USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY16 Final Performance Report
Due date: July 28, 2017

Cover Page

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Fiscal Year:	2016
USDA-ARS Agreement ID:	59-0206-4-021
USDA-ARS Agreement Title:	Molecular Genetics Approaches to Developing Scab Resistance.
FY16 USDA-ARS Award Amount:	$ 134,042
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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Project/Grant Reporting Period:	5/17/16 - 5/16/17
Reporting Period End Date:	05/16/17

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Molecular Genetics Approaches to Developing Scab Resistant Barley.	$ 71,221
GDER	Characterizing Trichothecene Resistance and Developing Scab Resistant Wheat.	$ 62,821
	FY16 Total ARS Award Amount	**$ 134,042**

Gary J. Muehlbauer July 20, 2017
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

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

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

Fusarium head blight (FHB; scab) caused by F*usarium graminearum* is a devastating disease of barley. 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 funded proposal are to develop germplasm resources and tools to increase FHB resistance in barley. The specific objectives of this project are (1) to characterize transgenic barley overexpressing *HvUGT13248*; and (2) fine map and characterize the chromosome 6H bin 7 FHB resistance QTL.

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

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. The transgenic barley is in the Golden Promise background that is not amenable to FHB screening in the field. 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 and increased seed.

Objective 2. Fine map and characterize the chromosome 6H bin 7 FHB resistance QTL. Barley QTL associated with Fusarium head blight resistance, reduced deoxynivalenol accumulation and increased grain protein 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 for FHB severity, DON accumulation and grain protein content. All data (FHB severity, DON accumulation, and grain protein content) have been collected from the 2016 field trial. From the 2016 field test, we identified recombinants that exhibit resistance that appears to be uncoupled from high grain protein content. In 2017, these same 13 recombinants were field tested again along with 26 additional recombinants. Data from the 2017 field for FHB severity has been collected and we will be obtaining DON accumulation and grain protein content in the near future.

Three additional activities related to this project include:

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. Three of the QTL on chromosome 3H, 5H and 6H appear to be novel. A manuscript is in preparation describing this work.

We analyzed RNA-Seq data from two NILs carrying FHB resistant alleles at chromosome 2H bin8 and chromosome 6H bin7, and their recurrent parents. We identified differentially expressed genes between the plants carrying the resistant and susceptible alleles. These genes are helping us understand and manipulate resistance in barley. We published the results of this study in BMC Genomics (Huang et al., 2016). The RNA-seq data were also used to identify SNPs between the NILs and recurrent parents for the fine mapping efforts of the chromosome 2H bin8 and 6H bin7 QTL, and for a fine mapping effort in Kevin Smith's (University of Minnesota) laboratory.

A major barley FHB QTL is 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 KASPar 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 and 2017. Lines that exhibit reduced FHB severity and early heading date were identified and need to be retested.

Specific objectives:

Objective 1. Characterize transgenic barley overexpressing *HvUGT13248*. As described above, we created transgenic barley lines overexpressing *HvUGT13248* and showed that they exhibit high levels of DON resistance in roots on DON-containing media. We backcrossed the *HvUGT13248* transgene into Rasmusson and selected lines for future study.

Objective 2. Fine map and characterize the chromosome 6H bin 7 FHB resistance QTL. We identified recombinants in the chromosome 6H bin7 region associated with FHB resistance and tested these lines in the summers of 2016 and 2017.

Significant results:

We identified genes that were differentially expressed between resistant and susceptible genotypes and these genes are being used to further understand the mechanisms of genetic resistance, tools to manipulate resistance, and a resource to identify SNPs for mapping. We mapped three novel QTL for FHB resistance on chromosome 3H, 5H and 6H. We have identified recombinants in the chromosome 6H bin7 and chromosome 2H bin8 regions and are in the process of fine mapping each region. We developed transgenic barley overexpressing *HvUGT13248* that will be an additional source of resistance to FHB.

Key Outcomes or other achievements:

We identified differentially expressed genes between resistant and susceptible genotypes and are using these genes in our gene discovery efforts and fine mapping work. We are in the process of fine mapping two QTL on chromosome 6H bin7 and chromosome 2H bin8. Preliminary

results indicate that we have recombinants that contain FHB resistance without the deleterious high grain protein content and late heading date alleles. Also, three novel QTL were detected that are associated with resistance to FHB. We developed transgenic barley overexpressing *HvUGT13248* that exhibits resistance to DON and introgressed the *HvUGT13248* transgene into Rasmusson.

3. **What opportunities for training and professional development has the project provided?** A Ph.D. student and a Postdoctoral Research Associate have worked on this project. Both have well-developed projects that are progressing nicely and the Ph.D. student will graduate in summer of 2017. Both have presented their work at the National Scab Forum and the postdoc presented his work at the International Barley Genetics Symposium. The postdoc and graduate student 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 in posters at the National Scab Forum, the International Barley Genetics Symposium and the 8th Canadian Workshop on Fusarium Head Blight. We also published a paper in BMC Genomics (Huang et al., 2016).

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 (*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 accumulation of trichothecenes and trichothecene-glucoside conjugates in transgenic wheat carrying *HvUGT13248*; and (3) test potential trichothecene resistance genes.

