USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY09 Final Performance Report
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Cover Page

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Fiscal Year:	2009
USDA-ARS Agreement ID:	59-0206-9-073
USDA-ARS Agreement Title:	Molecular Genetic Approaches to Develop Scab Resistace.
FY09- USDA-ARS Award Amount:	$ 118,942

USWBSI Individual Project(s)

USWBSI Research Category[*]	**Project Title**	**ARS Adjusted Award Amount**
BAR-CP	Characterize and Map Barley Genes that Respond to Fusarium graminearum Infection.	$ 48,399
BAR-CP	Identify Barley Genes that Respond to Deoxynivalenol.	$ 23,092
GDER	Rapidly Identify and Test Scab Resistance Genes.	$ 47,451
	Total Award Amount	**$ 118,942**

Principal Investigator Date

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[*] 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 Winter Wheat Region
  SWW – Southern Sinter 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. We also identified a large set of barley genes that respond to deoxynivalenol treatment (see below). We have 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 from a set of barley mapping population parents. Polymorphisms were identified and Sequenom assays have been developed to map 20 genes on the Oregon Wolfe Barley RIL population. The outcomes from this work will be markers for breeding programs, potential resistance genes, and increased understanding of the barley-*F. graminearum* interaction.

In collaboration with Kevin Smith and Brian Steffenson, we identified a set of 102 genotypes that exhibit variation for scab resistance and susceptibility. Previous disease screens of these lines categorized 79 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. More thorough analysis of the FHB field screening data will be conducted in the near future. The 102 lines were screened with DArT markers and we used that 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 sequenced 39 genes from this set of 25 genotypes. Analysis of the sequencing data will be conducted in the near future.

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 identified polymorphisms in mapping parents of 20 previously unmapped genes that are induced by *Fusarium graminearum* infection.
2. We genetically and phenotypically characterized a set of barley germplasm carrying resistance to FHB.

Impact:

1. We will use the polymorphisms to map genes that are induced by *Fusarium graminearum* infection and DON treatment. The map location of these markers will be used for breeding.
2. Phenotypic and genetic characterization of the barley germplasm will provide the opportunity to discover genes and novel sources of FHB resistance.

Project 2: *Identify Barley Genes that Respond to Deoxynivalenol.*

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 genes that respond to DON accumulation.

We conducted a microarray experiment to identify gene transcripts in barley that respond to DON accumulation (Gardiner et al., 2010). We identified 255 genes that respond to DON treatment. In collaboration with Gerhard Adam we also showed that DON is converted to the less toxic DON-3-O-glucoside in barley. Several genes encoding UDP-glucoyltransferases were identified with a potential role in trichothecene detoxification. We also noted that there were many induced genes that play a role in glutathione accumulation. We showed DON conjugation to glutathione *in vitro*, indicating a novel detoxification mechanism (Gardiner et al., 2010).

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 glutathione may play a role in trichothecene resistance.
2. We identified a set of UDP-glucosyltransferases that are induced by DON.
3. A manuscript was published describing the response to DON treatment in barley (Gardiner st al., 2010). All expression data is publically available on PLEXdb (http://www.plexdb.org/).

Impact:

1. We are exploring the role of glutathione on trichothecene resistance (see below).
2. We are exploring the role of UDP-glucosyltransferases on trichothecene resistance in plants (see below).
3. The manuscript and associated data will be useful for other studies examining the plant-trichothecene interaction.

Project 3: *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 it confers resistance to DON. Currently, we are testing the transgenic Arabidopsis for resistance to NIV. We are also developing transgenic wheat with this gene.

Multiple pieces of evidence suggest that glutathione is important for resistance to trichothecenes (Gardiner et al., 2010). We identified the induction of genes encoding cysteine synthase and glutathione-S-transferases in barley after application of DON. Cysteine synthase is a member of the cysteine biosynthetic pathway and cysteine is a component of the tripeptide glutathione. Other work in Gerhard Adam's lab showed that increasing the expression of cysteine synthase in yeast increased resistance to DON. In addition, we have evidence for the formation of DON-glutathione conjugates. Therefore, we think that increasing the level of glutathione in plants will provide increased resistance to trichothecenes. To examine the role that glutathione plays in trichothecene resistance, we cloned three glutathione-S-transferase genes and a cysteine synthase gene from barley. We developed transgenic Arabidopsis carrying the cysteine synthase gene and are in the process of developing transgenic Arabidopsis carrying the three glutathione-S-transferases. Testing of the transgenic plants will occur in the near future.

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 identified a barley UDP-glucosyltransferase that provided resistance in yeast to DON and NIV and in Arabidopsis to DON. This is the first plant gene identified that exhibits resistance to both DON and NIV. We published a paper (Schweiger et al., 2010) on the barley UDP-glucosyltransferase gene and yeast resistance.
2. We cloned three glutathione-S-transferase genes and a cysteine synthase gene.

Impact:

1. The barley UDP-glucosyltransferase gene provides a tool to develop transgenic wheat with resistance to DON. The Schweiger et al. (2010) manuscript will provide information to other scientists for future studies of trichothecene resistance.
2. The cysteine synthase and glutathione-S-transferase genes are tools to study the role of glutathione on trichothecene 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:

Jia, H., S. Cho and G.J. Muehlbauer. 2009. Transcriptome analysis of the wheat 3BS Fusarium head blight resistance QTL during *Fusarium graminearum* infection. Mol. Plant-Microbe Interact. 22:1366-1378.

