USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY05 Final Performance Report (approx. May 05 – April 06)
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Cover Page

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Fiscal Year:	**2005**
FY05 ARS Agreement ID:	**59-0790-4-116**
Agreement Title:	**Molecular Genetic Approaches to Develop Scab Resistance.**
FY05 ARS Award Amount:	**$ 126,585**

USWBSI Individual Project(s)

USWBSI Research Area[*]	**Project Title**	**ARS Adjusted Award Amount**
BIO	Developing and Characterizing Transgenic Wheat for Scab Resistance.	$ 60,853
BIO	Mechanisms and Essential Genes for Resistance to Fusarium Head Blight.	$ 65,732
	Total Award Amount	**$ 126,585**

Principal Investigator   Date

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

(Form – FPR05)

Project 1: *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. To enhance FHB resistance in wheat, we are developing transgenic wheat lines with potential resistance genes and testing these lines for scab resistance.

2. **List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):**
   Accomplishments:

1. We screened 21 transgenic wheat lines in the 2005 summer field trial (collaboration with R. Dill-Macky). We identified one, one, three, one and two lines carrying α-puro-thionin, thaumatin-like proteinl, ß-1,3-glucanase, ribosome inactivating protein and chitinase transgenes, respectively that exhibited statistically significant ($P<0.05$) reductions in FHB severity compared to the nontransformed Bobwhite control. The line carrying the ribosome inactivating protein exhibited levels of resistance that was equivalent to Alsen.
2. We screened 20 and seven transgenic wheat lines in the fall 2005 and spring 2006 greenhouse trials, respectively. We identified one line carrying a ribosome inactivating protein, one line carrying a glutathione-S-transferase and three lines carrying a lipid transfer protein that exhibited significant ($P<0.05$) reduction in FHB severity compared to the nontransformed Bobwhite control.
3. We developed five lines in the cv. Alsen genetic background that carried either the α-puro-thionin, thaumatin-like proteinl or ß-1,3-glucanase transgene. Each of these transgenes have shown a reduction in FHB severity under field conditions (see accomplishment 1). Each of these lines also carried the 3BS FHB resistance QTL. We conducted an initial screen of these lines in a spring 2005 greenhouse trial and they exhibited low FHB severity equivalent to Alsen. Further molecular and phenotypic analysis is ongoing to determine the impact of each transgene on Alsen.
4. We are in the process of screening 14 transgenic wheat lines in the field in the summer of 2006 (collaboration with R. Dill-Macky).
5. Thirteen wheat lines carrying a wheat glutathione-S-transferase (GST) transgene, ten wheat lines carrying a wheat lipid transfer protein transgene and three wheat lines carrying a rice pathogenesis-related 1 (PR-1) transgene were developed.
6. A construct carrying the barley germin-like gene GLP1 was developed and is currently being used in wheat transformation experiments.

Impact: **(Accomplishment 1)** We developed a set of eight transgenic wheat lines that exhibit statistically significant reductions in FHB severity under field conditions. One of these lines was equivalent to Alsen. These lines are available for breeding applications and further testing.
(Accomplishment 2) We identified a set of five transgenic wheat lines that performed well in the greenhouse. These lines will be further tested in the greenhouse and field.
(Accomplishment 3) We added proven transgenes to the Alsen background. These lines exhibited low levels of FHB severity and may enhance Alsen resistance to FHB.
(Accomplishment 4) We will have the results from the field test in August 2006.
(Accomplishment 5 and 6) We developed additional transgenic lines for FHB testing and a construct that will transformed into wheat.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?: The scientific community now has access to transgenic wheat lines that exhibit reduced FHB severity. These lines provide another source of variation for FHB resistance. In addition, the scientific community now has new information on the functional impact of a variety of genes on FHB resistance.

Project 2: *Mechanisms and Essential Genes for Resistance to Fusarium 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 the small grains wheat and barley. The wheat and barley transformation efforts have a limited number of genes that have the potential to reduce FHB. In addition, there is a limited amount of information pertaining to the molecular genetic interaction between the small grains and *Fusarium graminearum*. Our goal is to identify the mechanisms and essential genes for wheat and barley scab resistance.

2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):

Accomplishments:

1. Described for the first time the expression patterns of 22,000 barley genes during infection by *F. graminearum*. We identified 582 genes that were expressed due to *F. graminearum* infection. Genes encoding tryptophan biosynthetic and catabolic enzymes were identified as well as potential trichothecene transporters and catabolic enzymes. Three stages of infection were defined. All data are publicly available at BarleyBase (http://www.barleybase.org/).
2. Described for the first time the expression patterns of all *F. graminearum* genes during *in planta* infection of barley. Identified 421 *F. graminearum* genes that were specific to *in planta* infection. All data are publicly available at PLEXdb (http://www.plexdb.org/).
3. We are describing the impact of trichothecene mycotoxins on the expression patterns of 22,000 barley genes and 14,000 *Fusarium* genes during the barley-*F. graminearum* interaction. Identified potential barley and *Fusarium* genes that respond to trichothecene accumulation.
4. We are describing the gene expression patterns of 22,000 barley genes in three near-isogenic line pairs carrying resistant and susceptible alleles for two QTL on chromosome 2H and a QTL on chromosome 3H for Fusarium head blight resistance. From the expression data, two new markers were identified linked to the chromosome 3H QTL. Additional mapping is in progress.
5. We are describing the gene expression patterns of 61,000 wheat genes in a near-isogenic line pair carrying resistant and susceptible alleles for the chromosome 3BS FHB resistant QTL.

