U.S. Wheat and Barley Scab Initiative
FY02 Final Performance Report (approx. May 02 – April 03)
July 15, 2003

Cover Page

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Year:	**FY2002 (approx. May 02 – April 03)**
Grant Number:	**59-0790-9-055**
Grant Title:	**Fusarium Head Blight Research**
FY02 ARS Award Amount:	**$ 115,122**

Project

Program Area	Project Title	USWBSI Recommended Amount
BIO	Enhancement of scab resistance in wheat and barley by plant transformation.	$60,000
BIO	Mechanisms and essential genes for wheat and barley scab resistance.	$58,000
	Total Amount Recommended	**$118,000**

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Principal Investigator Date

Project 1: Enhancement of scab resistance in wheat and barley by plant transformation.

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 and 5 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 and RIP/tlp transgenes, respectively.

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 range from 30-45% scab severity, whereas our nontransformed Bobwhite controls range from 50-75% scab severity. We examined protein accumulation in the transgenic tlp-1 and ß-1,3-glucanase lines and observed significant increases in protein accumulation over the nontransformed controls. These transgenic lines are ready for more extensive field screening and incorporation into breeding material.

In one screen where 15 chitinase, 4 RIP, 3 chitinase/RIP and 6 chitinase/tlp-1 transgenic wheat lines were tested, six, one, one and one lines carrying expressed chitinase, RIP, chitinase/RIP and chitinase/tlp-1 transgenes, respectively, exhibited a significant reduction of 40% or less scab severity. Our non-transformed Bobwhite controls averaged between 50-75% scab severity. A further screen is ongoing, restesting these plants as well as initially testing 2 chitinase, 4 RIP, 1 chitinase/RIP, 5 chitinase/tlp-1, and five RIP/tlp-1 lines.

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. We obtained transgenic wheat carrying the *Tri101* gene from Dr. Ann Blechl (USDA-ARS, Albany, CA) and crossed this line with our wheat lines carrying tlp-1 and ß-1,3-glucanase. The tlp1/ ß-1,3-glucanase lines have been screened once and the data is currently being analyzed. The Tri101/tlp-1 and Tri101/ ß-1,3-glucanase transgenic plants will be examined for gene expression and scab resistance in the fall of 2003.

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. We showed that RIP inhibits the *in vitro* growth of *F. graminearum*. Assays with the other proteins are in progress.

We obtained two lines carrying the rice Nh1 gene (homolog to the Arabidopsis NPR1 gene) from Dr. Heidi Kaeppler (University of Wisconsin, Madison, WI). Currently, these lines are being screened in the greenhouse. Reconstruction of plant transformation plasmids carrying the NPR1 and Nh1 genes is in progress. These plasmids will provide increased expression and a method for more efficient detection of expression.

We are developing transgenic barley carrying the α-thionin and RIP genes. Multiple GUS staining plants are being regenerated. These plants will be advanced and screened for gene expression and FHB resistance.

We finished our work with the Sugarcane Bacilliform Badnavirus promoter (Al-Saady et al., submitted 5/03 to Molecular Breeding). We showed that this promoter functions in all tissues in wheat and barley. However, the level of expression in barley appears to be higher than wheat. Therefore, this promoter will be useful for developing transgenic barley.

Project 2: Mechanisms and essential genes for wheat and barley 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. 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?

We developed a large set of expressed sequence tags (ESTs) from barley and wheat spikes infected with *F. graminearum (Fg)*. Databases have been established with these ESTs and they provide the foundation resource for investigating the genes involved in resistance and for our future transformation efforts.

To initiate an understanding of the mechanisms and essential genes involved in resistance, we conducted a computerized comparison of the genes expressed in common between the wheat and barley libraries prepared from *Fg* -infected spikes. In addition, we compared our barley ESTs to approximately 15,000 ESTs from three barley cDNA libraries prepared from barley leaves inoculated with *Blumeria graminis* (*Bgh*; causal agent of powdery mildew). Using this strategy, we identified six sets of genes including: (i) stress-related genes involved in general responses to pathogen infection; (ii) genes expressed in barley infected with *Bgh* and *Fg*; (iii) genes expressed in both compatible and incompatible *Bgh*-barley interactions; (iv) genes expressed in barley and wheat infected with *Fg*; (v) genes selectively expressed in plant-microbe interactions; and (vi) *Fg* and *Bgh* genes that may be required for pathogenicity (Kruger et al., submitted 6/03 to Plant Physiology). These data are setting the groundwork for future studies on the mechanisms or FHB resistance.

