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

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

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Fiscal Year:	2021
USDA-ARS Agreement ID:	59-0206-0-135
USDA-ARS Agreement Title:	Molecular Genetics Approaches to Developing Scab Resistance
FY20 USDA-ARS Award Amount:	$162,190
Recipient Organization:	University of Minnesota Department of Agronomy and Plant Genetics 411 Borlaug Hall, 1991 Upper Buford Circle St. Paul, MN 55108
DUNS Number:	555917996
EIN:	41 -6007513
Recipient Identifying Number or Account Number, if any:	CON000000086331
Project/Grant Period:	5/17/21 - 5/16/23
Reporting Period End Date:	5/16/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Molecular Genetics Approaches to Developing Scab Resistant Barley	$85,575
GDER	Utilizing Genomics Resources to Develop Scab Resistant Wheat	$76,615
	FY21 Total ARS Award Amount	$162,190

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.

Gary J. Muehlbauer

Principal Investigator Signature

July 6, 2022

Date Report Submitted

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‡ 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: Molecular Genetics Approaches to Developing Scab Resistant Barley

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

The major goal of this project is to develop genetic tools for increasing FHB resistance in barley. There are three major objectives that will be addressed including: (1) characterize the impact of trichothecenes on infection and host responses; (2) identify resistant mutants; and (3) fine map and characterize the chromosome 2H bin8 and chromosome 6H bin7 FHB resistant QTL.

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. Characterize the impact of trichothecenes on infection and host responses. We are examining the infection pathways, host response, and DON and D3G levels of a barley transgenic overexpressing *HvUGT13248*, a *HvUGT13248* mutant and natural accessions inoculated with *F. graminearum*. Our overall goal is to identify resistance genes and mechanisms that can be genetically manipulated and used in breeding programs. We have shown that *HvUGT13248* rapidly conjugates DON with a glucoside group resulting in the nontoxic DON-3-Glucoside (D3G). *HvUGT13248* mutants had much reduced capacity to conjugate DON to D3G. We developed a type II resistance assay in barley and used it to screen a set of 10 barley genotypes that ranged from moderately resistant (e.g., Chevron), moderately susceptible (e.g., Morex) and highly susceptible (e.g., PI383933) and showed that only the PI383933 accession exhibited a susceptible reaction, indicating that most barley accessions exhibit type II resistance. We also used the type II assay to show that ergosterol and DON are largely confined to the inoculated florets in the wildtype plants but were found at higher concentrations in inoculated and adjacent florets in the *HvUGT13248* mutant. We examined *HvUGT13248* sequence data from 533 accessions and detected seven non-synonomous mutations in 25 accessions. Interestingly, in our root assay none of the mutations exhibited DON susceptibility compared to the controls. Using histological approaches, we showed that in *HvUGT13248* mutants the fungus travels through multiple rachis nodes and internodes and infects florets, whereas in wildtype plants fungal growth is restricted at the rachis node of the infected floret, indicating that the location of resistance is the rachis node. These results show that *HvUGT13248* is the primary type II resistance gene in barley. We have collected RNA-seq data and corresponding DON data from *HvUGT13248* mutant and wildtype plants after *F. graminearum* inoculation and are in the process of conducting the analysis.

Objective 2. Identify DON and FHB resistant mutants. In 2021, our field screen was not useful due to the dry and hot summer not being conducive to FHB. In 2022, we are screening 250 M3 lines from mutagenized population in the cv. Conlon. In the future we will begin screening M3 lines on DON-containing media.

Objective 3. Fine map and characterize the chromosome 2H bin8 and chromosome 6H bin7 FHB resistant QTL. With previous USWBSI funding we developed populations segregating for the chromosome 6H and 2H FHB QTL regions, genotyped approximately 2,000 individuals from each population with markers that flanked each QTL region, and selected recombinants (Kevin Smith collaboration). The recombinants were further genotyped with markers spanning the QTL region, recombination breakpoints identified, and phenotyped in the field in 2016-2019 for FHB, heading date and grain protein content. Lines that carry resistance uncoupled from the deleterious

traits were identified, and the FHB resistance allele containing regions were reduced to less than 1 cM and 8 cM for the chr 6H and 2H regions, respectively. A paper was published in *Theoretical and Applied Genetics* describing the fine mapping of the 6h bin7 region. We identified two DON QTL and an FHB QTL in our fine mapping of the 2H bin8 region. To confirm these QTL, the recombinants have been grown in the field in 2022 and will be phenotyped for FHB severity and DON accumulation.

Progress on related activities

Developing elite barley germplasm carrying the *HvUGT13248* transgene

We are introgressing the *HvUGT13248* transgene (originally in the Golden Promise background) into the elite cultivar 'Genesis'. We generated BC2F3 progeny and are screening the lines for transgene homozygosity. These lines will be tested in the greenhouse in the Fall 2022 – Winter 2023.

b) What were the significant results?

We showed that the rachis node is the site important for resistance. We showed that *HvUGT13248* conjugated DON to D3G. We have also shown that *HvUGT13248* is the primary gene conferring type II resistance in barley. Our mutant screening is just getting started so there are no results to report. We have fine mapped the 6H bin7 and 2H bin8 regions and have identified lines that carry resistance that are uncoupled from deleterious traits. We have also shown that both QTL regions are a complex of QTL for DON and FHB resistance.

c) List key outcomes or other achievements.

