USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY14 Final Performance Report
July 15, 2015

Cover Page

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Fiscal Year:	FY14
USDA-ARS Agreement ID:	59-0206-4-021
USDA-ARS Agreement Title:	Molecular Genetics Approaches to Developing Scab Resistance.
FY14 USDA-ARS Award Amount:	$ 136,784

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Molecular Genetics Approaches to Developing Scab Resistant Barley.	$ 70,873
GDER	Rapidly Identifying Scab Resistance Genes and Developing Scab Resistant Wheat.	$ 65,911
	FY14 Total ARS Award Amount	**$ 136,784**

Gary J. Muehlbauer

July 2, 2015

Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
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
WES-CP – Western 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 major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Fusarium head blight (FHB or scab), caused by *Fusarium graminearum*, is a disease that can devastate the small grains wheat and barley. There is a limited amount of information pertaining to the molecular genetic interaction between barley and *F. graminearum* and in particular the molecular mechanisms of resistance at key barley QTL. Also, the genes controlling the QTL have not been identified. In addition, few transgenic sources of resistance in barley have not been developed and tested. Our goals are three fold: (1) to examine gene expression in genotypes carrying either resistant or susceptible alleles at key QTL; (2) to map novel QTL for resistance and fine map previously identified resistance QTL; and (3) to characterize transgenic barley overexpressing a barley UDP-glucosyltransferase (*HvUGT13248*).

We conducted an experiment to examine gene expression at key QTL for FHB resistance. We used two near-isogenic line (NIL) pairs carrying resistant and susceptible alleles for FHB resistant QTL at chromosome 2H bin 8 (2hb8) and chromosome 6H bin 7 (6hb7). To genetically characterize these NILs, we collaborated with Shiaoman Chao (USDA-ARS, Fargo, ND) to SNP genotype the lines with a 9K SNP platform. The SNP analysis showed that the lines were highly isogenic. We inoculated spikes from the two pairs of NILs with *F. graminearum* and mock water and sampled spikes at 48 and 96 hours after inoculation. There are a total of 48 samples (four genotypes, two treatments, two timepoints and three replications) that we isolated RNA from. RNA-seq data has been obtained and the analysis has been conducted. A total of 2,083 differentially expressed transcripts in response to *F. graminearum* infection were detected in the four NILs. Comparative analysis of the 2hb8 NIL pair revealed that the 2hb8 resistant line exhibited a constitutive defense response in the absence of fungal infection and responded quicker than the 2hB8 susceptible line upon fungal infection. The 6hb7 resistant line displayed a more rapid induction of a set of defense genes than the 6hb7 susceptible line during the early stage of fungal infection and the transcript expression difference between the resistant and susceptible lines diminished during the late stages. Overlap of differentially accumulated genes were identified between the two resistant lines, suggesting that certain defense mechanisms may represent basal resistance to *F. graminearum* and were co-regulated by the two QTL. SNPs between the susceptible and resistant lines have been detected and are being used in the fine mapping of both QTL. Long noncoding RNAs (lncRNAs) have emerged as potential key regulators of transcription and are expressed during infection. A total of 12,366 lncRNAs were identified of which 604 were FHB responsive. The current transcriptomic analysis revealed the differential mechanisms conferred by two QTL in response to *F. graminearum* infection and identified genes and lncRNAs that were associated with FHB resistance.

Fine mapping previously identified QTL and mapping novel sources of resistance is underway. We are fine mapping the chromosome 6H bin 7 and chromosome 2H bin 8 QTL. We developed populations for both QTL by crossing near-isogenic lines carrying the resistant or susceptible alleles for each locus. For the chromosome 6H Bin 7 QTL, we genotyped 2,060 F2 individuals with markers that flank the QTL region and selected 399 recombinants. We genotyped the recombinants with 34 markers and delineated the recombination breakpoints. Fine mapping the

chromosome 2H bin 8 QTL is just starting. We developed a recombinant inbred line population from a cross between a highly susceptible barley genotype and a moderately susceptible line. This population will be used to detect minor effect QTL for FHB resistance that are not detectable due to detecting the major effect genes when using a resistant genotype as one of the parents. We are screening this population in St. Paul and Crookston, MN in the summer 2015 and will genotype the population by the end of 2015.

