

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY11 Final Performance Report
July 13, 2012**

Cover Page

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USDA-ARS Agreement ID:	59-0206-9-073
USDA-ARS Agreement Title:	Molecular Genetic Approaches to Develop Scab Resistance.
FY11 USDA-ARS Award Amount:	\$ 125,283

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Characterize and Map Barley Genes that Respond to Fusarium graminearum Infection.	\$ 61,868
GDER	Rapidly Identify and Test Scab Resistance Genes.	\$ 63,415
	Total ARS Award Amount	\$ 125,283

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Principal Investigator

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Date

* 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: *Characterize and Map Barley Genes that Respond to Fusarium graminearum Infection.*

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.

In collaboration with Drs. Kevin Smith and Brian Steffenson, we identified a set of 101 genotypes that exhibit variation for scab resistance and susceptibility. Previous disease screens of these lines categorized 78 as resistant and 23 as susceptible. To obtain FHB severity data from a single trial, we grew the complete set of lines in China. The disease severity data differentiate resistant from susceptible genotypes. The 101 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., accepted in TAG). This information will be useful for future mapping studies and breeding efforts. We also used the DArT data to identify a core set of 25 genotypes that served as a representative set for sequencing genes that have the potential to exhibit resistance or susceptibility. We completed the sequencing of 39 genes from this set of 25 genotypes. As part of this sequencing effort, we completely sequenced an UDP-glucosyltransferase (UGT) gene (~2.5 kb), that detoxifies DON via conversion to DON-3-O-glucoside, from 32 individuals. Transgenic yeast and *Arabidopsis* (Shin et al., 2012) carrying the barley UGT confer resistance to DON, and transgenic wheat confer resistance to FHB (see below). We identified a nonsynonymous mutation in the barley UGT gene in two of the most susceptible barley genotypes. In collaboration with Dr. Gerhard Adam, we examined the function of the mutant allele in yeast. We found that this mutation resulted in the reduced ability to convert DON to D3G, indicating that we may have identified a key component of FHB resistance in barley.

To obtain data to map resistance to DON in barley we screened a set of barley FHB mapping population parents for root growth on DON-containing media. We identified a differential response in the mapping population parents: Atahualpa (susceptible) and M81 (resistant), and Comp351 (resistant) and M98-102 (susceptible). The populations were increased and we are beginning to screen individuals of the Atahualpa x M81 mapping population for DON resistance.

To examine gene expression at key QTL for FHB resistance, we increased seed from three NIL pairs carrying the chromosome 2H bin 8 and 10, and chromosome 6H bin 7. To confirm we have the correct genotypes and to genetically characterize these NILs we submitted tissue from each NIL to Dr. Shiaoman Chao for SNP genotyping. Our plan is to use RNA-seq to examine gene expression in these NIL pairs during *Fusarium graminearum* infection.

- 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 determined DArT haplotypes in 101 FHB resistant and susceptible genotypes for four major barley FHB QTL.
2. In highly susceptible barley, we identified an allele of a barley UGT gene that exhibits reduced ability to convert DON to D-3G, indicating that this gene may be an important component of resistance.

Impact:

1. The haplotype work provided information regarding the genetic relationships between the FHB resistant genotypes, enabling us to select the genotypes that carry novel disease resistance loci and/or alleles for genetic mapping and breeding.
2. The identification of the mutant allele of the barley UGT gene indicates that this gene may be an important component of resistance and provides the rationale for selecting additional alleles of this gene that will confer increased resistance.

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.

We cloned eight genes from barley encoding UDP-glucosyltransferases (UGTs) that respond to DON accumulation and sent them to Dr. Gerhard Adam for functional assays in yeast to test for the ability of these enzymes to detoxify trichothecenes. One of these UGT genes confers resistance in yeast to DON (Schweiger et al., 2010). We developed transgenic Arabidopsis carrying this gene and showed that they confer resistance to DON (Shin et al., 2012). In contrast to the Arabidopsis UGT gene previous isolated, we showed that overexpression of the barley UGT gene in Arabidopsis did not exhibit deleterious phenotypes and did not conjugate brassinosteroids. Transgenic wheat carrying the barley UGT gene was developed and screened three times in the greenhouse. In these screens we observed a high level of type II resistance. In fact, we identified lines that exhibited higher levels of type II resistance than Sumai3. In collaboration with Dr. Ruth Dill-Macky, disease screens are being conducted in the field this summer. Currently, we are examining these transgenic wheat plants for their ability to convert DON to D-3G. Finally, we are collaborating with Dr. Jochen Kumlehn (IPK-Gaterslaben, Germany) to develop transgenic barley carrying this gene.

