goal to develop as quickly as possible effective control measures that minimize the threat of FHB, including the reduction of mycotoxins, to the producers, processors, and consumers of barley.

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

a) What were the major activities?

Objective 1: PI 350725 was crossed with ‘Quest,’ the first Midwestern six-rowed cultivar with partial FHB resistance. About 50 F_1 progeny were then backcrossed with ‘Quest’ to obtain the BC_1 generation. At least 5 crossed seeds were obtained per backcross. Then, about 90 BC_1 plants were backcrossed to ‘Quest’ to obtain the BC_2 generation. Through timely supplemental support from the USWBSI, the BC_2 generation was made into doubled haploids (DHs) by Patrick Hayes’ group at Oregon State University. This critical DH step shortened the time needed to obtain homozygous lines, allowing for earlier phenotyping in the field. From each BC_2 plant, 5 seeds were used for DH production. Due to the recalcitrant nature of this cross, only 170 doubled haploid progeny were obtained from Oregon State University. These DH progenies were increased in the fall/winter greenhouse season of 2015-2016 and were phenotyped at our late-planted FHB nursery in Crookston in 2016. Due to the lower than expected number of DH progeny, we also advanced remnant BC_2 seed by single seed descent, which will serve to expand the population for QTL mapping and validate the results found in the DH population. The number of BC_2 Recombinant Inbred Lines (RILs) generated was 328. The DH and RIL populations were phenotyped for FHB severity and DON concentration at St. Paul in 2017, in St. Paul and Crookston in 2018, and in Crookston in 2019. From these four environments, we have a robust phenotyping dataset for mapping quantitative trait loci (QTL) for resistance to the disease and accumulation of the mycotoxin.

The 50K iSelect single nucleotide polymorphism (SNP) array was used to genotype 161 DH lines and 317 RIL lines. SNP calls were manually edited to reduce missing data using GenomeStudio v2.0 and were filtered to remove markers with >10% missing data and >2% heterozygosity. Single marker analysis was performed using general linear model in Tassel v5.0 as a preliminary investigation of potentially significant QTL for FHB severity across all environments. DON accumulation data for 2019 was just recently obtained due to the pandemic. Composite interval mapping will be performed on the two populations to identify the chromosomal locations of loci conferring lower FHB and DON levels.

Objective 2: In 2020, a panel of the most resistant accessions was planted and evaluated for FHB reaction and DON accumulation in St. Paul and Crookston.

Objective 3: A panel of 528 previously untested barley landraces donated by the Leibniz-Institute für Pflanzengenetik und Kulturpflanzenforschung (IPK) in Germany and a panel of 80 barley landraces from the John Innes Centre in the United Kingdom were evaluated for FHB reaction and DON accumulation in Crookston in 2019.

Objective 4: All FHB and DON data taken on the most resistant selections were compiled for all but the 2020 field season.

b) What were the significant results?

Objective 1: At St. Paul (2017-18), the six-rowed resistant (Chevron), six-rowed susceptible (PI 383933), two-rowed resistant (CIho 4196), and two-rowed susceptible (ICB111809) accessions exhibited an average FHB severity and DON concentration of 11.1% and 1.9ppm; 55.5% and 14.0ppm; 10.6% and 2.4ppm, and 20.2% and 3.6ppm, respectively. At Crookston (2018), Chevron, PI 383933, CIho 4196, and ICB11180, exhibited a FHB severity and DON concentration of 2.8% and 3.3ppm; 92.1% and 13.3ppm; 2.1% and 2.3ppm, and 16.8% and 8.3ppm, respectively. With the mapping populations, two-rowed progeny ranged in FHB severity and DON concentration from 0.8 to 33.7% and from 0.11 to 15.0ppm in St. Paul and from 0.7 to 27.1% and from 0.6 to 28.1ppm in Crookston, respectively. These data indicate that DH and RIL progeny with low FHB severity and DON accumulation can be recovered from the PI 350725/Quest population. QTL analyses will be completed when data from the last trial in Crookston are collected and compiled. Agro-morphological traits such as heading date, height, and spike density can have a pronounced effect on FHB severity. Thus, these traits will also be scored to determine if they co-locate with QTLs for FHB reaction.