We collaborated with Jochen Kumlehn (IPK-Gatersleben, Germany) to develop 7 transgenic barley (cv. Golden Promise) lines carrying a barley UDP-glucosyltrasferase (*HvUGT132148*) transgene. Previously, we showed that overexpression of *HvUGT13248* in transgenic wheat resulted in increased FHB resistance. We identified lines that exhibited high levels of HvUGT13248 protein accumulation and showed that these plants exhibited a significant increase in resistance to DON. We backcrossed these lines to the six-row cultivar Rasmusson and are in the process of identifying lines that are six-row and exhibit high levels of HvUGT13248 protein accumulation. Lines will be screened for FHB resistance in the field in Summer 2016.

2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

Accomplishment:

1. We completed a large RNA-seq experiment to study gene expression in genotypes carrying resistant and susceptible alleles at two resistant QTL. SNPs have been detected for both QTL regions for the fine mapping effort. We are in the process of preparing a manuscript that describes this work.
2. We developed transgenic barley overexpressing the *HvUGT13248* transgene and backcrossed *HvUGT13248* into Rasmusson. These lines that will be ready for field testing in Summer of 2016.
3. Fine mapping of the chromosome 6H bin 7 QTL is underway and recombinants in the QTL region have been selected.
4. A population derived from crossing a highly susceptible genotype with a moderately susceptible genotype was developed and is being screened in the field in Crookston and St. Paul, MN.

Impact:

1. The RNA-seq data is providing a rich resource for gene discovery and markers for mapping the chromosome 2H bin 8 and chromosome 6H bin 7 QTL.
2. The transgenic barley overexpressing HvUGT13248 will be an additional source of resistance to FHB.

Project 2: *Rapidly Identifying Scab Resistance Genes and Developing Scab Resistant Wheat.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Fusarium head blight (FHB or scab), caused by *Fusarium graminearum*, is a disease that can devastate the small grains wheat and barley. During infection, *F. graminearum* produces deoxynivalenol (DON), a trichothecene mycotoxin. There is a limited amount of information pertaining to the molecular genetic interaction between the small grains and *Fusarium graminearum* and in particular the response to trichothecene accumulation. Our goals are to further characterize transgenic wheat expressing *HvUGT13248* and to identify and rapidly test genes that exhibit resistance to trichothecenes.

Six and four transgenic wheat lines carrying the barley UDP-glucosyltransferase gene (*HvUGT13248*) were developed in the Bobwhite and CB037 backgrounds, respectively. The Bobwhite and CB037 transgenics were screened in the greenhouse three and two times, respectively. In the greenhouse screens, the transgenic lines exhibited significantly reduced disease severity often times reaching the equivalent levels of Sumai3. In collaboration with Dr. Ruth Dill-Macky, field screens were conducted in 2012, 2013 and 2014 and lines were identified that exhibited significantly reduced FHB severity, very scabby kernels (VSK), and DON concentration. The level of FHB resistance that was observed was similar to commercially grown wheat cultivars. We collaborated with Franz Berthiller (University of Natural Resources and Life Sciences, Austria) to show that the mechanism of resistance in the *HvUGT1328* wheat transgenics is the conjugation of DON to DON-3-glucoside. We also showed that the wheat transgenics carrying *HvUGT13248* exhibit resistance to a nivalenol (NIV)-producing *F. graminearum* strain and a strain producing a new trichothecene NX-2. Currently, we are testing if the mechanism of NIV resistance is via the conjugation of NIV to NIV-3-glucoside. To determine if *HvUGT13248* introgressed in a type II genetic background will result in enhanced resistance, multiple Bobwhite transgenics were backcrossed to Rollag (type II resistant spring wheat conferred from *Fhb1*) and BC1 populations derived carrying the transgene and the resistant allele at *Fhb1*. These populations were screened in the greenhouse and no difference was detected between plants carrying the transgene and *Fhb1* and the type II resistant controls. We are also developing populations in the cv. Linkert background and currently we are selecting for lines that express *HvUGT13248*. Additional testing of these lines will be conducted in the summer of 2016.

