USDA-ARS | U.S. Wheat and Barley Scab Initiative

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

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USDA-ARS Agreement Title:	Evaluation and Genetic Characterization of Hordeum Germplasm for Resistance to FHB
FY20 USDA-ARS Award Amount:	$64,516
Recipient Organization:	University of Minnesota Department of Plant Pathology 495 Borlaug Hall, 1991 Upper Buford Circle St. Paul, MN 55108
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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	$64,516
	FY21 Total ARS Award Amount	$64,516

I am submitting this report as an: ☒ Annual Report ☐ Final Report

I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.

Brian J. Steffenson

Principal Investigator Signature

July 26, 2022

Date Report Submitted

[‡] BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
EC-HQ – Executive Committee-Headquarters
FST-R – Food Safety & Toxicology (Research)
FST-S – Food Safety & Toxicology (Service)
GDER – Gene Discovery & Engineering Resistance
HWW-CP – Hard Winter Wheat Coordinated Project
MGMT – FHB Management
  MGMT-IM – FHB Management – Integrated Management Coordinated Project
PBG – Pathogen Biology & Genetics
TSCI – Transformational Science
VDHR – Variety Development & Uniform Nurseries
  NWW –Northern Soft Winter Wheat Region
  SPR – Spring Wheat Region
  SWW – Southern Soft Red Winter Wheat Region

Project 1: Evaluation and Genetic Characterization of Hordeum Germplasm for Resistance to FHB

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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 dozens of 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 ~150 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?**
b) **What were the significant results?**
c) **List key outcomes or other achievements.**

Objective 1 activities: PI 350725 was crossed with ‘Quest,’ the first Midwestern six-rowed cultivar with partial FHB resistance. F_1 progeny were then backcrossed with ‘Quest’ to obtain the BC_1 generation. Then, 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 1 results: 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 1 key outcomes or other achievements: Lines having the lowest levels of FHB and DON are being used as parents in the breeding program. The results from this biparental mapping study were presented as a poster at the 2020 USWBSI forum.

Objective 2 activities: In 2021, a panel of the most resistant accessions was planted and evaluated for FHB reaction and DON accumulation in Crookston. This was the final year of evaluation for the panel. The final DON data will be obtained summer 2022.

Objective 2 results: Of the ~150 selected accessions evaluated in four environments over the past three years, several have exhibited consistently low levels of FHB and DON. We now have robust FHB and DON datasets on the selected panel of barleys. Best linear unbiased estimates (BLUEs) for each line were estimated for the panel of accessions, providing a single phenotypic value for each trait and each line across all environments. Forty-one accessions had BLUE values for FHB severity below that of the six-rowed resistant control Quest (21.4%) and 123 had BLUE values for DON accumulation below that of Quest (6.1 ppm). 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 association mapping and haplotype analysis. Association mapping was performed on the panel to identify genomic regions associated with FHB resistance and DON accumulation. Multiple QTLs for FHB and DON were identified that were independent of QTLs for key agro-morphological traits (heading date and height). Haplotypes of five markers were generated flanking the markers identified with the most significant marker-trait associations.

Objective 2 key outcomes or other achievements: The R package Haplostats was then used to evaluate haplotype frequency and haplotype effects in the population. It was discovered that the resistant six-rowed control line, Chevron, carried 11 of the most favorable haplotype alleles out of 24 key loci for resistance to FHB and DON accumulation. Other resistant accessions carried multiple favorable haplotype alleles in unique combinations across loci. These data were used to inform selection of parental lines to be used in the development of a multi-parent population with the goal of generating progeny carrying novel combinations maximizing favorable alleles across all identified loci. The R package PopVar was also used to predict the mean and genetic variance for FHB and DON of simulated bi-parental populations, as an additional heuristic for selecting potential parental combinations.

Objective 3 activities: A panel of 87 previously untested English spring barley landraces donated by the John Innes Centre were evaluated for FHB and DON accumulation in Crookston in 2021.

Objective 3 results: Twenty-four of the English landraces exhibited FHB severities below 15%. However, many of the accessions (30 of 87 or 34.5%) were extremely late-heading and therefore not comparable to adapted germplasm. For comparison, the resistant six-rowed and two-rowed controls averaged FHB severities of 10% and 5%, respectively.

Objective 3 key outcomes or other achievements: The 24 English landraces exhibiting moderate levels of resistance will be tested in future trials for resistance to FHB and DON accumulation. They will also be haplotyped for loci controlling these traits.

Objective 4 activities: All FHB and DON data taken on the most resistant selections were compiled for all previous field seasons. This screened germplasm also included an introgression library (S42IL) developed by Klaus Pillen's group in Germany with the malting barley variety Scarlett as the recurrent parent and a wild barley (*Hordeum vulgare* ssp. *spontaneum*) accession as the donor. These data were then used to determine which accessions were worthy of passing on to barley breeding programs for variety development. Agro-morphological traits including height and heading date were considered in addition to the FHB scores and DON concentrations.

Objective 4 results: We identified some promising accessions from the germplasm evaluation panel to pass along to the barley breeding programs. Moreover, six two-rowed lines from Pillen's introgression library performed consistently well with respect to low DON levels. These accessions also showed heading dates and plant heights within the desired range for breeding.

Objective 4: key outcomes or other achievements: Accessions with promising levels of resistance to FHB and DON accumulation will be submitted to barley breeding programs for variety development.

3. 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 included Michelle Jugovich, Molly Bergum, Michael Miller, Elena Angelo, and Anthony Shea. 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.

4. 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 defend her thesis and publish the complete work in a referred journal in 2022. We are also ready to submit our publication on a meta-analysis of the genetics of FHB resistance in barley.

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY21 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

Did you publish/submit or present anything during this award period?

Yes, I've included the citation reference in listing(s) below.
No, I have nothing to report.

Journal publications as a result of FY21 grant award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

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 as a result of FY21 grant award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

None

Other publications, conference papers and presentations as a result of FY21 grant award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.

R. Page, A.H. Sallam, T. Szinyei, O. Matny, J. Wodarek, B. Steffenson. 2022. Development of a multi-parent population to enhance FHB resistance in barley. 23rd North American Barley Researchers Workshop & 43rd Barley Improvement Conference. Presentation; 9/27/2022. Davis, CA, USA.
Status: Abstract Submitted
Acknowledgement of Federal Support: YES