USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY19 Final Performance Progress Report
Due date: July 29, 2021

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
EIN:	41 -6007513
Recipient Identifying Number or Account Number:	CON000000075171
Project/Grant Reporting Period:	5/17/19 - 5/16/21
Reporting Period End Date:	5/16/2021

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 27, 2021

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R – Research
S – Service (DON Testing Lab)
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

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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 these three items 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 for screening 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 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 were a collaboration with Ruth Dill-Macky. To obtain lines that exhibited a consistent height and to introgress the transgene into two-row germplasm, we initiated development of backcross lines in the two-rowed cultivar Genesis. Development of these lines is ongoing.

   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, conjugation to the non toxic D3G was dramatically reduced. These results indicate that *HvUGT13248* is the primary gene that functions to detoxify DON to D3G. Field trials with these lines in St. Paul and Crookston in 2019 and 2020 showed higher levels of disease in the mutants than in wild-type in 3 out of 4 trials. Additionally, DON to D3G conversion was reduced in the mutants compared to wild-type. Point inoculation experiments showed increased spread of disease in the mutants, suggesting a role of UGT13248 in type 2 resistance in barley. Rachis tissue of mutants contained more DON and ergosterol, used as a proxy for Fusarium growth, than wild-type. Fluorescence microscopy using a *Fusarium graminearum* strain containing Tri5prom::GFP and gpdAprom::dsRed identified both Fusarium growth and Tri5 promoter activity in rachis tissue, suggesting Fusarium spread along the spike might develop symptoms via the rachis.

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 conducted 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 a manuscript was submitted to Theoretical and Applied Genetics. 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. 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. From the 2016, 2018 and 2019 trials, lines that exhibit reduced FHB severity were identified. Preliminary QTL analysis identified two DON QTL and one FHB QTL and the DON and FHB QTL appear distinct. The Chevron alleles reduced DON accumulation at the DON QTL but inceased FHB severity at the FHB QTL. Further fine mapping work is in process.

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. These plants also showed reduced type 2 resistance, suggesting a role for *Hv*UGT13248 in type 2 resistance in barley.

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. Two DON QTL and a single FHB QTL were identified in the chromosome 2H bin 8 region.

c) List key outcomes or other achievements.

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 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 the non toxic D3G. Further, our HvUGT13248 mutants showed increased spread of disease in spikes and increased DON and Fusarium in rachis tissue, suggesting that full type 2 resistance in barley requires *Hv*UGT13248 and that inhibition of disease progression likely happens in the rachis and/or rachis node.

Objective 2. Fine map and characterize the chromosome 6H bin 7 and 2H bin 8 FHB resistance QTL. Our fine mapping results of the chromosome 6H bin 7 region 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. Fine mapping of the chromosome 2H bin 8 region has resulted in identifying lines that exhibit reduced FHB severity. Two DON and one FHB QTL were identified in this region.

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.

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, mutant, and fine mapping work were presented at the National Scab Forum in December 2020. A talk was presented at the 2020 Forum describing the role HvUGT13248 plays in resistance in barley.

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 these three items 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.

The UGT x Rollag BC1F3 population is being rescreened using PCR to verify *Fhb1*+ alleles and using RT-PCR to verify *UGT13248* expression. Once identified, the four genotypes will be grown in the greenhouse to assess FHB severity.

Objective 2. Characterize the ability of transgenic wheat carrying *HvUGT13248* to provide resistance to NX-2-producing *F. graminearum*. We inoculated the transgenic wheat plants carrying *HvUGT13248* with NX-2, 3ADON, 15ADON and DON. Our results showed increased glucosylation of DON in transgenic plants inoculated with 3ADON, 15ADON and DON and of NX-3 in plants inoculated with NX-2. This results indicate that *Hv*UGT13248 can glucosylate multiple trichothecene toxins produced by Fusarium. 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* detoxifies not only to DON and NIV, but also NX-2, 3ADON and 15ADON.

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 shown that *HvUGT13248* detoxifies DON, NIV, NX-2, 3ADON and 15ADON.

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 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.

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. A third postdoc joined the project in September 2020 and his ability to work in the lab was restricted.

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

All three postdocs (Gerit Bethke, Sean O'Mara 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?

We submitted a manuscript describing the fine mapping of the chromosome 6H bin7 QTL region, and have presented two poster abstracts and a talk at the National Fusarium Head Blight Forum 2020.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the **FY19 award period (5/17/19 - 5/16/21)**. 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 FY19 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 FY19 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

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 ☒No
   If yes, how many? Click to enter number here.

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?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

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 (5/17/19 - 5/16/21)**. 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
Not applicable to this project.	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

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

Publications, Conference Papers, and Presentations

Instructions: Refer to the 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 - 5/16/21**) 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:

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

Journal publications.

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. Genetic dissection of a pericentromeric region of barley chromosome 6H associated with Fusarium head blight resistance, grain protein content and agronomic traits.
Status: Submitted to Theoretical and Applied Genetics
Acknowledgement of Federal Support: Yes

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

Nothing to report.

Other publications, conference papers and presentations.

Bethke, G.,Y. Huang, G. Hensel, S. Wyant, X. Li, P. Morrell, J. Kumlehn, S. Salvi, F. Berthiller and Gary Muehlbauer. 2020. The barley glucosyltransferanse UGT13248 is required for deoxynivalenol conjugation and type 2 resistance to Fusarium head blight. National Fusarium Head Blight Forum Abstracts 2020 (Online meeting).
Status: Abstract published and poster presented
Acknowledgement of Federal Support: Yes

Huang, Y., L. Yin, A. Sallam, S. Heinen, L. Li, K. Beaubien, R. Dill-Macky, Y. Dong, B.J. Steffenson, K.P. Smith and G.J. Muehlbauer. 2020. Genetic dissection of quantitive trait loci associated with Fusarium head blight resistance, grain protein content and agronomic traits in the pericentromeric region of chromosome 6H in barley. National Fusarium Head Blight Forum Abstracts 2020 (Online meeting).

Status: Abstract published and poster presented

Acknowledgement of Federal Support: Yes

Muehlbauer, G.J. 2020. HvUGT13248 is required for type II resistance in barley. National Fusarium Head Blight Forum Talk 2020 (Online meting)

Status: Talk presented in the Barley CP session

Acknowledgement of Federal Support: Yes