USDA-ARS / USWBSI
FY04 Final Performance Report
July 15, 2005

Cover Page

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Year:	**FY2004** (approx. May 04 – April 05)
FY04 ARS Agreement ID:	**59-0790-4-116**
FY04 ARS Agreement Title:	**Molecular Genetic Approaches to Develop Scab Resistance.**
FY04 ARS Award Amount:	**$ 124,293**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Mechanisms and Essential Genes for Resistance to Fusrium Head Blight.	$ 58,293
BIO	Developing and Characterizing Transgenic Wheat for Scab Resistance.	$ 56,000
	Total ARS Award Amount	**$ 124,293**

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Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
VDUN – Variety Development & Uniform Nurseries

Project 1: Mechanisms and Essential Genes for Resistance to Fusrium Head Blight.

1. **What major problem or issue is being resolved and how are you resolving it?**

Fusarium head blight (FHB or scab) is a disease that can devastate wheat and barley. The wheat and barley transformation efforts have a limited number of genes that have the potential to reduce FHB. Our goal is to identify the mechanisms and essential genes for wheat and barley scab resistance.

2. What were the most significant accomplishments?

To identify mechanisms and genes that are involved in resistance, we established large-scale RNA profiling. We are using the Barley1 and Wheat1 Affymetrix GeneChip probe arrays to examine transcript accumulation in barley and wheat during *F. graminearum* infection. The Barley1 and Wheat1 GeneChips represent approximately 22,000 and 61,000 barley and wheat genes, respectively. Therefore these GeneChips are excellent resources for examining transcript accumulation in barley and wheat. We conducted four experiments. All experiments contained at least three replications of each genotype/treatment/timepoint combination. A variety of statistical and computer software packages were used to identify gene expression patterns that were statistically significant between treatments and genotypes. The general criteria we used for declaring a transcript statistically different was a $P<0.001$, >2-fold different transcript accumulation level, and a false discovery rate of approximately 5%.

In the 2003 progress report, we described the major findings of the experiment: Morex barley inoculated with *F. graminearum* and a water control and sampled at 24, 48, 72, 96 and 144 hours after inoculation. In short, we identified approximately 500 transcripts from Morex that were expressed during *F. graminearum* infection and these data provide the basis for a reference set of expressed genes for all of our other GeneChip experiments.

We conducted an experiment to examine the transcript accumulation differences in Morex barley inoculated with the *Tri5* mutant *F. graminearum* strain (trichothecene non producing), a wildtype *F. graminearum* strain (trichothecene producing) and water. We detected barley genes that were differentially expressed between the trichothecene producing and nonproducing strains of the fungus. We identified 113 genes that were upregulated during infection with the trichothecene producing strain, and 25 genes that were upregulated during infection with the trichothecene nonproducing strain.

We also conducted an experiment to examine the gene expression profile differences between a barley near–isogenic line (NIL) pair carrying susceptible and resistant alleles for a deoxynivalenol (DON) resistant QTL (chromosome 3H, BIN 6) during *F. graminearum* infection. We detected 17 genes that exhibited differences in transcript accumulation between the resistant and susceptible alleles. We mapped two of these genes to the chromosome 3H DON resistant QTL region.

We also conducted an experiment to examine the transcript accumulation differences between a wheat NIL pair carrying susceptible and resistant alleles for a FHB resistant QTL on chromosome 3BS during *F. graminearum* infection. We detected 45 genes that exhibited differences in transcript accumulation between the resistant and susceptible alleles.

We have initiated another experiment to examine the transcript accumulation differences between a barley NIL pair carrying susceptible and resistant alleles for a FHB resistant QTL (chromosome 2H, BIN 8) during *F. graminearum* infection.

Project 2: ***Developing and Characterizing Transgenic Wheat for Scab Resistance.***

1. What major problem or issue is being resolved and how are you resolving it?

Fusarium head blight (FHB or scab) is a disease that can devastate wheat and barley. To enhance FHB resistance in wheat and barley, we are developing transgenic wheat and barley carrying antifungal protein (AFP) genes and testing these lines for scab resistance.

