

USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY18 Performance Report
Due date: July 12, 2019

Cover Page

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Fiscal Year:	2018
USDA-ARS Agreement ID:	59-0206-8-203
USDA-ARS Agreement Title:	Molecular Genetics Approaches to Developing Scab Resistance.
FY18 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:	5/17/18 - 5/16/19
Reporting Period End Date:	05/16/19

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 Trichothece Resistance and Developing Scab Resistant Wheat.	\$ 81,696
	FY18 Total ARS Award Amount	\$ 170,503

Gary J. Muehlbauer

7/10/19

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 project?

Previous work in my laboratory has resulted in identifying a barley UDP-glucosyltransferase gene (*HvUGT13248*) that exhibits resistance to FHB and trichothecenes when expressed in transgenic wheat, and fine mapping a QTL for FHB resistance in barley on chromosome 6H bin 7 and chromosome 2H bin 8. The major goals of this grant were to develop germplasm resources and tools to increase FHB resistance in barley. The specific objectives are (1) to characterize transgenic barley overexpressing *HvUGT13248*; and (2) fine map and characterize the chromosome 6H bin 7 and 2H bin 8 FHB resistance QTL.

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

1) major activities

Objective 1. Characterize transgenic barley overexpressing *HvUGT13248*. We created transgenic barley lines overexpressing *HvUGT13248* and showed that they exhibit high levels of DON resistance in roots on DON-containing media. 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 has been replanted in the 2019. The field trials are a collaboration with Ruth Dill-Macky.

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 not conjugated to the non toxic D3G. These results indicate the *HvUGT13248* is the primary gene that functions to detoxify DON to D3G. Field trials with these mutants are underway in 2019.

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 F₂ 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 F₂ 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 F₄ plants were tested in field trials in St. Paul in 2016, 2017 and 2018 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 and 2018 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. The same lines have been planted in the field in 2019 and inoculations and disease scoring are ongoing.

A major barley FHB QTL is also located in the chromosome 2H bin8 region. To fine map this region, an F₂ 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 F₃ plants were phenotyped for FHB resistance, heading date, and DON accumulation in St. Paul in 2016, 2017 and 2018. Unfortunately, the disease pressure was low in 2017 and the data were unreliable. Lines that exhibit reduced FHB severity were identified in 2016 and 2018 and are currently being retested in field trials in 2019.

An additional activity related to this project included: A bi-parental F_{6:7} RIL population was developed from Rasmusson crossed to PI383933 and used to map QTL for FHB resistance, reduced DON accumulation and other agronomic traits. PI383933 is a highly susceptible landrace that exhibits early heading date, short stature and dense spikes. The population was phenotyped in St. Paul, MN and Crookston, MN in 2015 and in 2016 and genotyped with the iSELECT 9K barley chip. QTL analysis identified six QTL for FHB severity and DON accumulation on chromosomes 2H, 3H, 5H, 6H and 7H with the largest effect QTL located on chromosome 7H. A manuscript describing these results was published to *Frontiers in Plant Science* (Huang et al., 2018).

2) specific objectives

Objective 1. Characterize transgenic barley overexpressing *HvUGT13248*.

Objective 2. Fine map and characterize the chromosome 6H bin 7 and 2H bin 8 FHB resistance QTL.

3) 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. Backcross lines in the Rasmusson background containing the *HvUGT13248* transgene have been 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. The transgenic and mutant plants are being tested in the field for FHB resistance/susceptibility.

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. Fine mapping of the 2H bin 8 QTL is ongoing. Also, six FHB resistance QTL were detected in the Rasmusson x PI383933 mapping population.

4) 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. Backcross lines in the Rasmusson background containing the *HvUGT13248* transgene have been 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. The transgenic and mutant plants are being tested in the field for FHB resistance/susceptibility.

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. Also, six FHB resistance QTL were detected in the Rasmusson x PI383933 mapping population.

