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:	59-0206-8-203
USDA-ARS Agreement Title:	Molecular Genetics Approaches to Developing Scab Resistance
FY19 USDA-ARS Award Amount:	$ 170,503
Recipient Organization:	Regents of the University of Minnesota Suite 450 Sponsored FIN RPT-P100100001 Minneapolis, MN 55455-2003
DUNS Number:	555917996
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Recipient Identifying Number or Account Number:	CON000000075171
Project/Grant Reporting Period:	5/17/19 - 5/16/20
Reporting Period End Date:	5/16/2020

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Molecular Genetics Approaches to Developing Scab Resistant Barley	$ 88,807
GDER	Characterizing Trichothecene Resistance and Developing Scab Resistant Wheat	$ 81,696
	FY19 Total ARS Award Amount	**$ 170,503**

Gary J. Muehlbauer

July 22, 2020

Principal Investigator Date

* 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

Project 1: *Molecular Genetics Approaches to Developing Scab Resistant Barley*

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

The major goal of the project is to develop genetic tools for increasing FHB resistance in barley. There were two major objectives of the grant: (1) characterize transgenic barley overexpressing *HvUGT13248*; and (2) fine map and characterize the chromosome 2H bin8 and 6H bin 7 FHB resistance QTL.

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. Characterize transgenic barley overexpressing *HvUGT13248*. We created transgenic barley lines overexpressing *HvUGT13248* and showed that they exhibit high levels of deoxynivalenol (DON) resistance in roots on DON-containing media, and rapidly converted DON to the nontoxic DON-3-glucoside (D3G). To generate materials that can be screened in the field, we backcrossed the *HvUGT13248* transgene into Rasmusson and selected lines that are homozygous for the transgene. Transgenic plants carrying the *HvUGT13248* transgene in the Rasmusson background were planted in the field in the summer of 2018. Unfortunately, the level of disease was too low to discriminate between the non-transgenic controls and the transgenic lines. The field trial was replanted in 2019. In this trial, disease was appropriate to distinguish susceptible and resistant controls, however all plants containing the transgene were shorter than control plants and disease levels were high in all short genotypes. Thus, we could not distinguish between effects of the transgene expression and height effects on disease. The field trials are a collaboration with Ruth Dill-Macky. To obtain lines that exhibited a consistent height, we initiated development of backcross lines in the two-rowed cultivar Genesis.

An additional activity related to this project included: We identified mutations in *HvUGT13248* in the Morex genetic background and showed that plants carrying these mutations are susceptible to DON. We also showed that when DON was inoculated on these plants it was conjugated to the non-toxic D3G to a much lower extent. These results indicate the *HvUGT13248* is the primary gene that functions to detoxify DON to D3G. Field trials with these lines were conducted in St. Paul and Crookston in 2019 and the results showed higher level of disease in the mutants than in wild-type in Crookston, while no significant difference in disease development was apparent in St. Paul. However, DON to D3G conversion was still reduced in St. Paul, suggesting an effect of *HvUGT13248* even in environments where differences in disease progression are not apparent. In 2020, the field trials were replanted in St. Paul and Crookston and we are in the process of obtaining FHB severity, and DON and D3G accumulation data.

Objective 2. Fine map and characterize the chromosome 6H bin 7 and 2H bin 8 FHB resistance QTL. Barley QTL associated with Fusarium head blight resistance, reduced deoxynivalenol accumulation, early senescence and increased grain protein content (GPC)

colocalize on the short arm of chromosome 6H bin 7. To understand the complex genetics of this QTL, we are conducting a fine mapping project. We generated a large F_2 segregating population (~2,000 individuals) from crossing a near-isogenic line carrying the chromosome 6H bin 7 resistant allele in the cultivar Lacey genetic background to Lacey. SSR markers were used to identify recombinants in the chromosome 6H bin 7 region from the F_2 population, which were further genotyped with 34 SNP markers to identify 13 recombinant classes. Homozygous recombinants in the $F_{2:3}$ families were identified with SNP markers and homozygous F_4 plants were tested in field trials in St. Paul in 2016, 2017, 2018 and 2019 for FHB severity, DON accumulation and grain protein content (GPC). All data (FHB severity, DON accumulation, and GPC) have been collected from the trials. In 2017, the disease pressure was too low to obtain reliable FHB data. From the 2016, 2018 and 2019 field tests, we identified recombinants that exhibit resistance that appears to be uncoupled from the high GPC allele. Interestingly, high GPC is co-localized with reduced DON accumulation likely due to the early senescence conferred by the high GPC allele. An FHB QTL has been identified that appears to be independent of the GPC locus. This work was a collaboration with Kevin Smith. His group also conducted fine mapping of the same QTL region in an independent population and obtained similar results. We have combined the datasets and results, and are preparing a manuscript. To further fine map the FHB QTL that is independent of GPC, we are in the process of identifying additional recombinants in a new population of 2,000 F_2 individuals.

