USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY12 Final Performance Report
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Cover Page

PI:	Gary Muehlbauer
Institution:	University of Minnesota
Address:	Department of Agronomy and Plant Genetics 411 Borlaug Hall 1991 Upper Buford Circle St. Paul, MN 55108
E-mail:	muehl003@umn.edu
Phone:	612-625-6228
Fax:	612-625-1268
Fiscal Year:	FY12
USDA-ARS Agreement ID:	59-0206-9-073
USDA-ARS Agreement Title:	Molecular Genetic Approaches to Develop Scab Resistance.
FY12 USDA-ARS Award Amount:	$ 140,248[*]

USWBSI Individual Project(s)

USWBSI Research Category[**]	**Project Title**	**ARS Award Amount**
BAR-CP	Next Generation Approaches to Characterize Barley FHB Resistant QTL.	$ 73,096
GDER	Rapidly Identify and Test Scab Resistance Genes.	$ 67,152
	Total ARS Award Amount	**$ 140,248**

Principal Investigator Date

[*] Partial funding for this research is under ARS agreement # 59-0206-9-070

[**] MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
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: *Next Generation Approaches to Characterize Barley FHB Resistant QTL.*

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.* Our goal is to identify, characterize and map barley genes that respond to *F. graminearum* infection.

We have initiated an experiment to examine gene expression at key QTL for FHB resistance. We are using three near-isogenic line (NIL) pairs carrying resistant and susceptible alleles for FHB resistant QTL at chromosome 2H bin 8 and 10, and chromosome 6H bin 7. 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 three pairs of NILs with *F. graminearum* and mock water and sampled spikes at 48 and 96 hours after inoculation.

There are a total of 72 samples (six genotypes, two treatments, two timepoints and three replications) that we isolated RNA from. The RNA has been sent to the University of Minnesota Genomics Center for sequencing. The sequencing data will be obtained in July 2013. Disease severity, deoxynivalenol (DON) and ergosterol data have been obtained from the samples used for the RNA isolation. We also planted each of the lines in a replicated trial in the field in 2013. We plan to obtain disease severity, DON and ergosterol data from the field grown plants.

In collaboration with Brian Steffenson and Kevin Smith we identified a set of 78 barley genotypes carrying resistance to *F. graminearum.* These 78 resistant lines along with 23 susceptible lines were screened with DArT markers and we determined the haplotypes at three FHB QTL on chromosome 2H bins 8, 10 and 13-14 and a QTL on chromosome 6H bin 7 (Huang et al., 2012). This information will be useful for future mapping studies and breeding efforts. We also used the DArT data to identify a core set of genotypes that serve as a representative set for sequencing genes that have the potential to exhibit resistance or susceptibility. From previous microarray experiments we identified 39 genes that were induced during infection. We completed the sequencing of these 39 genes from a core set of 24 genotypes. Also, as part of this sequencing effort, we completely sequenced a barley UDP-glucosyltransferase (HvUGT13248; see below) gene (~2.5 kb), which detoxifies DON via conversion to DON-3-O-glucoside, from 30 individuals. Upon close inspection of the haplotypes from this sequencing effort, none of these genes showed a clear association with resistance or susceptibility.

2. List the most important accomplishment and its impact (i.e. how is it 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 established a large RNA-seq experiment to study gene expression in the host (barley) and pathogen (*F. graminearum*) during infection. We are complementing the RNA-seq data with disease severity, DON and ergosterol data from growth chamber and field grown plants.
2. We determined DArT haplotypes in 101 FHB resistant and susceptible genotypes for four major barley FHB resistant QTL. We published a paper in Theoretical and Applied Genetics (Huang et al., 2012)
3. We determined that the haplotypes for genes that are induced by *F. graminearum* are not associated with resistance.

Impact:

1. The DArT haplotype work of the four FHB resistant QTL provided information regarding the genetic relationships between the FHB resistant and susceptible genotypes, enabling breeders to select the genotypes that carry novel disease resistance loci and/or alleles for genetic mapping and breeding.

Project 2: *Rapidly Identify and Test Scab Resistance Genes.*

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 DON accumulation. Our goal is 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, a field screen was conducted in 2012 and lines were identified that exhibited significantly reduced FHB severity. The level of FHB resistance that was observed was similar to Sumai3. 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. To determine if HvUGT13248 introgressed into a type II genetic background will result in enhanced resistance, multiple Bobwhite transgenics were backcrossed to Rollag (type II resistant spring wheat) and BC1 populations derived. These populations have been screened in the greenhouse and molecular analysis is ongoing. Finally, we collaborated with Dr. Jochen Kumlehn (IPK-Gaterslaben, Germany) to develop transgenic barley overexpressing HvUGT13248. We have seven transgenic barley lines that we have shown express the transgene.

   To rapidly identify additional DON resistance genes, we transformed Arabidopsis with putative DON resistance genes from barley. 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. To date, testing of the transgenic Arabidopsis has not resulted in the identification of DON resistant plants.

