

**U.S. Wheat and Barley Scab Initiative  
 FY01 Final Performance Report (approx. May 01 – April 02)  
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**Cover Page**

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<b>Grant Title:</b>	<b>Fusarium Head Blight Research</b>
<b>FY01 ARS Award Amount:</b>	<b>\$ 97,040</b>

**Project**

<b>Program Area</b>	<b>Project Title</b>	<b>Requested Amount</b>
Biotech	Enhancement of Scab Resistance in Wheat and Barley by Plant Transformation	\$ 60,000
Biotech	Mechanisms and essential genes for wheat and barley scab resistance.	\$ 96,043
	<b>Total Amount Requested</b>	<b>\$ 156,043</b>

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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 genes. Transgenic plants carrying antifungal protein genes will be tested for scab resistance.

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

We developed 25, 25, 31, 24 and 15 transgenic wheat lines carrying expressed wheat  $\alpha$ -thionin, barley thaumatin-like protein 1 (tlp-1), barley  $\beta$ -1,3-glucanase, barley ribosome-inactivating protein (RIP), and barley chitinase transgenes, respectively. In addition, we developed 10, 11 and 11 transgenic wheat lines expressing a combination of chitinase/RIP, chitinase/tlp-1, and chitinase/tlp-1, respectively. Therefore, we have developed transgenic wheat carrying all antifungal protein genes currently in my lab. In addition, we have developed transgenic plants with combinations of antifungal protein genes that exhibit different potential mechanisms of resistance.

We have conducted FHB screens on the transgenic lines expressing the  $\beta$ -1,3-glucanase, tlp-1 and  $\alpha$ -thionin transgenes. In general, the non-transgenic controls (Bobwhite and nontransformed siblings of the transgenic events) exhibited between 70-90% FHB severity. We identified three lines expressing the  $\beta$ -1,3-glucanase that exhibited a statistically significant reduction in FHB severity compared to the non-transgenic controls in three screens. In addition, we identified one line carrying  $\alpha$ -thionin and two lines carrying tlp-1 that exhibited a statistically significant reduction in FHB severity compared to the non-transgenic controls in three out of the four screens conducted. In general, the transgenic lines that exhibit enhanced resistance range from 25-45% FHB severity.

We have developed polyclonal antibodies to the barley tlp-1, barley  $\beta$ -1,3-glucanase, barley RIP and barley chitinase proteins. The transgenic lines exhibiting reduced FHB severity that expressed the tlp-1 and  $\beta$ -1,3-glucanase transgenes were examined for protein accumulation using Western blot analysis. Our preliminary data indicates that the lines that exhibited a reduction in FHB severity accumulated the corresponding proteins to high levels.

Our results indicate that we have developed transgenic wheat that exhibit reduced FHB severity. Currently, we are increasing the seed for our promising lines and we will evaluate them in the fall of 2002 and in the field in the summer of 2003. In addition, we plan to evaluate transgenic wheat carrying the expressed RIP and chitinase transgenes as well as the lines carrying combinations of expressed transgenes in the fall and spring of 2002.

To identify additional promoters that can be used for wheat and barley transformation, we developed transgenic wheat and barley carrying the sugarcane badnavirus (ScBV) promoter fused to the  $\beta$ -glucuronidase (GUS) gene. The ScBV promoter expresses the GUS gene in all tissues of wheat and barley. The expression level appears to be greater in barley than wheat. Our data indicate that the ScBV promoter will be useful for driving AFP gene expression in 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 cDNA library from Sumai 3 wheat spikes spray-inoculated with *F. graminearum*. We funded Dr. Olin Anderson's laboratory (USDA-ARS, Albany, CA) from FY 2001 USWBSI funding to sequence 10,000 ESTs from this library. To date, approximately 10,000 ESTs have been generated by Dr. Anderson's laboratory (<http://wheat.pw.usda.gov/NSF/>). From the initial 5,000 ESTs, we identified 3,552 unigenes and gave a functional annotation to each unigene based on sequence similarity to proteins using the BLASTX algorithm. We identified four sets of genes including: (1) biotic and abiotic stress response genes; (2) *F. graminearum* genes that are potentially pathogenesis specific; (3) library specific genes; and (4) genes that may be specifically expressed in resistant plant-pathogen interactions (Kruger et al 2002). To conduct the barley EST work, we funded Dr. Rod Wing at Clemson University to sequence 5,000 ESTs from a cDNA library prepared from the barley cv. Morex spikes spray inoculated with *F. graminearum*. We annotated these ESTs using the BLASTX algorithm. Both the wheat and barley ESTs have been deposited in genbank. The wheat and barley EST databases are the 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 *F. graminearum*-infected spikes. In addition, we compared our barley ESTs to 15,000 ESTs from three barley cDNA libraries prepared from barley leaves inoculated with *Blumeria graminis* (causal agent of powdery mildew). In these comparisons, we are identifying genes that are expressed in common in the same plant infected by two different pathogens and in the same plant infected with different pathogens. Interestingly, some of these genes that are found to be in common in these comparisons are not found in over 2 million plant ESTs deposited in genbank, indicating that we have identified rarely-expressed genes involved in plant-pathogen interactions that may play a role in resistance. Currently, we are examining the expression of this select group of genes in wheat infected with *F. graminearum*.

