

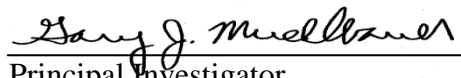
**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
July 15, 2011**

Cover Page

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Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0206-9-073
USDA-ARS Agreement Title:	Molecular Genetic Approaches to Develop Scab Resistace.
FY10 USDA-ARS Award Amount:	\$ 109,320

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Characterize and Map Barley Genes that Respond to <i>Fusarium graminearum</i> Infection.	\$ 61,868
GDER	Rapidly Identify and Test Scab Resistance Genes.	\$ 47,452
	Total ARS Award Amount	\$ 109,320


7/6/11

 Principal Investigator 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 and map barley genes that respond to *F. graminearum* infection.

Previously, we conducted a large set of microarray experiments in barley and wheat aimed at identifying genes that are involved in resistance or susceptibility to FHB and respond to deoxynivalenol treatment. We showed that there was no overlap in gene expression in three FHB resistance QTL NIL pairs (Jia et al., 2011). We carefully examined these gene lists and identified a set of genes that have the potential to play a role in the barley-*F. graminearum* interaction.

To identify polymorphisms for mapping, we sequenced 39 genes that are upregulated in the barley-*Fusarium graminearum* interaction from a set of barley mapping population parents. We identified polymorphisms from 20 genes and used Sequenom assays to map 17 genes on the Oregon Wolfe Barley RIL population.

In collaboration with 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 (bin 8, bin 10 and bin 13-14). This information will be useful for future mapping studies and breeding efforts. Association mapping using the DArT and phenotype data identified eight QTL, four of which were in previously identified FHB resistance QTL. 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 39 genes from this set of 25 genotypes. As part of this sequencing effort, we completely sequenced an UDP-glucosyltransferase gene (~2.5 kb) that detoxifies DON in yeast and *Arabidopsis* and that confers resistance to FHB in transgenic wheat (see below) from 32 genotypes. We examined the association of the sequence data from the UDP-glucosyltransferase gene and found no associations with resistance.

To obtain data to map resistance to DON we screened a set of barley FHB mapping population parents 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 are being increased this summer for mapping the response to DON in the fall.

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 the genetic relationships between 101 FHB resistant and susceptible genotypes.
2. We determined the DArT haplotypes in 101 FHB resistant and susceptible genotypes for three major barley FHB QTL.
3. We sequenced 39 genes from 25 FHB resistant and susceptible genotypes that respond to *Fusarium graminearum* infection and/or DON.
4. We mapped 17 barley genes that respond to *Fusarium graminearum* infection and/or DON.

Impact:

1. The genotyping, sequencing and 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 map locations of the genes that are expressed during *Fusarium graminearum* could be used for breeding efforts and genetic studies.

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 that respond to DON accumulation and sent them to Gerhard Adam for functional assays in yeast to test for the ability of these enzymes to detoxify trichothecenes. One of these UDP-glucosyltransferase genes confers resistance in yeast to DON and nivalenol (NIV), another trichothecene mycotoxin (Schweiger et al., 2010). This is the first plant gene identified that exhibits resistance to both DON and NIV. We developed transgenic Arabidopsis carrying this gene and showed that they confer resistance to DON, DAS and NIV. Transgenic wheat carrying this gene exhibits a high level of FHB resistance. Compared to Sumai 3, six lines exhibited higher levels of type II resistance. Additional disease screens will be conducted in the fall and spring. We are collaborating with Jochen Kumlehn (IPK-Gaterslaben, Germany) to develop transgenic barley carrying this gene.

Multiple pieces of evidence suggest that glutathione is important for resistance to trichothecenes (Gardiner et al., 2010). We identified the induction of five genes encoding glutathione-S-transferases (GSTs) in barley after application of DON. In addition, we have evidence for the formation of DON-glutathione conjugates. To examine the role that glutathione plays in trichothecene resistance, we cloned five barley GSTs and developed transgenic Arabidopsis carrying these genes. Preliminary tests revealed that three of these lines confer resistance to DON. The other two lines will be tested in the near future.