Our results show that the rachis node is the site important for FHB resistance in barley. We showed that *HvUGT13248* conjugated DON to D3G. We have also shown that *HvUGT13248* is the primary gene conferring type II resistance in barley. Our mutant screening is just getting started so there are no results to report. We have fine mapped the 6H bin7 and 2H bin8 regions and have identified lines that carry resistance that are uncoupled from deleterious traits. We have also shown that both QTL regions are a complex of QTL for DON and FHB resistance.

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

Three postdocs have worked on this project. Each of the postdocs meet with me regularly and attend and present results in weekly lab meetings. Two of the postdocs presented posters at the 2021 National Scab Forum.

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

The fine mapping of the chromosome 6H QTL region was published in Theoretical and Applied Genetics. Two posters were presented at the 2021 National Scab Forum.

Project 2: Utilizing Genomics Resources to Develop Scab Resistant Wheat

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

The major goal of this project is to develop genetic tools for increasing FHB resistance in wheat. There are two major objectives that will be addressed including: **(**1) Identify and characterize mutations for increased trichothecene and FHB resistance in wheat; and (2) Identify mutants with increased trichothecene and FHB resistance in wheat.

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. Identify and characterize mutations for increased trichothecene and FHB resistance in wheat. Our plan is to use the mutagenized Kronos population (Krasileva et al., 2017) and identify mutations in candidate susceptibility genes and test plants carrying those mutations for FHB and trichothecene resistance. Kronos is a tetraploid exhibiting susceptibility to FHB. This objective is a targeted approach to identify susceptibility genes that when mutated result in resistant plants. Using previously generated and published transcriptome data from wheat and barley inoculated with *F. graminearum* and literature searches of papers published on plant-pathogen interactions, we identified a gene family that is a good candidate for studying in more detail. We plan to begin to work with mutagenized lines that contain mutations in members of this gene family.

Objective 2. Identify mutants for increased tricothecene and FHB resistance in wheat. We will phenotypically screen a random selection of 500 individuals from the Kronos population. To date, we have screened 288 M3 lines in the greenhouse and 100 lines in the field. In the field trial in the summer of 2021, the disease severity was too low to discriminate between susceptible and more resistant lines. To date, from the greenhouse screen we have identified 20 lines that decreased severity and 22 that exhibit increased susceptibility. We will rescreen these lines along with additional lines in the greenhouse in the fall and spring.

Other related activities

Developing elite wheat germplasm carrying the *HvUGT13248* transgene

We are introgressing the *HvUGT13248* transgene (Bobwhite background) into the elite cultivar 'Rollag'. Rollag carries the *Fhb1* resistance gene. Thus, we are developing lines that contain four genotypic combinations: *Fhb1*/*Fhb1*,*UGT*+/*UGT*+; *Fhb1*/*Fhb1*,UGT-/UGT-; +/+,*UGT*+/*UGT*+; +/+,UGT-/UGT-. We generated BC1F1 progeny and are screening the lines for *HvUGT13248* expression and the presence of *Fhb1*. BC1F2 plants will be screened for *HvUGT13248* and *Fhb1* in the Fall to select the four genotypes for characterization. These lines will be tested in the greenhouse in the Winter 2023.

Characterize the ability of transgenic wheat carrying *HvUGT13248* to provide resistance to NX-2 producing *F. graminearum*.

We inoculated transgenic plants carrying *HvUGT13248* with NX-2, 3ADON, 15ADON and DON and showed that each of the toxins are conjugated with a glucoside group. This was a collaboration with Franz Berthiller and Gerhard Adam.

b) What were the significant results?

We identified a gene family that meets our criteria for studying in more detail. To date, from the greenhouse screen we have identified 20 lines that exhibit reduced severity and 22 that exhibit increased susceptibility.

c) List key outcomes or other achievements.

Preliminary results resulted in 20 and 22 lines that were identified that exhibit increased resistance and susceptibility, respectively. A gene family was identified that has potential for being involved in resistance.

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

A postdoc has worked on this project. He meets with me regularly and attends and presents results in weekly lab meetings.

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

We have not generated enough results that have been validated to disseminate them.

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).

Huang, Y., L. Yin, A.H. Sallam, S. Heinen, L. Li, K. Beaubien, R. Dill-Macky, Y. Dong, B.J. Steffenson, K.P. Smith and G.J. Muehlbauer. 2021. Genetic dissection of a pericentromeric region of barley chromosome 6H associated with Fusarium head blight, grain protein content and agronomic traits. *Theor. Appl. Genet.* 134:3963-3981. https://doi.org/10.1007/s00122-021-03941-9; acknowledgment 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).

Bethke, G., Y. Huang, G. Hensel, S. Wyant, X. Li , S. Heinen, S. McCormick, P. Morrell, Y. Dong, J. Kumlehn, S. Salvi, F. Berthiller, G.J. Muehlbauer. 2021. The barley UDP-glycosyltransferase *UGT13248* is required for deoxynivalenol conjugation and type 2 resistance to Fusarium head blight. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf

Huang, Y., S. Heinen, B. Steffenson, K.P. Smith and G.J. Muehlbauer. 2021. Fine mapping of FHB and DON quantitative trait loci on chromosome 2H in barley. *Proceedings of the 2021 National Fusarium Head Blight Forum*; Virtual. December 6-7, 2021. Retrieved from: https://scabusa.org/forum/2021/2021NFHBForumProceedings.pdf

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.

None