USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report – NCE for FY18
Due date: July 12, 2019

Cover Page

Principle Investigator (PI):	Gary Muehlbauer
Institution:	University of Minnesota
E-mail:	muehl003@umn.edu
Phone:	612-625-6228
Fiscal Year:	2017 (NCE for FY18)
USDA-ARS Agreement ID:	59-0206-4-021
USDA-ARS Agreement Title:	Molecular Genetics Approaches to Developing Scab Resistance.
FY17 USDA-ARS Award Amount:	$ 133,782
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:	CON000000048178
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.	$ 71,082
GDER	Characterizing Trichothecene Resistance and Developing Scab Resistant Wheat.	$ 62,700
	FY17 Total ARS Award Amount	$ 133,782

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 (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. The major goals of this grant were to develop germplasm resources and tools to increase FHB resistance in barley. The specific objectives are (1) fine map and characterize the chromosome 6H bin 7 FHB resistance QTL; (2) identify novel QTL associated with FHB resistance; and (3) field test transgenic barley overexpressing HvUGT13248.

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

1) major activities

Objective 1. Fine map and characterize the chromosome 6H bin7 FHB resistance QTL. Barley QTL associated with Fusarium head blight resistance, reduced deoxynivalenol accumulation, early senescence and increased grain protein content (GPC) colocalize on 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 and 2018 for FHB severity, DON accumulation and 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 appear 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 high GPC. The same lines have been planted in the field in 2019 and inoculations and disease scoring are ongoing.

Objective 2. Identify novel QTL associated with FHB resistance. We developed a RIL population by crossing the highly susceptible accession PI383933 with the moderately susceptible cultivar Rasmusson and mapped QTL associated with FHB, 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 in *Frontiers in Plant Science*.

Objective 3. Field test 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 the 2019.

2) specific objectives

Objective 1. Fine map and characterize the chromosome 6H bin 7 FHB resistance QTL.
Objective 2. Identify novel QTL associated with FHB resistance.
Objective 3. Field test transgenic barley overexpressing *HvUGT13248*.

3) significant results

Objective 1. Fine map and characterize the chromosome 6H bin 7 FHB resistance QTL. We identified recombinants in the chromosome 6H bin7 and are in the process of fine mapping the region. 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.

Objective 2. Identify novel QTL associated with FHB resistance. QTL analysis in the Rasmusson x PI383933 mapping population 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.

Objective 3. Field test 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. The transgenic and mutant plants are being tested in the field for FHB resistance/susceptibility.

4) key outcomes or other achievements

Objective 1. Objective 1. Fine map and characterize the chromosome 6H bin 7 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 association with decreased DON accumulation, likely due to the early senescence in plants carrying the high GPC allele.

Objective 2. Identify novel QTL associated with FHB resistance. We identified six FHB resistant and DON accumulation QTL in the Rasmusson x PI383933 mapping population. A paper was published describing these results in *Frontiers in Plant Sciences*.

Objective 3. Field test 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 and are currently being tested in the field. Plants carrying mutations in the *HvUGT13248* have been identified and we showed that these plants are susceptible to DON. The transgenic and mutant plants are being tested in the field for FHB resistance/susceptibility.

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 wheat exhibiting trichothecene and FHB resistance. Previously, my laboratory developed transgenic wheat carrying a barley UDP-glucosyltransferase (*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) develop elite wheat cultivars with FHB resistance; (2) characterize the ability of transgenic wheat expressing *HvUGT13248* to provide resistance to a broad spectrum of trichothecenes; and (3) test potential trichothecene resistance genes.

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

1) major activities

Objective 1. Develop elite wheat cultivars expressing *HvUGT13248* that confer FHB resistance. We backcrossed the *HvUGT13248* transgenic line into the cultivar Linkert and identified 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; however, the disease severity was too low to discriminate the transgenic plants from the non transgenic controls. These lines were planted in the field in 2019. This work is a collaboration with Ruth Dill-Macky.

Objective 2. Characterize the ability of transgenic wheat expressing *HvUGT13248* to provide resistance to a broad spectrum of trichothecenes. We showed that transgenic wheat expressing *HvUGT13248* exhibits high levels of resistance to DON-producing *F. graminearum* strains due to the conjugation of DON to DON-3-O-glucoside (Li et al., 2015). We also showed that these same transgenic wheat lines exhibit high levels of type II resistance to NIV-producing *F. graminearum* and the transgenic wheat quickly converts NIV to NIV-3-O-glucoside. A paper was published that described the NIV detoxification results (Li et al., 2017). These lines also exhibit resistance to three other trichothecenes (3,15-di-ANIV, NX-2, and 3-ADON).

Objective 3. Test potential trichothecene resistance genes. To rapidly identify additional DON resistance genes, we transformed Arabidopsis with putative DON resistance genes

from barley and tested the transgenics on DON-containing media. We transformed Arabidopsis with a zinc finger protein, two ABC transporters, two cytochrome P450s, one epoxide hydrolase, three glutathione-S-transferases and a cysteine synthase. We did not identify any genes that resulted in increased DON resistance.

2) specific objectives

Objective 1. Develop elite wheat cultivars expressing *HvUGT13248* that confer FHB resistance.

Objective 2. Characterize the ability of transgenic wheat expressing *HvUGT13248* to provide resistance to a broad spectrum of trichothecenes.

Objective 3. Test potential trichothecene resistance genes.

3) significant results

Objective 1. Develop elite wheat cultivars expressing *HvUGT13248* that confer FHB resistance. We developed transgenic wheat in elite cultivars that may provide enhanced resistance to FHB and will provide the genetic materials to study the potential interactions between *HvUGT13248* and *Fhb1*. 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 expressing *HvUGT13248* to provide resistance to a broad spectrum of trichothecenes. We showed that *HvUGT13248* provides resistance to DON and NIV and have evidence that it exhibits resistance to a broad range of trichothecene mycotoxins including: 3,15-di-ANIV, NX-2, and 3-ADON.

Objective 3. Test potential trichothecene resistance genes. There were no significant results to report as we did not identify any new genes that exhibited trichothecene resistance.

4) key outcomes or other achievements

Objective 1. Develop elite wheat cultivars expressing *HvUGT13248* that confer FHB resistance. The transgene, *HvUGT13248*, has been introgressed into two elite cultivars and we tested those lines in the greenhouse and 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 expressing *HvUGT13248* to provide resistance to a broad spectrum of trichothecenes. We showed that transgenic wheat lines expressing *HvUGT13248* exhibit high levels of type II resistance to NIV-producing *F. graminearum* and the transgenic wheat quickly converts NIV to NIV-3-O-glucoside. A paper was published that described the NIV detoxification results (Li et al., 2017). These lines also exhibit resistance to three other trichothecenes (3,15-di-ANIV, NX-2, and 3-ADON).

Objective 3. Test potential trichothecene resistance genes. There were no significant results to report as we did not identify any new genes that exhibited trichothecene resistance.

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.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17-NCE 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 FY17-NCE 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 FY17-NCE period?**

   NO

   If yes, how many?

3. **Have any post docs who worked for you during the FY17-NCE 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 FY17-NCE 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?

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 FY17-NCE 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 FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17-NCE grant period. 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 presented at the FHB Forum:

> Conley, E.J., and J.A. Anderson. 2017. 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. Zargaran, 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)

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)