USDA-ARS / USWBSI
FY03 Final Performance Report (approx. May 03 – April 04)
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Cover Page

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Year:	**FY2003 (approx. May 03 – April 04)**
FY03 ARS Agreement ID:	**59-0790-9-055**
FY03 ARS Agreement Title:	**Molecular genetic approaches to develop scab resistance.**
FY03 ARS Award Amount:	**$ 146,341**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Mechanisms and essential genes for resistance to Fusarium head blight.	$ 78,048
BIO	Developing and characterizing transgenic wheat for scab resistance.	$ 68,293
	Total Amount Recommended	**$ 146,341**

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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 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 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 Affymetrix GeneChip, which is represented by 22,786 genes and therefore is an excellent resource for examining gene expression. We sampled four replications of spikes from the barley cultivar Morex at 1, 2, 3, 4, and 6 days after *F. graminearum* and water inoculation. A fifth replication at 1 and 3 days after *F. graminearum* and water inoculation was also conducted. We examined RNA profiles from these timepoints during infection. We identified approximately 15,000 genes that were expressed at least at one time point during the experiment. Genes that were statistically significantly induced or repressed ($P<0.001$; 2-fold difference) upon *F. graminearum* infection were identified based on a comparison to the water controls. Based on these criteria, we identified 582 genes that were significantly induced or repressed at least in one time point of the experiment. Only four genes were identified as significantly repressed and this was at 6 days after inoculation (dai). There were no genes significantly induced or repressed that were identified at the 1 dai time point. A cluster analyses of the 578 induced genes was conducted and 296 genes were found to exhibit quantitative differences in gene expression between infected (high expression) and water controls (expressed but lower than the infected plants). The other 282 genes exhibited qualitative differences in gene expression between the water controls (no expression) and the infected (induced expression) plants. To validate these transcript accumulation patterns from the GeneChip, we performed RNA gel blot analysis on 25 differentially expressed genes. Transcript accumulation data via RNA gel blot analysis were consistent with the GeneChip data.

Using the gene expression profiles, we made many interesting observations and/or interpretations including: (1) We identified genes that are induced in a qualitative and quantitative fashion due to infection. (2) We identified four predominant transcript accumulation patterns during infection including: induced at 2, 3, 4, and 6 dai; induced at 3, 4 and 6 dai; induced at 4 and 6 dai; and induced only at 6 dai. (3) Based on the four transcript accumulation patterns, we propose that there are four stages (non pathogenic, and early, middle and late) of *F. graminearum* infection on barley. These stages provide the theoretical basis for a better understanding of the plant response to infection. (4) We identified genes that are specifically expressed in each of these stages and possibly represent important physiological changes in the host during infection. (5) We identified six up regulated genes in the tryptophan biosynthetic pathway. This observation demonstrates a specific biochemical host response to infection. (6) We identified transcript accumulation in 13 fungal genes at 3, 4 and 6 dai. The identification of fungal gene expression on the Barley1 GeneChip is likely due to the fact that some of the barley ESTs used for the chip design were derived from cDNA libraries prepared from barley infected with *F. graminearum* and *Blumeria graminis*.

GeneChip experiments with two barley near-isogenic line (NIL) pairs (chromosome 3H and 2H) and one wheat NIL pair (chromosome 3BS) carrying QTL for FHB resistance have been initiated.

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 tested 25, 25, 31, 17, 8, 4, 11, 5 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 and lipid transfer protein (LTP) transgenes, respectively.

As described in our progress 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 screens. These transgenic lines are being screened in the field at Crookston, MN in the summer of 2004.

We conducted at least three screens with 17 chitinase, 8 RIP, 4 chitinase/RIP, 11 chitinase/tlp-1 and 5 RIP/tlp-1 transgenic wheat lines. 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 scab severity compared to the non transgenic controls. The transgenic lines exhibiting a significant reduction in disease exhibited between 6-45% scab severity. Three chitinase lines exhibited as low as 6-8% scab severity. Our non-transformed Bobwhite controls averaged between 50-75% scab severity. Western blot analyses of the transgenic lines are ongoing. Our results indicate that the transgenice lines are over expressing the appropriate protein.