2. List the most important accomplishment and its impact (i.e. how is it 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 developed transgenic wheat carrying a barley UDP-glucosyltransferase (HvUGT13248). In greenhouse and field screens we showed that these transgenics exhibit a high level of type II FHB resistance and low disease severity, respectively.
2. We demonstrated that the mechanism of resistance conferred by HvUGT13248 is the conjugation of DON to DON-3-glucoside.
3. We backcrossed the HvUGT13248 transgene into the Type II resistant cv. Rollag background.

Impact:

1. The transgenic wheat carrying HvUGT13248 exhibited an equivalent level of resistance to Sumai3, indicating that this gene is useful to confer a high level of resistance in wheat. We have initiated backcrossing of the transgene into Rollag.
2. The demonstration of HvUGT13248 conjugating DON to DON-3-glucoside provides a key piece of information for future strategies to detoxify DON.

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 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Publications:

Huang, Y., B.P. Millet, K.A. Beaubian, S.K. Dahl, B.J. Steffenson, K.P. Smith and G.J. Muehlbauer. 2013. Haplotype diversity and population structure in cultivated and wild barley evaluated for *Fusarium* head blight responses. Theor. Appl. Genet. 126:619–636.

Schweiger, W., B. Steiner, C. Ametz, G. Siegwart, G. Wiesenberger, F. Berthiller, M. Lemmens, H. Jia, G. Adam, G. Muehlbauer, D. Kreil, and H. Buerstmayr. Transcriptomic characterization of two major Fusarium resistance QTL, *Fhb1* and *Qfhs.ifa-5A*, identify novel candidate genes. Mol. Plant Path. DOI: 10.1111/mpp.12048.

Presentations:

"Developing Fusarium head blight resistant wheat", at the National Scab Forum, Orlando, FL.

Abstracts:

Kovalsky Paris, M.P., W. Schweiger, S. Shin, G. Muehlbauer, C. Hametner, R. Krska, F. Berthiller and G. Adam. 2012. A barley UDP-glucosyltranferase forming a novel zearalenone-glucoside. National Scab Forum Abstracts.

Hofstad, A., H. Jia, B.P. Millett, E. Akhunov and G.J. Muehlbauer. 2012. Identifying FHB resistance genes in wheat using a next generation sequencing approach. National Scab Forum Abstracts.

Huang, Y., S. Shin, B.P. Millett, X. Li, G. Adam, S. McCormick, K.P. Smith, B.J. Steffenson and G.J. Muehlbauer. 2012. Identification and characterization of barley genes that provide resistance to trichothecenes. National Scab Forum Abstracts.

Koeritz, E.J., A.M. A.M. Elakkad, L.S. Dahleen, R. Skadsen, T. Abebe, J. Shah, V.J. Nalam, G. Klossner, N. Tumer, R. Di, G.J. Muehlbauer, X. Li, S. Shin and R. Dill-Macky. 2012. Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2012 field nursery report. National Scab Forum Abstracts.

Li, X., S. Shin, R. Dill-Macky, F. Berthiller, T. Clemente, S. McCormick and G.J. Muehlbauer. 2012. Transgenic wheat carrying a barley UDP-glucosyltranferase exhibits high levels of Fusarium head blight resistance. National Scab Forum Abstracts.

Muehlbauer, G.J., S. Shin, X. Li, J. Boddu, S. Heinen, J.A. Torres-Acosta, M.P.K. Paris, W. Schweiger, T. Clemente, R. Dill-Macky, S. McCormick, M. Lemmens, F. Berthiller and G. Adam. 2012. Developing Fusarium head blight resistant wheat. National Scab Forum Abstracts.

Schweiger, W., M.P. Kovalsky Paris, G. Wiesenberger, F. Berthiller, M. Lemmens, S. Shin, J.A. Torres-Acosta, G.J. Muehlbauer and G. Adam. 2012. Functional genomics of UDP-glucosyltransferases: heterologous expression in yeast to test for deoxynivalenol detoxification capability of candidate genes. National Scab Forum Abstracts.

Schweiger, W., B. Steiner, C. Ametz, G. Siegwart, G. Wiesenberger, F. berthiller, M. Lemmens, H. Jia, G. Adam, G.J. Muehlbauer, D.P. Kreil and H. Buerstmayr. 2012. Transcriptomic characterization of the Fusarium resistance QTL *FHB1* and *QFHS-IFA.5A*. National Scab Forum Abstracts.

Hofstad, A.N., H. Jia, B.P. Millett, E. Akhunov, and G. Muehlbauer. 2013. Identifying FHB resistance genes in wheat using a next generation sequencing approach. Plant and Animal Genome XXI Abstracts.

Kovalsky Paris, M.P., W. Schweiger, G. Wiesenberger, JA. Torres Acosta, H. Michlmayr, S. Newmister, M. Lemmens, A. Malachova, S. Shin, G. Muehlbauer, T. Weigl-Pollack, P. Fruhmann, H. Mikula, C. Hametner, B. Kluger, R. Schuhmacher, R. Krska, J. Fröhlich, I. Rayment, F. Berthiller, and G. Adam. 2013. *In planta* inactivation of Fusarium mycotoxins. European Fusarium Symposium Abstracts.