3. **What opportunities for training and professional development has the project provided?** Two Postdoctoral Research Associates have worked on this project. Both attended the 2018 National Scab Forum and presented their work in the poster session (Bethke et al., 2018; Huang et al., 2018), one presented in the Flash and Dash session. Both postdocs meet with me regularly, and participate in weekly lab meetings.
4. **How have the results been disseminated to communities of interest?** We presented our work at a poster at the National Scab Forum. A paper was published in *Frontiers in Plant Science* describing mapping FHB resistance in the Rasmusson x PI383933 population (Huang et al., 2018).

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

1. What are the major goals and objectives of the 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? *Address items 1-4) below for each goal or objective.*

1) 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 inoculations and scoring are just beginning.

Objective 2. Characterize the ability of transgenic wheat carrying *HvUGT13248* to provide resistance to NX-2-producing *F. graminearum*. We are in the process of testing the transgenic plants carrying *HvUGT13248* for resistance to NX-2, a recently identified trichothecene mycotoxin.

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 on this topic in December 2018 (Gatti et al., 2018, *Frontiers in Plant Science*).

2) specific objectives

- Objective 1. Test elite wheat cultivars carrying *HvUGT13248* for FHB resistance.**
- Objective 2. Characterize the ability of transgenic wheat carrying *HvUGT13248* to provide resistance to NX-2-producing *F. graminearum*.**
- Objective 3. Characterize wheat UDP-glucosyltransferase genes.**

3) 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 is ongoing.

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

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

A Postdoctoral Research Associate worked on this project. She attended the 2018 National Scab Forum and was a co-author on a poster. She meets with me regularly, and participates in weekly lab meetings.

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

We presented our work on a poster (Dill-Macky et al., 2018) at the National Scab Forum.

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Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY18 award period. 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 FY18 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 FY18 award period? NO**

If yes, how many?

3. **Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant taken faculty positions with universities? NO**

If yes, how many?

4. **Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies? NO**

If yes, how many?

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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 FY18 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

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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY18-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY18 grant. Only include citations for publications submitted or presentations given during your award period (5/17/18 - 5/16/19). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each reference/citation, you must indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in publication/presentation. See example below for a poster presentation with an abstract:

Conley, E.J., and J.A. Anderson. 2018. Accuracy of Genome-Wide Prediction for Fusarium Head Blight Associated Traits in a Spring Wheat Breeding Program. In: Proceedings of the XXIV International Plant & Animal Genome Conference, San Diego, CA.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), NO (abstract)

Journal publications.

Huang, Y., M. Haas, S. Heinen, B.J. Steffenson, K.P. Smith, and G.J. Muehlbauer. 2018. QTL Mapping of *Fusarium* head blight and correlated agromorphological traits in an elite barley cultivar Rasmusson. *Frontiers in Plant Science* doi: 10.3389/fpls.2018.01260
Status: Published
Acknowledgement of Federal Support: Yes

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

Other publications, conference papers and presentations.

Bethke, G., X. Li, Y. Huang, G. Hensel, J. Kumlehn, S. Salvi and G. Muehlbauer. 2018. Towards understanding the function of the barley UDP-glucosyltransferase UGT13248 in disease resistance. National Scab Forum Poster Abstract, St. Louis, MO.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), NO (abstract)

Dill-Macky, R., A.M. Elakkad, B. Zargar, G.J. Muehlbauer, G. Bethke, J. McLaughlin, N. Tumer and D. Funnell-Harris. 2018. Testing transgenic spring wheat and barley for reaction to *Fusarium* head blight. National Scab Forum Poster Abstract, St. Louis, MO
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), NO (abstract)

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Huang, Y., S. Heinen, B. Steffenson, K.P. Smith and G.J. Muehlbauer. 2018 Fine mapping of FHB quantitative trait loci on chromosome 6H and 2H in barley. National Scab Forum Poster Abstract, St. Louis, MO

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (poster), NO (abstract)