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

Major activities:

Objective 1. Develop elite wheat cultivars with FHB resistance. We backcrossed the *HvUGT13248* transgenic line into the cultivar Linkert and identified six homozygous lines with transgene expression, and ten lines without transgene expression. We also developed backcross lines of *HvUGT13248* transgenics in the cv. Rollag genetic background and identified four lines of each of the four genotypes, namely *UGT*+/*Fhb1*+, *UGT*-/*Fhb1*+, *UGT*+/*Fhb1*-, and *UGT*-/*Fhb1*- from four different transgenic events (a total of 64 lines). These lines were screened in the greenhouse in the Fall 2016. 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. These backcross lines are a resource for enhanced FHB resistance in elite wheat cultivars, and provide an opportunity to study the potential interactions between *HvUGT13248* and *Fhb1* QTL.

Objective 2. Characterize the accumulation of trichothecenes and trichothecene-glucoside conjugates in transgenic wheat carrying *HvUGT13248*. 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. 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.

Specific objectives:

Objective 1. Develop elite wheat cultivars with FHB resistance. We developed elite wheat cultivars that contain *HvUGT13248* and tested the lines in the greenhouse in 2016.

Objective 2. Characterize the accumulation of trichothecenes and trichothecene-glucoside conjugates in transgenic wheat carrying *HvUGT13248*. We showed that *HvUGT13248* exhibits resistance to a broad spectrum of trichothecenes.

Objective 3. Test potential trichothecene resistance genes. Our testing of various potential trichothecene resistance genes did not result in identifying new resistance genes.

Significant results:

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 initial greenhouse test showed that the combination of HvUGT13248 and *Fhb1* in the same background exhibited stable and higher resistance than *Fhb1* alone. We have also shown that *HvUGT13248* provides resistance to a broad range of trichothecene mycotoxins including: DON, NIV, 3,15-di-ANIV, NX-2, and 3-ADON.

Key outcomes or other achievements:

We developed transgenic wheat lines that exhibit resistance to FHB and to a broad spectrum of trichothecenes. 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.

3. **What opportunities for training and professional development has the project provided?** A Ph.D. student has worked on this project. He is progressing nicely on this project and plans to graduate in the summer of 2017. He has presented his work at the National Scab Forum, participates in weekly lab meetings, and meets regularly with me.

4. **How have the results been disseminated to communities of interest?**
We published a manuscript in Journal of Experimental Botany (Li et al., 2017) describing some of this work. We have also presented our work in posters at the National Scab Forum and a talk at the 8th Canadian Workshop on Fusarium Head Blight.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY16 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 FY16 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 FY16 award period?** NO

   If yes, how many?

3. **Have any post docs who worked for you during the FY16 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 FY16 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?

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 FY16 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *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 FY16-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY16 grant. Only include citations for publications submitted or presentations given during your award period (5/17/16 - 5/16/17). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

Li, X., H. Michlmayr, W. Schweiger, A. Malachova, S. Shin, Y. Huang, Y. Dong, G. Wiesenberger, S. McCormick, M. Lemmens, P. Fruhmann, C. Hametner, F. Berthiller, G. Adam and G.J. Muehlbauer. 2017. A barley UDP-glucosyltransferase inactivates nivalenol and provides Fusarium head blight resistance in transgenic wheat. *J. Exp. Bot.* 68:2187-2197.
Status: Published
Acknowledgement of Federal Support: YES

Huang, Y., L. Li, K.P. Smith and G.J. Muehlbauer. 2016. RNA-Sequencing revealed differential resistance mechanisms of two barley near-isogenic line pairs to Fusarium head blight and identified long noncoding RNAs responsive to *Fusarium graminearum* infection. *BMC Genomics* 17:387.
Status: Published
Acknowledgement of Federal Support: YES

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

Nothing to report.

Other publications, conference papers and presentations.

Poster Abstracts:

Huang, Y., L. Li, K.P. Smith and G.J. Muehlbauer. 2016. Differential transcriptomic responses to *Fusarium graminearum* infection in two barley Fusarium head blight resistant QTL. Intl. Barley Genetics Symposium Poster Abstract, Minneapolis, MN
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: Yes (poster)

Huang, Y., L. Li, K.P. Smith and G.J. Muehlbauer. 2016. Characterization of small RNAs from Fusarium-inoculated spike tissues. In: S. Canty, A. Clark, S. Vukasovich and D. Van Sanford (Eds.), *Proceedings of the 2016 National Fusarium Head Blight Forum*. St. Louis, MO: U.S. Wheat & Barley Scab Initiative.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster)

Li, X., H. Michlmayr, W. Schweiger, A. Malachova, S. Shin, Y. Huang, Y. Dong, G. Wiesenberger, S. McCormick, M. Lemmens, P. Fruhman, C. Hametner, F. Berthiller, G. Adam and G.J. Muehlbauer. 2016. A barley UDP-glucosyltransferase provides resistance to nivalenol and nivalenol-producing *Fusarium graminearum.* In: S. Canty, A. Clark, S. Vukasovich and D. Van Sanford (Eds.), *Proceedings of the 2016 National Fusarium Head Blight Forum*. St. Louis, MO: U.S. Wheat & Barley Scab Initiative.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster)

Presentations:
Muehlbauer, G.J. 2016. Genomic approaches to developing FHB resistance in wheat and barley. 8th Canadian Workshop on Fusarium Head Blight (Plenary talk), Ottawa, Canada
Status: Presented
Acknowledgement of Federal Support: YES (presentation)