We also developed transgenic wheat with combinations of transgenes through genetic crossing. We crossed our tlp-1 lines with our ß-1,3-glucanase lines, and we crossed a line carrying the *F. sporotrichoides Tri101* gene (gift from Dr. Ann Blechl, USDA-ARS, Albany, CA) to our wheat lines carrying tlp-1 and ß-1,3-glucanase. The tlp1/ß-1,3-glucanase lines have been screened twice and one line has exhibited statistically significant reduction in scab severity in both screens. The *Tri101*/tlp-1 and *Tri101*/ß-1,3-glucanase lines will be examined for gene expression and scab resistance in the fall of 2004.

To assess the *in vitro* inhibition of our AFPs, we expressed RIP, ß-1,3-glucanase, tlp-1, α-thionin and chitinase in an *E. coli* expression vector. All five AFPs significantly reduce the growth of the fungus in our *in vitro* assays.

We obtained two lines carrying the rice Nh1 gene (homolog to the Arabidopsis *NPR1* gene) from Dr. Heidi Kaeppler (University of Wisconsin, Madison, WI). We screened these lines twice in the greenhouse and the molecular analysis of the second screen is ongoing.

We developed a plant transformation construct carrying the wheat glutathione-S-transferase gene. We are working on developing new constructs for the Arabidopsis *NPR1* and rice *Nh1* gene.

We developed a transformation system for the early flowering, FHB susceptible wheat variety Apogee. Apogee will serve as a model wheat cultivar for a variety of genetic studies and rapid *in planta* analysis of transgenes.

We developed 14 and one transgenic barley lines carrying the α-thionin and RIP genes, respectively. These lines are being advanced and will be screened for gene expression and 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 you grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Publications:

Canci, P.C., L.M. Nduulu, R. Dill-Macky, G.J. Muehlbauer, D.C. Rasmusson and K.P. Smith. 2003. The genetic relationship between kernel discoloration resistance and grain protein in barley. *Crop Sci*. 43:1671-1679.

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*. (In press)

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*. (In press)

Manuscripts in preparation:

Kruger, W.M., S. Cho and G.J. Muehlbauer. Microarray analysis of barley genes expressed in response to infection by *Fusarium graminearum.* To be submitted to *Plant Physiology.*

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.*

Abstracts:

Kruger, W., Cho, S. and G.J. Muehlbauer. 2003. Identification of genes upregulated in barley in response to inoculation with *Fusarium graminearum*. National Fusarium head blight forum abstracts, p. 19.

Mackintosh, C.A., D.F. Garvin, L.E. Radmer, S.L. Jutila, A.C. Cyrus, J.E. Mason and G.J. Muehlbauer. 2003. A model cultivar for transformation of wheat to improve resistance to Fusarium head blight. National Fusarium head blight forum abstracts, p. 25.

Mackintosh, C.A., L.E. Radmer, S.L. Jutila, A.C. Cyrus, G.D. Baldridge, R.J. Zeyen and G.J. Muehlbauer. 2003. A transgenic approach to enhancing the resistance of wheat to Fusarium head blight. National Fusarium head blight forum abstracts, p. 26.

Mackintosh, C.A., L.E. Radmer, S.L. Jutila, A.C. Cyrus, L.A. Smith, M.N. Wyckoff, S.J. Heinen, G.D. Baldridge, R.J. Zeyen and G.J. Muehlbauer. 2003. Over-expression of antifungal proteins increases the resistance of wheat to Fusarium head blight. National Fusarium head blight forum abstracts, p. 27.

Nduulu, L.M., A. Mesfin, G.J. Muehlbauer and K.P. Smith. 2003. Fine mapping of Fusarium head blight resistance and heading date QTL in barley. National Fusarium head blight forum abstracts, p. 30.

Kruger, W. and G.J. Muehlbauer. 2004. RNA profiling of barley inoculated with *Fusarium graminearum*. Plant and Animal Genome Abstracts, p. 270.

Baluch, S.D., G.J. Muehlbauer, K.P. Smith, D.A. Somers and B.J. Steffenson. 2004. Fine mapping the *VRS1* region of chromosome 2(2H) of barley and analyzing a set of NILs for the *VRS1* region for FHB severity. International Triticeae Mapping Initiative Workshop Abstracts.