A major barley FHB QTL is also located in the chromosome 2H bin8 region. To fine map this region, an F_2 population was generated from near-isogenic lines in the M69 genetic background carrying the resistant allele crossed to M69, a susceptible line. Two KASP SNP markers were used to genotype ~2,000 plants to identify recombinants. To determine the general location of the breakpoints, the recombinants were genotyped with another 33 SNP markers within the introgressed region. Homozygous F_3 plants were phenotyped for FHB resistance, heading date, and DON accumulation in St. Paul in 2016, 2017, 2018 and 2019. Unfortunately, the disease pressure was low in 2017 and the data were unreliable. Lines that exhibit reduced FHB severity were identified in 2016, 2018 and 2019.

b) What were the significant results?

Objective 1. Characterize transgenic barley overexpressing *HvUGT13248.* We developed transgenic barley overexpressing *HvUGT13248* that exhibits DON resistance in roots in DON-containing media and that detoxified DON to the nontoxic D3G. Backcross lines in the cultivar Rasmusson containing the *HvUGT13248* transgene have been developed and backcross lines in the two rowed cultivar Genesis are being developed. Plants carrying mutations in the *HvUGT13248* have been identified and we showed that these plants are susceptible to DON due to a deficiency in converting DON to non-toxic D3G.

Objective 2. Fine map and characterize the chromosome 6H bin 7 and 2H bin 8 FHB resistance QTL. Our fine mapping results indicate that we have recombinants that contain

FHB resistance without the deleterious high GPC allele. We also have evidence that the high GPC allele is associated with decreased DON accumulation, likely due to the early senescence in plants carrying the high GPC allele.

c) List key outcomes or other achievements.

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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, the work on this project was impacted by the COVID-19 pandemic. The two postdocs working on this project needed to work from home for approximately three months. Fortunately, they had analysis and paper writing so there was still progress on the objectives of the project. However, progress requiring lab work was hindered including: developing transgenic lines for screening, and identifying additional recombinants in the 6H Bin 7 region.

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

Both postdocs (Gerit Bethke and Yadong Huang) meet with me regularly, and participate and present their work in weekly lab meetings.

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

Posters describing the transgenic and mutation work, and the fine mapping work were presented at the National Scab Forum in December 2019.

Project 2: *Characterizing Trichothecene Resistance and Developing Scab Resistant Wheat*

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

Fusarium head blight (FHB, scab), a fungal disease of small grain crops caused by *Fusarium graminearum*, threatens to reduce wheat and barley to economically unviable crops in the United States. During infection the fungus produces trichothecene mycotoxins such as deoxynivalenol (DON) that have been shown to increase fungal virulence. To complement the current breeding efforts, a major goal of my laboratory is to develop and characterize transgenic wheat exhibiting trichothecene and FHB resistance. Previously, my laboratory developed transgenic wheat carrying a barley UDP-glucosyltransferase gene (*HvUGT13248*) and showed that these transgenics exhibit high levels of FHB resistance via conjugation of DON to DON-3-O-glucoside (D3G). There are three major objectives in the proposed work including: (1) test elite wheat cultivars carrying *HvUGT13248* for FHB resistance; (2) characterize the ability of transgenic wheat carrying *HvUGT13248* to provide resistance to NX-2-producing *F. graminearum*; and (3) characterize wheat UDP-glucosyltransferase genes.

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. Test elite wheat cultivars carrying *HvUGT13248* for FHB resistance. We backcrossed the *HvUGT13248* transgenic line into the cultivar Linkert and identified homozygous lines with transgene expression, and lines without transgene expression. We also developed backcross lines of *HvUGT13248* transgenics in the cv. Rollag genetic background and identified lines of each of the four genotypes, namely *UGT+*/*Fhb1+*, *UGT-*/*Fhb1+*, *UGT+*/*Fhb1-*, and *UGT-*/*Fhb1-*. These lines were screened in the greenhouse in the Fall 2016 and spring 2017. In the Rollag background, lines carrying the combination of *HvUGT13248* and *Fhb1* exhibited stable and higher resistance than *Fhb1* alone. In the Linkert background, lines carrying *HvUGT13248* exhibit higher resistance than lines that did not carry the transgene. The lines were planted in the field in 2018 and the disease pressure was not high enough to distinguish the transgenic lines from non-transgenic controls. We planted the lines in the field in 2019 and the results showed height differences in the Linkert background made it difficult to distinguish between effects of the UGT transgene and height effects on disease development. For the plants in the Rollag background plants carrying both the *HvUGT13248* transgene and *FHB1* showed the lowest disease levels while variation in disease existed in the other combinations of these two genes. More replicates will be necessary to draw clear conclusions of disease development in the field.