To rapidly identify additional DON resistance genes we are transforming Arabidopsis with putative DON resistance genes from barley. We isolated barley genes encoding two zinc finger proteins, two ABC transporters, three cytochrome P450s, one epoxide hydrolase and seven glutathione-S-transferases that were upregulated after DON application. Transgenic Arabidopsis have been developed for seven GSTs, one zinc finger protein, and two ABC transporters. To date, testing of the transgenic Arabidopsis carrying five GSTs resulted in a lack of resistance. Development and testing of additional transgenic plants is underway.

- 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 and in three greenhouse screens showed that this gene confers a high level of type II FHB resistance.
2. The transgenic Arabidopsis and wheat carrying the barley UGT did not exhibit any deleterious phenotypes.
3. Transgenic Arabidopsis have been developed for seven GSTs, one zinc finger protein and two ABC transporters and will be screened for DON resistance in the near future.

Impact:

4. The transgenic wheat carrying the barley UGT exhibited a higher level of resistance than Sumai3, indicating that this gene is useful to confer a high level of resistance in wheat.
5. The lack of deleterious phenotypes in the transgenic Arabidopsis and wheat carrying the barley UGT indicates that this gene will be a useful source of FHB resistance.

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:

Shin, S., J.A. Torres-Acosta, S.J. Heinen, S. McCormick, M. Lemmens, P.M.K. Paris, F. Berthiller, G. Adam, and G.J. Muehlbauer. 2012. Transgenic *Arabidopsis thaliana* expressing a barley UDP-glucosyltransferase exhibit resistance to the mycotoxin deoxynivalenol. *J. Exp. Botany* (In press).

Huang, Y., B.P. Millet, K.A. Beaubian, S.K. Dahl, B.J. Steffenson, K.P. Smith, and G.J. Muehlbauer. Haplotype diversity and population structure in cultivated and wild barley evaluated for *Fusarium* head blight responses. *Theoretical & Applied Genetics* (Accepted with revisions 6-12).

Schweiger, W., B. Steiner, C. Ametz, F. Berthiller, M. Lemmens, H. Jia, G. Adam, G.J. Muehlbauer, D.P. Kreil, and H. Buerstmayr. Transcriptomic characterization of the *Fusarium graminearum* resistance quantitative trait loci *Fhb1* and *Qfhs-ifa.5A* in wheat near-isogenic lines. *New Phytologist* (Submitted 6-12)

Presentations:

“Genomics approaches to Triticeae Improvement”, at the Plant Biological Sciences Colloquium, St. Paul Campus

“The role of trichothecenes in the Triticeae-*Fusarium graminearum* interactions” at the American Phytopathological Society Meeting, Providence, RI

Abstracts:

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

Schweiger, W., M.P. Kovalsky Paris, G. Wiesenberger, T. Nussbaumer, K.F.X. Mayer, F. Berthiller, R. Krska, P. Fruhmann, J. Fröhlich, M. Lemmens, J.A. Torres-Acosta, S. Shin, G. Muehlbauer, and G. Adam. 2012. Functional characterization of *Brachypodium distachyon* UDP-glucosyltransferases and candidate deoxynivalenol detoxification genes from crop plants.

Muehlbauer, G.J., J. Boddu, S. Gardiner, S. Shin, H. Jia, S. Cho, S. McCormick, W. Schweiger, M. Lemmons, F. Berthiller, C. Hametner, P.M. Kovalsky Paris, J.A. Torres-Acosta and G. Adam. 2012. The role of trichothecenes in the Triticeae-*Fusarium graminearum* interactions. American Phytopathological Society Meeting Abstracts.

Hofstad, A., H. Jia, B.P. Millett and G.J. Muehlbauer. 2011. Identifying FHB resistance genes in wheat using a next-generation sequencing approach. National Fusarium Head Blight Forum Abstracts.

Shin, S., A. Torres-Acosta, M. Lemmens, P. Paris, F. Berthiller, G. Adam, S. McCormick and G.J. Muehlbauer. 2011. Identifying and characterizing barley genes that protect against trichotheceenes. National Fusarium Head Blight Forum Abstracts.

Huang, Y., B. P. Millett, K.A. Beaubian, S.K. Dahl, B.J. steffenson, K.P. Smith and G.J. Muehlbauer. 2011. Sequence diversity and haplotype analysis of Fusarium head blight-responsive genes in diverse wild and cultivated barley. National Fusarium Head Blight Forum Abstracts.

Shin, S., A. Torres-Acosta, F. Berthiller, W. Schweiger, G. Adam, S. McCormick and G.J. Muehlbauer. 2011. Identifying and characterizing barley genes that protect against trichotheceene mycotoxins. American Society of Plant Biologists Meeting Abstracts.