To identify mechanisms and genes that are involved in resistance we are establishing cDNA macroarray (RNA profiling) analysis in my laboratory. We have PCR amplified approximately 80% of the 3,552 wheat unigenes. Currently, we are amplifying the remaining clones and will begin to spot them on nylon membranes in the near future. We plan to study the expression of all 3,552 genes during *F. graminearum* infection of wheat. In the short term, we have developed a small macroarray of 300 cDNA clones that encode defense response genes. We have sampled Sumai3 and wheaton wheat spikes from three experiments. The spikes in these experiments were inoculated with a tricothecene-producing strain of *F. graminearum*, a non-tricothecene producing strain, deoxynivalenol or water. We have just initiated hybridizations of these membranes with cDNA synthesized from RNA isolated from the sampled plants.

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:**

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

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.

Muehlbauer, G.J. and D.A. Somers. 2001. Genetically modified barley and the brewing industry. *Technical Quarterly, Master Brewers Association of the Americas* 38:145-154.

Yu, G-Y, and G.J. Muehlbauer. 2001. Benzothiadiazole-induced gene expression in wheat spikes does not provide resistance to *Fusarium* head blight. *Physiol. Mol. Plant Path.* 59:129-136.

Kolb, F.L., G-H. Bai, G.J. Muehlbauer, J.A. Anderson, K.P. Smith and G. Fedak. 2001. Host plant resistance genes for *Fusarium* head blight: mapping and manipulation with molecular markers. *Crop Sci.* 41:611-619.

Pritsch, C., C.P. Vance, W.R. Bushnell, D.A. Somers, T.M. Hohn and G.J. Muehlbauer. 2001. Systemic expression of defense response genes in wheat spikes as a response to *Fusarium graminearum* infection. *Physiol. Mol. Plant Path.* 58:1-12.

Pritsch, C., G.J. Muehlbauer, W.R. Bushnell, D.A. Somers and C.P. Vance. 2000. Fungal development and induction of defense response genes during early infection of wheat spikes by *Fusarium graminearum*. *Mol. Plant-Microbe Interact.* 13:159-169.

de la Peña, R.C., K. Smith, F. Capettini, G.J. Muehlbauer, M. Gallo-Meagher, R. Dill-Macky, D.A. Somers and D.C. Rasmusson. 1999. Quantitative trait loci associated with resistance to *Fusarium* head blight and kernel discoloration in barley. *Theor. Appl. Genet.* 99:561-569.

**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 Wm. R. Bushnell eds. American Phytopathological Society Press, St. Paul, MN. (In press)

**Conference proceedings:**

Muehlbauer, G.J., L. Smith, N. Al-Saady, G. Baldrige, and R. Zeyen. 2001. Barley transformation. *33<sup>rd</sup> Barley Improvement Conference Proceedings*. San Antonio, TX, p. 21-27.

**Abstracts:**

Mackintosh, C.A., M.N. Wyckoff, L.A. Smith, R.J. Zeyen, G. Baldrige and G.J. Muehlbauer. 2002. Transgenic approaches to improve the resistance of wheat to *Fusarium* head blight. *10<sup>th</sup> IAPTC&B Congress Abstracts (In press)*

Muehlbauer, G.J. 2002. Molecular genetic interactions between wheat and *Fusarium graminearum*. *Plant, Animal and Microbe Genome Abstracts*, p. 91.

