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

FY21 FINAL Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	59-0206-0-135
USDA-ARS Agreement Title:	Molecular Genetics Approaches to Developing Scab Resistance
Principle Investigator (PI):	Gary Muehlbauer
Institution:	University of Minnesota
Institution UEI:	KABJZBBJ4B54
Fiscal Year:	2021
FY21 USDA-ARS Award Amount:	$162,190
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Period of Performance:	5/17/21 - 5/16/23
Reporting Period End Date:	5/16/2023

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 a: ☒ 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 19, 2023

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: 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 DON and FHB resistant mutants; and (3) fine map and characterize the chromosome 2H bin8 and chromosome 6H bin7 FHB resistant QTL.

a) What was accomplished under these goals or objectives?

What were the major activities?

Objective 1. Characterize the impact of trichothecenes on infection and host responses. We examined the infection pathways, host response, and DON and D3G levels in barley transgenics overexpressing *HvUGT13248*, a *HvUGT13248* mutant, and natural accessions inoculated with *F. graminearum*. Our overall goals are to identify resistance genes and mechanisms that can be genetically manipulated and used in breeding programs, to determine if *HvUGT13248* is required for type II resistance in barley, and to determine the timing and location of trichothecene biosynthesis and host resistance. We showed 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 found no significant difference in type II resistance between barley genotypes, indicating that all barleys carry type II resistance. We also used the type II assay to examine the location of ergosterol and DON in wildtype and *HvUGT13248* mutants and found that DON and ergosterol 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 521 accessions and detected eleven non-synonomous mutations. Interestingly, in our DON root assay none of the mutations exhibited DON susceptibility compared to the controls, indicating that most if not all barleys contain a functional *UGT13248* gene. Using a transgenic strain of *F. graminearum* that provides the opportunity to track fungal growth, 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. Using a transgenic *F. graminearum* strain that provides the ability to track trichothecene biosynthesis, we observed trichothecene biosynthesis at the rachis node at four days after inoculation. These results show that *HvUGT13248* is the primary type II resistance gene in barley. A publication is in revision at *Plant Physiology* that describes this work.

We have collected RNA-seq data and corresponding DON and ergosterol data from *HvUGT13248* mutant and wildtype plants after *F. graminearum* inoculation and are in the process of conducting the analysis. As expected, the mutant compared to wildtype accumulated more DON and ergosterol. To date, we have identified a large suite of UDP-glucosyltransferases, fungal effector proteins, and other barley genes that we are following up on.

To characterize the ability of HvUGT13248 to detoxify a broad set of trichothecenes, we conducted two replicates of an experiment to characterize a set of barley sister genotypes (transgenic Genesis UGT+ and UGT-, transgenic Rasmussen UGT+ and UGT-, and a Morex UGT13248 TILLING mutant and wildtype) to examine resistance to FHB infection using four chemotypes of *Fusarium graminearum* (3ADON, 15ADON, NIV, and NX-2). Preliminary results

indicate that lines expressing the transgene show less variability in FHB symptoms compared to the wildtype controls. The mutant lacking the native UGT shows increased susceptibility to FHB infection to all four chemotypes. Spike tissues from the first replication have been sent to Franz Berthiller for toxin 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 screened 250 M3 lines from a mutagenized population in the cv. Conlon. In 2023, we are screening 1,000 M3 lines (500 in Crookston and 500 in St. Paul). From the screening work, we have identified six lines that may exhibit increased FHB susceptibility and possibly 2-3 lines that exhibit decreased susceptibility. In 2022 and 2023, disease severity has been low, likely due to increased heat and drought conditions, making it difficult to identify mutants that clearly reduce disease severity.

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, 2018-2022 for FHB severity and DON accumulation. 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 (Huang et al., 2021) 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 St. Paul and Crookston in 2022 and 2023 and were phenotyped for FHB severity, DON accumulation, heading date and height. Three DON QTL (Qdon-2H-1, Qdon-2H-2 and Qdon-2H-3) and one FHB QTL (Qfhb-2H-1) were identified. The Qdon-2H-1 was found only in the SP2016 trial and the Chevron allele contributed resistance. The Qdon-2H-2 and Qfhb-2H-1 were coincident and the Chevron allele contributed susceptibility. The Qdon-2H-3 was about 1.2 cM away from Qdon-2H-2 and the Chevron allele contributed resistance. One height QTL was identified and not coincident with the FHB or DON QTL. The Chevron allele increased height.

We worked with Brian Steffenson to assemble a meta-analysis of the barley QTL that are associated with FHB resistance, reduced DON accumulation and agro-morphological traits. A publication is in press at *Plant Breeding* (Sallam et al., 2023) describing this work.

Progress on related activities

Developing elite barley germplasm carrying the *HvUGT13248* transgene

We introgressed the *HvUGT13248* transgene (originally in the 'Golden Promise' background) into the elite cultivars 'Genesis' and 'Rasmusson'. We identified sister lines in both genetic backgrounds that either carry or do not carry the transgene. These genetic stocks are being used to understand the role of *HvUGT13248* in disease resistance.

b) What were the significant results?

