USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Annual Performance Progress Report
Due date: July 29, 2021

Cover Page

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Fiscal Year:	2020
USDA-ARS Agreement ID:	59-0206-0-182
USDA-ARS Agreement Title:	Evaluation and Genetic Characterization of Hordeum Germplasm for Resistance to FHB
FY20 USDA-ARS Award Amount:	$ 72,932
Recipient Organization:	Regents of the University of Minnesota Suite 450 Sponsored FIN RPT-P100100001 Minneapolis, MN 55455-2003
DUNS Number:	555917996
EIN:	41 -6007513
Recipient Identifying Number or Account Number:	CON000000086367
Project/Grant Reporting Period:	5/15/20 - 5/14/21
Reporting Period End Date:	5/14/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Evaluation and Genetic Characterization of Hordeum Germplasm for Resistance to FHB	$ 72,932
	FY20 Total ARS Award Amount	**$ 72,932**

_____   7/29/2021
Principal Investigator   Date

FY20 Annual Performance Progress Report
PI: Steffenson, Brian
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Project 1: *Evaluation and Genetic Characterization of Hordeum Germplasm for Resistance to FHB*

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 these three items 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. Phenotypic evaluations for the DH population were conducted in Crookston, MN in 2016, 2018, and 2019 and in Saint Paul, MN in 2017 and 2018. The RIL population was screened in Crookston in 2018 and 2019 and in Saint Paul in 2017 and 2018. From these five and four environments utilized for the DH and RIL populations, respectively, 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. DON accumulation data for 2019 was obtained in 2020 due to a delay from the pandemic. QTL IciMapping v4.1 was used for detection of marker-trait associations.

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 29 previously untested Irish barley landraces donated by the Ireland Department of Agriculture, Food and the Marine genebank were evaluated for FHB and DON accumulation in Crookston in 2020.

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: Within the mapping populations, best linear unbiased estimates (BLUEs) for each line were estimated, providing a single phenotypic value for each trait and each line across all environments. BLUE values for FHB severity and DON concentration for the resistant six-rowed check, Chevron, were 4.9% and 6.6 ppm, respectively. BLUE values for FHB severity and DON concentration for the resistant two-rowed check, CIho 4196 were 10.3% and 7.4 ppm, respectively. BLUE values for FHB severity and DON

concentration in the DH population ranged from 4.4 to 43.9% and from 7.8 to 29.9 ppm, respectively. BLUE values for FHB severity and DON concentration in the RIL population ranged from 7.2 to 46.3% and from 9.7 to 30.6 ppm, 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. In the DH population, six QTL were detected for reduced FHB severity across all environments. PI 350725 contributed the resistance allele for four of these FHB QTL. One FHB QTL was identified in two of five environments, with the resistance allele being contributed by Quest. In the RIL population, four QTL were detected for reduced FHB severity across all environments. PI 350725 contributed the resistance allele for two of these FHB QTL. One FHB QTL was identified in two of four environments, with the resistance allele contributed by Quest. In the DH population, five QTL were detected for reduced DON accumulation across all environments. PI 350725 contributed the resistance allele for three of these DON QTL. One DON QTL was identified in three of five environments, with the resistance allele contributed by Quest. In the RIL population, four QTL were detected for reduced DON accumulation across all environments. PI 350725 contributed the resistance allele for one of these DON QTL. One DON QTL was identified in three of four environments, with the resistance allele contributed by Quest. Agro-morphological traits such as heading date, height, and spike density can have a pronounced effect on FHB severity. Thus, these traits were also be scored to determine if they co-locate with QTLs for FHB reaction. In both populations, the major effect QTL explaining the majority of variation were coincident with QTL for height and heading date, suggesting pleiotropic effects of major QTL. Across the two populations, comparison of the physical positions of the markers flanking significant QTL revealed three QTL influencing FHB resistance and/or DON accumulation residing within the same or in overlapping marker intervals.

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. Evaluations and analyses of these accessions will continue on into 2021 to obtain a very robust dataset.

Objective 3: Only four of the Irish heritage accessions showed FHB severities below 15% and many of the accessions were extremely late-heading. For comparison, the resistant six-rowed and two-rowed controls had FHB severities of 3.2% and 4.5% respectively.