Gardiner, S.A., J. Boddu, F. Berthiller, C. Hametner, R. Stupar, G. Adam, and G.J. Muehlbauer. 2010. Transcriptome analysis of the barley-deoxynivalenol interaction: evidence for a role of glutathione in deoxynivalenol detoxification. Mol. Plant-Microbe Interact. 23:962-976.

Schweiger, W., J. Boddu, S. Shin, B. Poppenberger, F. Berthiller, M. Lemmens, G.J. Muehlbauer, and G. Adam. 2010. Validation of a candidate deoxynivalenol-inactivating UDP-glucosyltransferase from barley by heterologous expression in yeast. Mol. Plant-Microbe Interact. 23:977-986.

Massman, J., B. Cooper, R. Horsley, S. Neate, R. Dill-Macky, S. Chao, Y. Dong, P. Schwarz, G.J. Muehlbauer and K.P. Smith. 2010. Genome-wide association mapping of Fusarium head blight resistance in contemporary barley breeding germplasm. Mol. Breeding. (In press).

Jia, H., B.P. Millett, S. Cho, H. Bilgic, K.P. Smith, W.W. Xu, and G.J. Muehlbauer. Quantitative trait loci conferring resistance to Fusarium head blight in barley respond differentially to *Fusarium graminearum* infection. Submitted to Functional & Integrative Genomics.

Shin, S., W. Schweiger, S. McCormick, S. Heinen, A. Cole, G. Adam and G.J. Muehlbauer. Overexpression of a barley UDP-glucosyltransferase in Arabidopsis confers resistance to trichothecenes. In preparation.

Abstracts:

Muehlbauer, G.J., J. Boddu, S. Gardiner, S. Shin, H. Jia, S. Cho, W. Kruger, W. Schweiger, F. Berthiller, C. Hametner and G. Adam. 2010. The role of trichothecenes in the *Triticeae-Fusarium* interaction. Plant and Animal Genome Meeting Abstracts.

Adam, G., F. Berthiller, C. Hametner, S.A. Gardiner, J. Boddu, R. Stupar and G.J. Muehlbauer. 2010. Formation of DON-glutathione conjugates: a role for glutathione-S-transferases in DON resistance of barley? Society for Mycotoxin Research, 32nd Mycotoxin Workshop Abstracts, Copenhagen, Denmark.

Adam, G., F. Berthiller, C. Hametner, C, S.A. Gardiner, J. Boddu, R. Stupar and G.J.Muehlbauer. 2010. A role for glutathione-mediated detoxification in deoxynivalenol resistance of barley? 11th European Fusarium Seminar, Radzikow, Poland.

Schweiger, W., P. Kovalsky, F. Berthiller, M. Lemmens, S. Shin, J. Boddu, G.J. Muehlbauer and G. Adam. 2010. Functional testing of barley UDP-glucosyltransferase for the ability to inactivate deoxynivalenol and nivalenol by expression in yeast. 11th European Fusarium Seminar, Radzikow, Poland.

Schweiger, W., S. Shin, J. Boddu, B. Steiner, M. Lemmens, H. Buerstmayr, G.J. Muehlbauer, and G. Adam. 2009. Functional testing of crop plant and *Brachypodium distachyon* UDP-glucosyltransferases in yeast: identification of a barley UGT inactivating the Fusarium

mycotoxins deoxynivalenol and nivalenol. XIV International Congress on Molecular Plant-Microbe Interactions Meeting Abstracts. Quebec City, Quebec.

Dahleen, L., R. Dill-Macky, J. Shah, G. Muehlbauer, R. Skadsen, M. Manoharan, T. Abebe and J. Jurgenson. 2009. Transgenic field trials for FHB resistance and related research in wheat and barley. In: Ouellet, T and Leger, D. (eds.). Proceedings of the 6th Canadian Workshop on Fusarium Head Blight. Nov. 1-4, 2009. Ottawa, ON, Canada. Page 38.

Millett, B.P., K.A. Beaubian, S.K. Dahl, B.J. Steffenson, K.P. Smith, and G.J. Muehlbauer. 2009. Characterization of Fusarium head blight responsive genes in diverse wild and cultivated barley. National Fusarium Head Blight Forum Abstracts.

Shin, S.H., J. Boddu, A. Cole, W. Schweiger, G. Adam, and G.J. Muehlbauer. 2009. Identifying and characterizing barley genes that protect against trichothecenes. National Fusarium Head Blight Forum Abstracts.

Muehlbauer, G.J., J. Boddu, S. Gardiner, S.H. Shin, H. Jia, S. Cho, W. Kruger, and F. Berthiller. 2009. Unraveling the *Triticeae-Fusarium graminearum* interaction. National Fusarium Head Blight Forum Abstracts.

Dill-Macky, R., K.J. Wennberg, T.C. Scanlan, G.J. Muehlbauer, S. Shin, D. Shah, J. Kaur, and L.S. Dahleen. 2009. Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2009 field nursery report. National Fusarium Head Blight Forum Abstracts.

Berthiller, F., W. Schweiger, B. Steiner, H. Buerstmayr, M. Lemmens, R. Krska, J. Boddu, S. Shin, G.J. Muehlbauer, C. Hametner, J.A. Torres Acosta, G. Wiesenberger, and G. Adam. 2009. Host resistance to *Fusarium* metabolites: relevance of masked mycotoxins for resistance breeding and toxicology. National Fusarium Head Blight Forum Abstracts.