We showed that the rachis node is the site important for resistance and that *HvUGT13248* conjugated DON to D3G. We have also shown that *HvUGT13248* is the primary gene conferring type II resistance in barley. From our screening of a Conlon mutagenized population, we identified

six lines with increased susceptibility and 2-3 lines that exhibit decreased susceptibility. 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. The barley mutants may provide insight into the barley-*F. graminearum* interaction and provide genetic stocks for developing resistant barley cultivars. 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.

2. 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 Online National Scab Forum and one postdoc presented two posters at the 2022 National Scab Forum.

3. 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*. A paper is in press at *Plant Breeding* describing a meta-analysis of the barley QTL associated with FHB resistance, DON accumulation and agro-morphological traits. Another paper describing the role of *HvUGT13248* in FHB resistance is in revision at *Plant Physiology*. Two posters were presented at the 2021 Online National Scab Forum and two posters was presented at the 2022 National Scab Forum. Muehlbauer presented a talk at IPK-Gatersleben, Germany.

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?

a) What were the major activities?

Objective 1. Identify and characterize mutations for increased trichothecene and FHB resistance in wheat. We are using a mutagenized Kronos population (Krasileva et al., 2017) and identifying mutations in candidate susceptibility/resistance genes and testing plants carrying those mutations for FHB and trichothecene resistance/susceptibility. 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 and to identify resistance genes that when mutated result in susceptible 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 that is a good candidate for studying in more detail. We have identified Kronos lines that contain mutations in this gene, screened three mutant families and all three exhibited increased susceptibility. We plan to rescreen these lines and the remainder of the Kronos lines predicted to carry a mutation in this gene.

Objective 2. Identify mutants for increased tricothecene and FHB resistance in wheat. We have used point inoculation assays to screen over 400 M3 lines (10 plants/line) in the greenhouse and we have identified 30 lines that exhibit decreased severity and 35 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-; *Fhb1*-/*Fhb1*-,*UGT*+/*UGT*+; *Fhb1*-/*Fhb1*-,*UGT*-/*UGT*-. We generated BC1F1 progeny and are screening the lines for the presence and absence of *HvUGT13248* expression and the presence and absence of *Fhb1* to select the four genotypes for characterization. These lines will be tested in the greenhouse in the Winter 2023-2024.

Characterize the ability of transgenic wheat carrying *HvUGT13248* to provide resistance to NX-2, 3ADON, 15ADON and DON 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 30 lines that exhibit reduced severity and 35 that exhibit increased susceptibility.

c) List key outcomes or other achievements.

Preliminary results revealed 30 and 35 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?

x Yes, I've included the citation reference in listing(s) below.

☐ No, I have nothing to report.

Journal publications as a result of FY21 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. doi.org/10.1007/s00122-021-03941-9. Acknowledged of federal support: yes.

Sallam, A., M. Haas, Y. Huang, Y. Dong, Z. Tandukar, G. Muehlbauer, K. Smith, and B. Steffenson. 2023. Meta-analysis of the genetics of resistance to FHB in barley and considerations for breeding. *Plant Breeding* (In press). Acknowledged of federal support: yes.

Bethke, G.*, Y. Huang*, G. Hensel, S. Wyant, X. Li, S. Shin, S. Heinen, M.B. Quin, S. McCormick, P. Morrell, Y. Dong, S. Salvi, J. Kumlehn, F. Berthiller, and G.J. Muehlbauer. 2022. *UGT13248* confers type II resistance to *Fusarium graminearum* in barley. *Plant Physiol.* (In revision). *indicates co-first authors. Acknowledged of federal support: yes.

Books or other non-periodical, one-time publications as a result of FY21 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).

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

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

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

Bethke, G., Y. Huang, G. Hensel, S. Wyant, X. Li, S. Heinen, S. McCormick, P. Morrell, Y. Dong, J. Kumlehn, S. Salvi, F. Berthiller, and 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

Bethke, G., Y. Huang, G. Hensel, S. Heine, C. Liu, S. Wyant, X. Li, S. McCormick, P. Morrell, Y. Dong, J. Kumlehn, S. Salvi, F. Berthiller, and G.J. Muehlbauer. (2022). The UDP-Glycosyltransferase UGT13248 is Required for Type 2 Resistance to Fusarium Head Blight in Barley. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf

Huang, Y., A. Haaning, G. Bethke, S. O'Mara, Y. Dong and G.J. Muehlbauer. (2022). Microscopy and RNA-Seq analysis of fusarium head blight Infection in a barley mutant deficient in deoxynivalenol detoxification. Proceedings of the 2022 National Fusarium Head Blight Forum; Tampa, FL. December 4-6, 2022. Retrieved from: https://scabusa.org/forum/2022/2022NFHBForumProceedings.pdf