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

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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 PI 350725/Quest population. Further investigation into the identified QTL donated from PI 350725 will reveal if any have utility in breeding or if they relate to previously identified QTL. 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. 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 and/or restrictions, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.**

We were fortunate to be able to successfully complete our FHB field trials in 2020 and to plant our 2021 trial in May. In 2020, there were long delays in receiving our DON data coming from Yanhong Dong's laboratory. This is no reflection on her because I am well aware of the restrictions she faced at the University of Minnesota. In addition, the extra step of now having to grind our own samples ahead of submitting them to Yanhong's lab also resulted in some delay. Moreover, with the university shut-down and restrictions of personnel coming onto campus, our progress in performing QTL analyses were delayed.

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 over the past years. The current research project is part of the 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, and graduate students Mitchell Ritzinger and Eva Henningsen. There were also several undergraduate students who assisted on this project in various capacities, the recent ones 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 Ph.D. thesis. She presented her research as a poster at the 2020 USWBSI forum. She plans to publish the complete work in a referred journal in 2021. We are also ready to submit our publication on a meta-analysis of the genetics of FHB resistance in barley.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (5/15/20 - 5/14/21). 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 FY20 award period?**
   ☐Yes   ☒No
   If yes, how many?   Click to enter number here.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY20 award period?**
   ☐Yes   ☒No
   If yes, how many?   Click to enter number here.

3. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☐Yes   ☒No
   If yes, how many?   Click to enter number here.

4. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   ☐Yes   ☒No
   If yes, how many?   Click to enter number here.

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Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with <u>full or partial</u> support through the USWBSI during the <u>FY20 award period (5/15/20 - 5/14/21)</u>. 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	FHB Rating (0-9)	Year Released
Nothing to report.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

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

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Publications, Conference Papers, and Presentations

Instructions: Refer to the PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (5/15/20 - 5/14/21**) 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. See example below for a poster presentation with an abstract:

> Winn, Z.J., Acharya, R., Lyerly, J., Brown-Guedira, G., Cowger, C., Griffey, C., Fitzgerald, J., Mason R.E., and Murphy, J.P. (2020, Dec 7-11). Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat (p. 12). In: Canty, S., Hoffstetter, A. and Dill-Macky, R. (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum.* https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Su, W.H., Yang, C., Dong, Y., Johnson, R., Page, R., Szinyei, T., Hirsch, C.D. and Steffenson, B.J. 2021. Hyperspectral imaging and improved feature variable selection for automated determination of deoxynivalenol in various genetic lines of barley kernels for resistance screening. Food Chemistry. 343. 128507.
https://doi.org/10.1016/j.foodchem.2020.128507
Status: Published
Acknowledgement of Federal support: YES

Su, W.H., Zhang, J., Yang, C., Page, R., Szinyei, T., Hirsch, C.D. and Steffenson, B.J., 2021. Automatic Evaluation of Wheat Resistance to Fusarium Head Blight Using Dual Mask-RCNN Deep Learning Frameworks in Computer Vision. Remote Sensing. 13(1). 26.
https://doi.org/10.3390/rs13010026
Status: Published
Acknowledgement of Federal support: YES

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

Nothing to report.

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Other publications, conference papers and presentations.

Page, R., Szinyei, T., Martin, M., Sallam, A.H., Matny, O., Wodarek, J., Dong, Y., Hayes, P., and
   Steffenson, B. (2020, Dec. 7-11). Quantitative trait loci associated with resistance to
   Fusarium head blight and DON accumulation in barley populations derived from
   moderately resistant six- and two-rowed parents. In: S. Canty, A. Hoffstetter, and R.
   Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum* (p. 9),
   https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (abstract and poster)

Su, W., Zhang, J., Yang, C., Page, R., Szinyei, T., Hirsch, C.D., and Steffenson, B. (2020, July 12-
   15). Evaluation of Mask RCNN for learning to detect Fusarium head blight in wheat
   images. ASABE Annual International Virtual Meeting.
   https://doi:10.13031/aim.202000816
Status: Conference paper published
Acknowledgement of Federal Support: YES