We have also isolated barley genes encoding a zinc finger protein, an ABC transporter and two cytochrome P450s that were upregulated after DON application. For two of the genes (cytochrome P450 and the zinc finger protein) we have developed transgenic Arabidopsis plants. For the other two genes (ABC transporter and cytochrome P450) we have developed the plant transformation construct and will begin developing transgenic Arabidopsis in the near future.

We selected an Arabidopsis mutant that exhibits tolerance to DAS. We are in the initial stages of mapping the mutant before beginning a map-based cloning strategy.

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 showed that transgenic Arabidopsis carrying a barley UDP-glucosyltransferase confers resistance to DON, DAS and NIV.
2. We developed transgenic wheat carrying a barley UDP-glucosyltransferase and showed that this gene confers a high level of type II FHB resistance.
3. We developed transgenic Arabidopsis carrying GSTs, a zinc finger protein, and a cytochrome P450.
4. The transgenic Arabidopsis carrying barley GSTs appear to exhibit DON resistance.

Impact:

1. The barley UDP-glucosyltransferase is the first gene that has been shown to provide resistance to type A (DAS) and type B (DON, NIV) trichothecenes.
2. The transgenic wheat are the first transgenic lines that I am aware of that exhibit type II resistance that is superior to Sumai 3. This could result in a novel source of resistance for wheat.
3. The ability of the barley GSTs to provide DON resistance provides the rationale for developing transgenic wheat carrying these genes.

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

Jia, H., B. Millett, S. Cho, H. Bilgic, K.P. Smith and G.J. Muehlbauer. 2011. Transcriptome analysis of three barley near-isogenic line pairs carrying contrasting alleles for different *Fusarium* head blight resistance QTLs. *Functional & Integrative Genomics* 11:95-102.

Shin, S., S.J. Heinen, A. Cole, W. Schweiger, G. Adam and G.J. Muehlbauer. Transgenic *Arabidopsis* plants expressing a barley UDP-glucosyltransferase show tolerance to trichothecene mycotoxins. In preparation.

Presentations

“Exploiting barley breeding germplasm for QTL detection” at the 6th Canadian Barley Symposium, Saskatoon, Saskatchewan, Canada.

“Integrating breeding and genomics in the public sector: the barley CAP example”, at the Plant Breeding Coordinating Committee Annual Meeting, Johnston, IA.

“Genomics approaches to Triticeae improvement” at Oregon State University, Corvallis, OR.

“Genome-wide association studies in barley: gene discovery and applications” at the University of Missouri, Columbia, MO.

“Genome-wide association studies in barley: gene discovery and applications” at the University of Georgia, Athens, GA.

Abstracts

Schweiger, W., M. Kovalsky, F. Berthiller, M. Lemmens, S. Shin, J. Boddu, G.J. Muehlbauer and G. Adam. 2011. Functional testing of barley and *Brachypodium distachyon* UDP-glucosyltransferases for their ability to inactivate *Fusarium* toxins by heterologous expression in yeast. Gordon Conference Abstracts “Mycotoxin and Phycotoxins”, Waterville, ME.

Huang, Y., B.P. Millett, K.A. Beaubian¹, S.K. Dahl, B.J. Steffenson, K.P. Smith and G.J. Muehlbauer. 2010. Characterization of *Fusarium* head blight-responsive genes in diverse wild and cultivated barley. National Scab Forum, Milwaukee, WI.

Shin, S.H., S.J. Heinen, W. Schweiger, G. Adam and G.J. Muehlbauer. 2010. Identifying and characterizing barley genes that protect against trichothecenes. National Scab Forum, Milwaukee, WI.

Steiner, B., A. Limmongkon, K. Schiessl, M. Lemmens¹, H. Jia, G. Muehlbauer, A. Posekany, D.P. Kreil and H. Brstmayr. 2010. Gene expression analysis of related wheat lines with contrasting levels of head blight resistance after *Fusarium graminearum* inoculation. National Scab Forum, Milwaukee, WI.