Impact: **(Accomplishments 1 and 2)** The barley and *Fusarium* GeneChip data that we have generated and placed on the web are providing a rich resource of information for Fusarium workers worldwide. The results from our experiments are also providing a resource of genes that are involved in barley and *Fusarium* during the barley-*Fusarium* interaction.

(Accomplishment 3) Identified barley and *Fusarium* genes that respond to trichothecene accumulation. The barley genes may be involved in protecting the plant from trichothecene accumulation. Data will soon be released on the web.
(Accomplishments 4 and 5) We are identifying genes that differentiate the resistant from susceptible interactions, and may be involved in providing resistance, for important QTL in barley and wheat. All data will soon be released on the web. We used the data to map two new markers in the barley chromosome 3H QTL region.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?: The scientific community now has access to an unprecedented amount of expression data that defines the barley and wheat-*F. graminearum* interactions. These gene expression data will be used to test the functional significance of particular genes in transgenic plants and in the viral induced gene silencing system (collaboration with S. Scofield), and to develop molecular markers for breeding applications.

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:
Boddu, J., S. Cho, W.M. Kruger and G.J. Muehlbauer. 2006. Transcriptome analysis of the barley-*Fusarium graminearum* interaction. *Mol. Plant-Microbe Interact.* 19:407-417.

Güldener, U., K. Seong, J. Boddu, S. Cho, F. Trail, J-R. Xu, G.J. Muehlbauer and H.C. Kistler. 2006. Development of a *Fusarium graminearum* Affymetrix GeneChip for profiling fungal gene expression *in vitro* and *in planta*. *Fungal Genetics and Biology* 43:316-325.

Mackintosh, C.A., D.F. Garvin, L.E. Radmer, S.J. Heinen and G.J. Muehlbauer. 2006. A model wheat cultivar for transformation to improve resistance to Fusarium Head Blight. *Plant Cell Reports* 25:313-319.

Mackintosh, C.A., J. Lewis, L.E. Radmer, S. Shin, S.J. Heinen, L.A. Smith, M.N. Wyckoff, R. Dill-Macky, C.K. Evans, S. Kravchenko, G.D. Baldridge, R.J. Zeyen and G.J. Muehlbauer. Overexpression of defense response genes in transgenic wheat enhances resistance to Fusarium Head Blight. Submitted to Plant Cell Reports (7-06).Cuomo, C.A., U. Güldener, J-R. Xu, F. Trail, B.G. Turgeon, S. Calvo, D. DeCaprio, S. Gnerre, L-J. Ma, E. Mauceli, G. Muehlbauer, Y-L. Chang, K. Hilburn, R. Goswami, K-Y. Seong, L. Gale, A. DiPietro, J. Walton, J. Kennell, M. Rep, S.E. Baker, J.K. Magnuson, K. Hammond-Kosack, L.J. Harris, T. Ouellette, M.I.G. Roncero, S. Kroken, D. Nelson, T. Ward, K. O'Donnell, C. Waalwijk, G. Adam, R. Mitterbauer, G. Mannhaupt, M. Münsterkötter, I. Tetko, V. Stümpflen, M. Oesterheld, H-W. Mewes, B.W. Birren and H.C. Kistler. Genome sequence of cereal pathogen reveals adaptation to host–pathogen arms race. In preparation for submission to Science.

Nduulu, L.M., A. Mesfin, G.J. Muehlbauer and K.P. Smith. Fusarium Head Blight Resistance and Heading Date in Barley are controlled by tightly linked but separate loci. In preparation for submission to Theoretical and Applied Genetics.

Boddu, J., S. Cho, H.C. Kistler and G.J. Muehlbauer. Transcriptome analysis of trichothecene-induced gene expression in barley. In preparation for submission to Molecular Plant-Microbe Interactions.

Proceedings:
Nduulu, L., G.J. Muehlbauer and K.P. Smith. 2005. Fine mapping of a QTL region associated with Fusarium head blight, kernel discoloration, protein concentration and heading date on barley chromosome 6H. U.S. Wheat and Barley Scab Initiative Forum Proceedings, p. 75-78.

Abstracts:
Boddu, J., S. Cho, H.C. Kistler and G.J. Muehlbauer. 2006. Trichothecene-influenced gene expression during the barley-*F. graminearum* interaction. Plant and Animal Genome Abstracts p. 282.

Boddu, J., S. Cho, H.C. Kistler and G.J. Muehlbauer. 2005. Gene profiling studies in trichothecene-influenced barley-*Fusarium graminearum* interaction. U.S. Wheat and Barley Scab Initiative Forum Abstracts p. 17.

Lewis, J.N., C.A. Mackintosh, S.H. Shin, L.A. Smith, M.N. Wyckoff, A. Elakkad, K. Wennberg, S.J. Heinen, L.E. Radmer, G.D. Baldridge, R.J. Zeyen, C.K. Evans, S. Kravchenko, R. Dill-Macky and G. J. Muehlbauer. 2005. Overexpression of antifungal proteins inceases resistance of wheat to Fusarium head blight. U.S. Wheat and Barley Scab Initiative Forum Abstracts p. 103.

Bilgic, H., S. Cho, L. Nduulu, K. Smith and G.J. Muehlbauer. 2006. Microarray analysis of gene expression in barley during *Fusarium graminearum* infection. Society of In Vitro Biology Meeting Abstracts p. 42-A.