To identify mechanisms and genes that are involved in resistance, we have established large-scale RNA profiling in my laboratory. We are using the barley Affymetrix GeneChip, which is represented by 22,786 genes and therefore is an excellent resource for examining gene expression. We sampled four replications of Morex spikes at 1, 2, 3, 4, 6, 8, and 10 days after *Fg* and water inoculation. We isolated RNA from spike tissue and hybridized the GeneChip with RNA from 1 and 3 days after *Fg* and water inoculation. At 1 day after inoculation, there were few genes that exhibited differential expression in *Fg* inoculated plants compared to the water control plants. However, at three days after *Fg* inoculation, we identified 150 genes that were significantly up regulated 2-fold or more compared to the water controls. We also identified 10 genes that were significantly down regulated at least 2-fold or more compared to the water controls. Many of the up regulated genes are the classic pathogenesis-related genes but there are a large number of unknown genes and genes that are of regulatory and/or physiological importance during plant-pathogen interactions. These genes will be studied further and potential resistance genes will be incorporated into our transformation efforts

We also developed and utilized wheat cDNA macroarray technology using approximately 350 genes. We generated material from Sumai3 and Wheaton wheat spikes inoculated with a tricothecene-producing *Fg* strain (tox+), a tricothecene non-producing *Fg* strain (tox-), deoxynivalenol (DON) or water. We identified large sets of genes induced and repressed in all treatments and observed several interesting trends, including: early gene induction in tox+ inoculated plants compared to the tox- and DON treatments; and earlier repression of gene expression in Wheaton versus Sumai3. To examine the expression of more genes in one experiment we have chosen to conduct all future RNA profiling experiments with the Affymetrix GeneChip. We know that approximately 50% of the genes on the barley GeneChip will work with wheat (Wise et al., submitted 6/03 to Plant Physiology).

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:

Kruger, W.M., C. Pritsch. S. Chao and G.J. Muehlbauer. 2002. Functional and comparative bioinformatic analysis of expressed genes from wheat spikes infected with *Fusarium graminearum*. *Mol. Plant-Microbe Interact*. 15:445-455.

Mesfin, A., K.P. Smith, R. Dill-Macky, C.K. Evans, R. Waugh, C.D. Gustus and G.J. Muehlbauer. 2003. Quantitative trait loci for Fusarium head blight resistance in barley in a two-rowed by six-rowed population. *Crop Sci.* 43:307-318.

Canci, P.C., L. Nduulu, R. Dill-Macky, G.J. Muehlbauer, D.C. Rasmusson and K.P. Smith. The genetic relationship between kernel discoloration resistance and grain protein in barley. *Crop Sci*. (In press).

Manuscripts submitted:

Canci, P.C., L.M. Nduulu, G.J. Muehlbauer, R. Dill-Macky, D.C. Rasmusson and K.P. Smith. Validation of quantitative trait loci for fusarium head blight and kernel discoloration resistance in barley. Submitted to Molecular Breeding (4/03).

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. Tissue specificity of the sugarcane bacilliform virus promoter in oat, barley and wheat. Submitted to Molecular Breeding (5/03).

Kruger, W.M., R.P. Wise, T.A. Close, D-W. Choi, Y. Yu, R.A. Wing and G.J. Muehlbauer. *In silico* analysis of genes expressed in a broad spectrum of Triticeae-pathogen interactions. Submitted to Plant Physiology (6/03).

Book Chapter:

Muehlbauer, G.J. and Wm. R. Bushnell. Transgenic Approaches to Resistance. *In:* "Fusarium Head Blight of Wheat and Barley", K.J. Leonard and W.R. Bushnell (ed.) American Phytopathological Society Press, St. Paul, MN.

Meeting presentations:

Kruger, W.M. and G.J. Muehlbauer. 2002. Using EST databases for functional and comparative bioinformatics. Tri-Annual North American Barley Researchers' Workshop Abstracts.

Nduulu, L.M., A. Mesfin, G.J. Muehlbauer and K.P. Smith. 2002. Evaluation of near-isogenic lines for Fusarium Head Blight resistance QTL in barley. Tri-Annual North American Barley Researchers' Workshop Abstracts.

Mackintosh, C.A, S.J. Heinen, M.N. Wyckoff, L.A. Smith, G.D. Baldridge, R.J. Zeyen and G.J. Muehlbauer. 2002. Transgenic approaches to improve the resistance of wheat to Fusarium head blight. U.S. Wheat and Barley Scab Forum Abstracts.

Nduulu, L.M., A. Mesfin, G.J. Muehlbauer and K.P. Smith. 2002. Effect of Chevron alleles at two Fusarium head blight resistance QTL determined using near-isogenic lines. U.S. Wheat and Barley Scab Forum Abstracts.

Kruger, W.M. and G.J. Muehlbauer. 2003. Identification of genes specifically expressed in plant-microbe interactions. Plant and Animal Genome Abstracts, p. 77.

Mackintosh, C.A., S.J, Heinen, L.A. Smith, M.N. Wyckoff, R.J. Zeyen, G.D. Baldridge and G.J. Muehlbauer. 2003. Overexpression of anti-fungal protein genes increases resistance of transgenic wheat to Fusarium head blight. Plant and Animal Genome Abstracts, p. 257.

Muehlbauer, G.J. and W.M. Kruger. 2003. Using EST databases to examine barley-pathogen interactions. Plant and Animal Genome Abstracts, p. 15.

Invited presentation:

"Molecular genetics of Triticeae-Fusarium interactions" at Scottish Crop Research Institute, Invergowrie, Scotland