Kruger, W.M., C. Pritsch, S. Chao, T.J. Close, R. Wing and G.J. Muehlbauer. 2002. Functional and comparative bioinformatic analysis of expressed genes from wheat and barley infected with *Fusarium graminearum*. *Plant, Animal and Microbe Genome Abstracts*, p. 91.

Muehlbauer, G.J. 2001. Using molecular genetics to enhance wheat and barley scab resistance. *U.S. Wheat and Barley Scab Forum Abstracts*, p. 22.

Kruger, W.M., C. Pritsch and G.J. Muehlbauer. 2001. Characterization of expressed sequence tags from *Fusarium graminearum*-infected wheat. *International Molecular Plant Microbe Interactions Meeting Abstracts*, poster #253.

Mesfin, A., P. Canci, R. Dill-Macky, K. Smith and G.J. Muehlbauer. 2001. Barley chromosome 2: Does it carry *Fusarium* head blight resistance? *Plant and Animal Genome Abstracts* p 165.

Kruger, W., C. Pritsch, R. Skaggs and G.J. Muehlbauer. 2001. Characterization of wheat expressed sequence tags from *Fusarium graminearum* infected Sumai3. *Plant and Animal Genome Abstracts* p. 70.

Mesfin, A., G.J. Muehlbauer, D.C. Rasmusson, R. Dill-Macky, T. Walsh, C.D. Gustus and K.P. Smith. 2000. Identification of QTLs for scab resistance in barley. *U.S. Wheat and Barley National Scab Forum* p. 38.

Wyckoff, M., L. Smith, G. Baldrige, R. Zeyen and G.J. Muehlbauer. 2000. Genetic engineering wheat for scab resistance. *U.S. Wheat and Barley National Scab Forum* p. 61.

Canci, P.C., K.P. Smith, R. Dill-Macky, G.J. Muehlbauer and D.C. Rasmusson. 2000. Validation of *Fusarium* head blight and kernel discoloration QTLs in barley. *Agronomy Abstracts* p. 105.

Mesfin, A., G.J. Muehlbauer, D.C. Rasmusson, R. Dill-Macky, T. Walsh, C.D. Gustus, K.P. Smith. 2000. Identification of QTLs for scab resistance in barley. *Agronomy Abstracts* p. 185.

Smith, L., M. Wyckoff, G. Baldrige, R. Zeyen and G.J. Muehlbauer. 2000. Antifungal protein gene expression in transgenic wheat. *Agronomy Abstracts* p. 186.

Alexander, N.J., S.L. Ziegenhorn, G.J. Muehlbauer, S.P. McCormick and B. Kurtz. 1999. Isolation of tricothecene resistant genes from the wheat cultivar Frontana. *National Scab Forum Abstracts*, p. 18.

Yu, G-Y. and G. J. Muehlbauer. 1999. BTH-induced gene expression in wheat spikes does not provide resistance to scab. *National Scab Forum Abstracts*, p. 44.

Muehlbauer, G.J., C. Pritsch, D. Somers, W.R. Bushnell, T. Hohn and C. Vance. 1999. Wheat spike-*Fusarium graminearum* molecular interactions. *National Scab Forum Abstracts*, p. 28.

Kolb, F.L., G-H. Bai, G.J. Muehlbauer, J.A. Anderson, K.P. Smith and G. Fedak. 1999. Host plant resistance genes for *Fusarium* head blight: mapping and manipulation with molecular markers. *Agronomy Abstracts* p. 83.

Muehlbauer, G.J. and L. Smith. 1999. Developing transgenic barley carrying antifungal protein genes. *Barley Workers Meeting Abstracts*, p. 50.

#### **Invited Talks:**

“Molecular genetic interactions between wheat and *Fusarium graminearum*”. 2002 Plant, Animal and Microbe Genome Meeting, San Diego, CA.

“Using molecular genetics to enhance wheat and barley scab resistance”. 2001 U.S. Wheat and Barley Scab Forum, Cincinnati, OH.

“Genetic engineering for improved wheat scab resistance”. 2001 University of Minnesota Extension Service Crop Pest Management Short Course, St. Paul Campus

“Biotechnology in wheat and barley, research and development”. 2001 Wheat Growers Convention-Prairie Grains Conference, Fargo, ND

“Barley Transformation”. 2001 Barley Improvement Conference, San Antonio, TX.

“Using molecular genetics to enhance scab resistance in wheat”. 2001 Hard Red Winter Wheat Meeting, Kansas City, MO.