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. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

Accomplishment:

1. We showed that transgenic wheat expressing *HvUGT13248* exhibits resistance to nivalenol and NX-2 producing Fusarium strains.
2. We backcrossed the *HvUGT13248* transgene into the cv. Linkert and are in the process of selecting lines expressing HvUGT13248.

Impact:

1. In field trials, transgenic wheat expressing *HvUGT13248* exhibited an equivalent level of resistance to commercially grown FHB resistant cultivars, indicating that this gene is useful to confer a high level of resistance in wheat.
2. We showed that the wheat transgenics expressing *HvUGT13248* exhibited resistance to nivalenol- and NX-2-producing *F. graminearum*, indicating that HvUGT13248 acts on a broad spectrum of trichothecenes.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY14 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 FY14 award period?** Yes

   If yes, how many? Anna Hofstad finished her M.S. degree in December 2013.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY14 award period?** No

   If yes, how many?

3. **Have any post docs who worked for you during the FY14 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 FY14 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?

Currently, I have a postdoctoral research associate and a Ph.D. student working on FHB that is funded through the USWBSI.

Include below a list of all germplasm or cultivars released with full or partial support of the USWBSI during the FY14 award period. List the release notice or publication. Briefly describe the level of FHB resistance. *If not applicable because your grant did NOT include any VDHR-related projects, enter N/A below.*

N/A

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the FY14 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Peer-reviewed Publications:

Hofstad, A.N., T. Nussbaumer, E. Akhunov, S. Shin, K.G. Kugler, H.C. Kistler, K.F.X. Mayer, and G.J. Muehlbauer. Examining the transcriptional response of the wheat *Fhb1* gene to *Fusarium graminearum* infection and deoxynivalenol treatment. The Plant Genome (Accepted with revisions).

Li, X., S. Shin, S. Heinen, R. Dill-Macky, F. Berthiller, T. Clemente, S. McCormick and G.J. Muehlbauer. Transgenic wheat carrying a barley UDP-glucosyltransferase exhibits high levels of *Fusarium* head blight resistance by detoxifying deoxynivalenol. Molecular Plant-Microbe Interactions (Accepted with revisions).

Abstracts:

Dill-Macky, R., A.M. Elakkad, G.J. Muehlbauer, X. Li, L.S. Dahleen, R.W. Skadsen, P.P. Bregitzer, J.E. McLaughlin and N.E. Tumer. 2014. Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2014 field nursery report. National Scab Forum Abstracts, St. Louis, MO.

Huang, Y., L. Li, K.P. Smith and G.J. Muehlbauer. 2014. RNA-seq characterization of two barley Fusarium head blight resistant QTL. National Scab Forum Abstracts, St. Louis, MO.

Li, X., S. Shin, S. Heinen, R. Dill-Macky, F. Berthiller, T. Clemente, S. McCormick, S. Chao, and G.J. Muehlbauer. 2014. Transgenic wheat and barley carrying a barley UDP-glucosyltransferase exhibit high levels of Fusarium head blight resistance. National Scab Forum Abstracts, St. Louis, MO.

Muehlbauer, G.J., X. Li, S. Shin, Y. Huang, J. Boddu, W. Schweiger, S. McCormick, R. Dill-Macky, T. Clemente, F. Berthiller, S. Chao and G. Adam. 2014. Developing transgenic wheat and barley that exhibit resistance to Fusarium graminearum via glucoside conjugation of trichothecene mycotoxins. National Scab Forum Abstracts, St. Louis, MO.