2. **What were the most significant accomplishments?**

We developed and are testing 25, 25, 31, 17, 12, 4, 11, 7, 14, 2, 1 and 4 transgenic wheat plants carrying expressed α-thionin, thaumatin like protein-1 (tlp-1), ß-1,3-glucanase, chitinase, ribosome inactivating protein (RIP), chitinase/RIP, chitinase/tlp-1, RIP/tlp-1, lipid transfer protein (LTP), *Tri101*/tlp-1, *Tri101*/ß-1,3-glucanase and tlp-1/ß-1,3-glucanase transgenes, respectively.

We identified two lines that exhibited enhanced resistance to FHB under field conditions. As described in our 2002 progress report, four, one and two lines carrying the ß-1,3-glucanase, α-thionin and tlp-1 transgenes, respectively, exhibited statistically significant reductions in scab severity compared to the non transformed controls in multiple greenhouse screens. These transgenic lines were screened in collaboration with Dr. Ruth Dill-Macky in the field at Crookston, MN in the summer of 2004. Our results show that one line carrying the tlp-1 transgene and one line carrying the ß-1,3-glucanase transgene exhibited statistically significant reductions ($P<0.05$) in mean FHB severity (%), VSK (%) and deoxynivalenol accumulation (ppm) compared to the non transformed Bobwhite controls. The lines carrying the α-thionin transgene and two other lines carrying the ß-1,3-glucanase transgene exhibited statistically significant reductions ($P<0.05$) in mean FHB severity (%) compared to Bobwhite controls. In general, the mean FHB severity in the transgenic plants exhibiting enhanced resistance was between 50-68% while Bobwhite exhibited 81%.

As described in our 2003 progress report, eight, one, one, three and three lines carrying expressed chitinase, RIP, chitinase/RIP, chitinase/tlp-1 and RIP/tlp-1 transgenes, respectively, exhibited a statistically significant reduction in FHB severity compared to the non transgenic controls in multiple greenhouse screens. Our Western blots show that the appropriate protein is accumulating in these lines. Most of these lines are in our 2005 field test (see below).

A field screen is currently being conducted in collaboration with Dr. Dill-Macky at Crookston, MN. The lines in the field test were screened in the summer of 2004 (four, one and two lines carrying the ß-1,3-glucanase, α-thionin and tlp-1 transgenes, respectively) and recently identified lines exhibiting promising results in our greenhouse tests (seven, one, one, two and three lines carrying chitinase, RIP, chitinase/RIP, chitinase/tlp-1 and RIP/tlp-1 transgenes, respectively).

We conducted two greenhouse screens of four, two, one, four, four and two lines carrying tlp-1/ß-1,3-glucanase, *Tri101*/tlp-1, *Tri101*/glucanase, LTP, RIP and RIP/tlp-1. To obtain accurate disease severity on plants expressing the transgenes, we are currently assessing transgene expression.

We crossed the lines carrying the tlp-1, ß-1,3-glucanase and α-thionin transgenes, that exhibited enhanced FHB resistance in our 2004 summer field test, to the partially resistant cultivar Alsen. F_1 progeny are currently growing.

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

Published manuscripts related to project:

Canci, P.C., L.M. Nduulu, G.J. Muehlbauer, R. Dill-Macky, D.C. Rasmusson and K.P. Smith. 2004. Validation of quantitative trait loci for fusarium head blight and kernel discoloration resistance in barley. *Mol. Breed*. 14:91-104

Al-Saady, N.A., K.A. Torbert, L. Smith, I. Makarevitch, G. Baldridge, R.J. Zeyen, G.J. Muehlbauer, N.E. Olszewski and D.A. Somers. 2004. Tissue specificity of the sugarcane bacilliform virus promoter in oat, barley and wheat. *Mol. Breed*. 13:331-338.