Objective 2: Of the ~200 selected accessions evaluated in four environments over the past three years, several have exhibited consistently low levels of FHB and DON.

Objective 3: Of the 528 IPK landraces screened at Crookston in 2019, 60 exhibited FHB severities below 10%. For comparison, the resistant six-rowed and two-rowed controls had FHB severities of 10.4% and 13.8%, respectively. DON data for this panel has not yet been obtained.

Objective 4: We now have robust FHB and DON datasets on the selected panel of barleys. Of the 38 accessions evaluated in seven environments, eight had a mean relative FHB severity (i.e. percentage of infected kernels as compared to the susceptible six-rowed control Stander averaged over all environments) below 50% and 33 had a mean relative DON accumulation (i.e. percent of DON accumulation in ppm of Stander) below 50%. Of the 155 lines evaluated in at least four environments, 33 had a mean relative FHB severity below 50% and 130 had a mean relative DON accumulation below 50%. This panel of select resistant accessions (176 lines) was genotyped with the 50k Illumina Infinium iSelect genotyping array for barley to provide a comprehensive set of markers to be used for future haplotype analysis of previously reported FHB resistance QTL.

c) List key outcomes or other achievements.

When the QTL analysis is completed, we anticipate identifying novel resistance alleles in the PI350725/Quest population. The extensive phenotyping of the select panel has revealed several accessions with consistently low levels of FHB and DON. These will be distributed to breeders in fall 2020. Several new accessions with resistance to FHB were identified in the IPK panel and will be evaluated again in future field trials. Haplotype analyses will be completed for all elite resistant germplasm, providing key insights into the genetic basis of resistance.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

Yes, due to the university shut-down, progress in performing QTL analyses on the computer was severely restricted. Also, DON data coming from Yanhong Dong’s laboratory was delayed for several months. We are still waiting on some data.

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

Our USWBSI-funded research has provided an excellent training opportunity for many scientists. The immediate past research comprised a portion of the Ph.D. dissertation of Dr. Matthew Haas, and the current research project is part of the M.S. (now Ph.D.) thesis of my graduate student Rae Page. Other participants in this research included post-doctoral research associates Ahmad Sallam and Oadi Matny, Researcher 2 scientists Tamas Szinyei, Matthew Martin, and Ryan Johnson, and graduate student Mitchell Ritzinger. There were also many undergraduate students who assisted on this project in various capacities, the three current ones being Michelle Jugovich, Molly Bergum and Michael Miller. All of 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.

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

The current research project is part of Rae Page's thesis. She presented her research as a poster at the 2019 USWBSI forum. She plans to publish the complete work in a referred journal in 2021.

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Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (5/2/19 - 5/1/20). The term “support” below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student’s stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY19 award period?**
   No

   If yes, how many?

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY19 award period?**
   No

   If yes, how many?

3. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   No

   If yes, how many?

4. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   No

   If yes, how many?

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY19 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.

Name of Germplasm/Cultivar	**Grain Class**	**FHB Resistance** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**

Add rows if needed.

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY19-FPR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY19 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (5/2/19 - 5/1/20**) should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/ presentation.

Journal publications.

Books or other non-periodical, one-time publications.

Other publications, conference papers and presentations.

Huang, Y., Yin, L., Sallam, A., Heinen, S., Beaubien, K., Dill-Macky, R., Dong, Y., Steffenson, B., Smith, K. P., and Muehlbauer, G. J. 2019. "Genetic analysis of Fusarium head blight severity, malting quality and agronomic traits in the centromeric region of chromosome 6H in barley." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 51), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Qiu, R., Yang, C., Moghimi, A., Zhang, M., Steffenson, B. J., and and Hirsch, C. D. 2019. "Detection of Fusarium head blight in wheat using a deep neural network and color imaging." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 76), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Page, R., Steffenson, B., Szinyei, T., Martin, M., Matny, O., Sallam, A., Wodarek, J., and Dong, Y. 2019. Evaluation of select barley accessions for resistance to Fusarium head blight and DON accumulation in multi-year, multi-environment trials in the Upper Midwest. In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 108), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.

Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)