Objective 2. Characterize the ability of transgenic wheat carrying *HvUGT13248* to provide resistance to NX-2-producing *F. graminearum*. We have completed the inoculation of the transgenic wheat plants carrying *HvUGT13248* with NX-2, 3ADON,

15ADON and DON and are in the process of preparing the samples for biochemical analysis. This objective is a collaboration with Franz Berthiller and Gerhard Adam.

Objective 3. Characterize wheat UDP-glucosyltransferase genes. We identified the wheat UDP-glucosyltransferase genes to isolate but then learned that another group was already working on this topic and published a paper in December 2018 (Gatti et al., 2018, Frontiers in Plant Science).

b) What were the significant results?

Objective 1. Test elite wheat cultivars carrying *HvUGT13248* for FHB resistance. We developed transgenic wheat in elite cultivars that provide resistance to FHB and combined HvUGT13248 with *Fhb1* to test for enhanced resistance. Our greenhouse tests showed that the combination of *HvUGT13248* and *Fhb1* in the same background exhibited stable and higher resistance than *Fhb1* alone.

Objective 2. Characterize the ability of transgenic wheat carrying *HvUGT13248* to provide resistance to NX-2-producing *F. graminearum.* We have also shown that *HvUGT13248* provides resistance to DON and NIV and examination of resistance to NX-2, 3ADON and 15ADON is ongoing.

Objective 3. Characterize wheat UDP-glucosyltransferase genes. There are no significant results to report as another group has completed this work

c) List key outcomes or other achievements.

Objective 1. Test elite wheat cultivars carrying *HvUGT13248* for FHB resistance. We developed transgenic wheat in elite cultivars that provide resistance to FHB and combined HvUGT13248 with *Fhb1* to test for enhanced resistance. Our greenhouse tests showed that the combination of *HvUGT13248* and *Fhb1* in the same background exhibited stable and higher resistance than *Fhb1* alone.

Objective 2. Characterize the ability of transgenic wheat carrying *HvUGT13248* to provide resistance to NX-2-producing *F. graminearum.* We have also shown that *HvUGT13248* provides resistance to DON and NIV and examination of resistance to NX-2, 3ADON and 15ADON is ongoing.

Objective 3. Characterize wheat UDP-glucosyltransferase genes. There are no key outcomes or other achievements to report as another group has completed this work.

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, the work on this project was impacted by the COVID-19 pandemic. The two postdocs working on this project needed to work from home for approximately three months. Fortunately, they had analysis and paper writing so there was still progress on the objectives of the project. However, progress requiring lab and field work was hindered including: biochemical analysis of toxins inoculated on the transgenic wheat carrying *HvUGT13248* and testing the impact of *Fhb1* and *HvUGT13248* in the same background.

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

Both postdocs (Gerit Bethke and Yadong Huang) meet with me regularly, and participate and present their work in weekly lab meetings.

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

There is nothing to report.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (5/17/19 - 5/16/20). 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?**
   Yes

   If yes, how many? Two

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?**
   None.

   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

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 (5/17/19 - 5/16/20**) 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. See example below for a poster presentation with an abstract:

> De Wolf, E., D. Shah, P. Paul, L. Madden, S. Crawford, D. Hane, S. Canty, R. Dill-Macky, D. Van Sanford, K. Imhoff and D. Miller. 2019. "Impact of Prediction Tools for Fusarium Head Blight in the US, 2009-2019." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 12), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications. Nothing to report.

Books or other non-periodical, one-time publications. Nothing to report.

Other publications, conference papers and presentations.

Poster abstracts:

Bethke, G., Y. Huang, X. Li, G. Hensel, S. Salvi, J. Kumlehn, F. Berthiller and G.J. Muehlbauer. 2019. "The UDP-glucosyl transferase UGT13248 catalyzes DON to DON-3-glucose conversion in barley and affects DON accumulation in spikes." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 44), Milwaukee, WI; December 8-10.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Huang, Y., L. Yin, A. Sallam, S. Heinen, K. Beaubein, Y. Dong, B. Steffenson, K.P. Smith and G.J. Muehlbauer. 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.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)