Submitted manuscripts related to project:

Boddu, J., S. Cho W.M. Kruger and G.J. Muehlbauer. Transcriptome analysis of the barley-*Fusarium graminearum* interaction. Submitted to Mol. Plant-Microbe Interact.

Mackintosh, C.A., D.F. Garvin, L.E. Radmer, S.J. Heinen and G.J. Muehlbauer. A model wheat cultivar for transformation to improve resistance to Fusarium Head Blight. Submitted to Plant Cell Reports.

Manuscripts in preparation related to project:

Mackintosh, C.A., S.J. Heinen, L.A. Smith, M.N. WycKoff, G.D. Baldridge, R.J. Zeyen and G.J. Muehlbauer. Overexpression of anti-fungal proteins enhances the resistance of wheat to Fusarium Head Blight. To be submitted to *Crop Science.*

Conference Proceedings related to project:

Muehlbauer, G.J., D.F. Garvin, J. Boddu and S. Cho. 2005. Applications of microarrays to barley research. Proceedings of the 35th Barley Improvement Conference, Charleston, SC.

Muehlbauer, G.J., D.F. Garvin, K. Smith, J. Boddu and S. Cho. 2005. Applications of GeneChips for barley improvement. Proceedings of the North American Barley Researchers Workershop, Red Deer, Alberta, Canada.

Abstracts related to project:

Nduulu, L., A. Mesfin, G.J. Muehlbauer and K.P. Smith. 2004. Fine mapping of Fusarium head blight resistant and heading date coincident QTL on chromosome 2H in barley. American Society of Agronomy Meeting Abstracts, p. 117

Muehlbauer, G.J., J. Boddu, W. Kruger and S. Cho. 2004. Microarray analysis of barley infected with *Fusarium graminearum.* Proc. of the 2nd International Symposium on Fusarium Head Blight p. 245.

Kistler, H.C., B. Birren, S. Calvo, C. Cuomo, L.R. Gale, U. Gueldener, L-J. Ma, G. Muehlbauer, K. O'Donnell, F. Trail, T. Ward, J-R. Xu and members of the *Gibberella zeae* International Genomics Initiative. 2004. Genomics of *Fusarium graminearum.* Proc. of the 2nd International Symposium on Fusarium Head Blight p. 4.

Nduulu, L.M., A. Mesfin, G.J. Muehlbauer and K.P. Smith. 2004. High resolution mapping of Fusarium head blight resistance and heading date QTL on chromosome 2H of barley. Proc. of the 2nd International Symposium on Fusarium Head Blight p. 246.

Mackintosh, C.A., J.M. Lewis, S.J. Heinen, L.E. Radmer, R. Dill-Macky, C.K. Evans, G.D. Baldridge, R.J. Zeyen and G.J. Muehlbauer. 2004. Overexpression of antifungal proteins increases the resistance of wheat to Fusarium head blight. Proc. of the 2nd International Symposium on Fusarium Head Blight p. 243.

Baluch, S.D., K.P. Smith, G.J. Muehlbauer, D.A. Somers and B.J. Steffenson. 2004. Investigating FHB QTL associated with the *Vrs1* (row-type) locus on chromosome 2(2H) of *Hordeum vulgare*. Proc. of the 2nd International Symposium on Fusarium Head Blight p. 12.

Chang, Y-L., S. Cho, H.C. Kistler, H-C. Shen and G.J. Muehlbauer. 2004. Physical mapping of the *Fusarium graminearum* genome. Proc. of the 2nd International Symposium on Fusarium Head Blight p. 558.

Cho, S., K. Smith, L. Nduulu and G.J. Muehlbauer. 2004. Microarray analysis of scab resistant QTL-specific gene expression in barley in response to *Fusarium graminearum*. Proc. of the 2nd International Symposium on Fusarium Head Blight p. 37.

Chang, Y-L., S. Cho, C. Kistler, H.-C. Sheng and G.J. Muehlbauer. 2005. Bacterial artificial chromosome-based physical map of *Gibberella zeae* (*Fusarium graminearum*). Plant and Animal Genome Meeting Abstracts p. 94.