U.S. Wheat and Barley Scab Initiative
FY00 Final Performance Report (approx. May 00 – April 01)
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Cover Page

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Grant Number:	**59-0790-9-055**
Grant Title:	**Fusarium Head Blight Research**
2000 ARS Award Amount:	**$87,805**

Project

Program Area	Project Title	Requested Amount
Biotechnology	Enhance scab resistance in wheat and barley by plant transformation.	$60,000.00
Biotechnology	Mapping scab QTL in barley and development of markers for germplasm enhancement.	$40,000.00
	Requested Total	$100,000.00[1]

Principal Investigator Date

[1] Note: The Requested Total and the Award Amount are not equal.

Project 1: Enhance 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 scab resistance, we are developing transgenic wheat and barley carrying antifungal protein genes. In addition, we are testing the sugarcane badnavirus (ScBV) promoter for future wheat and barley transformation.

2. What were the most significant accomplishments?

Transgenic wheat carrying overexpressed wheat α-thionin, barley PR-5, barley ß-1,3-glucanase, and barley Type I ribosome-inactivating protein (RIP) genes were developed and characterized. Efforts to develop transgenic wheat carrying an overexpressed barley chitinase are in progress. Transgene expression was monitored using RT-PCR and RNA gel blot analysis. Twenty five, 25, 31 and 8 lines were developed that expressed the α-thionin, PR-5, ß-1,3-glucanase and RIP genes, respectively. FHB severity was assessed for all four sets of transgenic plants. The plants carrying the α-thionin, ß-1,3-glucanase and PR-5 transgenes were screened two times in the T_2 generation. The plants carrying the RIP transgene were screened once in the T_2 generation. The plants carrying the α-thionin and PR-5 transgenes were also screened in the T_3 generation. Averaging over the FHB disease screens, two, four, seven and one line exhibited less than 40% FHB severity for plants carrying the α-thionin, PR-5, ß-1,3-glucanase and RIP transgenes, respectively. Control plants (e.g., nontransformed siblings and Bobwhite) exhibited 60-80% FHB severity. These data indicate that we have developed transgenic wheat carrying the α-thionin, ß-1,3-glucanase and PR-5 transgenes that exhibit reduced FHB severity. The plants carrying the RIP transgene will need to be screened further. To further verify our results, another disease screen for FHB resistance is currently in progress for plants carrying the α-thionin, ß-1,3-glucanase and PR-5 transgenes.

Transgenic barley carrying the barley RIP transgene were developed. Twelve T_1 plants carrying the expressed RIP gene were identified using RT-PCR. T_2 generation lines will be screened this fall.

Transgenic wheat and barley were developed carrying the sugarcane badnavirus (ScBV) promoter fused to the ß-glucuronidase (GUS) gene. Barley plants carrying the ScBV-GUS plasmid express GUS in all tissues tested including: flowers, seeds, leaves, stems and roots. These data indicate that the ScBV promoter will be useful for expressing antifungal protein genes in all tissues of the barley plant. Wheat plants carrying the ScBV-GUS plasmid are currently being characterized.

Project 2: Mapping scab QTL in barley and development of markers for germplasm enhancement.

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

Fusarium head blight (FHB or scab) is a fungal disease that can devastate barley. To enhance the breeding effort in barley, we are identifying and utilizing molecular markers associated with scab resistance.

2. What were the most significant accomplishments?

To identify the location of QTL for resistance to FHB, we are mapping new sources of FHB resistance in barley. We developed a 128 $F_{4:6}$ line population from a cross between Fredrickson (moderately resistant to FHB) and Stander (susceptible to FHB). Using this population, we developed an RFLP and SSR linkage map consisting of 144 markers (89 RFLP and 45 SSR). We evaluated the F/S population in four field and two greenhouse environments. Our QTL mapping results revealed three QTL located on chromosome 2. One QTL is associated with heading date, one QTL is associated with the two-row/six row locus and the other QTL was identified in the greenhouse environment.

Before QTL are used in breeding programs they should be validated. Previous mapping results from the Chevron/M69 population (de la Pena, 1999) indicated that there were 10 and 4 QTL for resistance to FHB and a related disease kernel discoloration (KD), respectively. The major QTL for resistance to FHB and KD are located on chromosome 2 and 6. We identified two lines (MNS93 and M92-299) that carried resistance to FHB and KD. Two populations were developed by crossing MNS93 and M92-299 with Stander and M81, respectively. We developed RFLP and SSR linkage maps for these populations and evaluated these populations for FHB and KD resistance. QTL analysis validated the two major QTL for FHB and KD resistance located on chromosome 2 and 6.

Based on the QTL validation experiments, we selected several markers linked to FHB QTL on chromosomes 2 and 6 to use in a marker assisted selection (MAS) experiment. We developed two populations from crosses between two breeding lines carrying Chevron FHB alleles and the variety Lacey. Using a set of SSR markers that are linked to the QTL on chromosomes 2 and 6, we selected 25 plants from each of four marker genotype classes (homozygous Chevron chr. 2, homozygous Chevron chr. 6 , homozygous Chevron chr. 2 and 6, and homozygous Lacey chr. 2 and 6) for one F_2 population and two marker genotypic classes (homozygous Chevron chr. 2, homozygous Lacey chr. 2) for another F_2 population. For each population we also selected 25 plants at random as a control. We advanced these lines to the F_4 generation and evaluated them for heading date and FHB severity in a disease nursery in St. Paul in the summer of 2001. In addition, because the QTL on chromosome 6 is associated with grain protein, we have planted the first population in a trial in Crookston, MN that will be used to assess the affect of MAS on grain protein.

For further genetic and biological characterization of the Chevron-derived QTL for resistance to FHB and KD located on chromosome 2 and 6, we are developing near-isogenic lines for these QTL. BC_3F_3 lines are available carrying the chromosome 2 and 6 regions and

these lines will be advanced to the BC_3F_4 generation and evaluated in the summer of 2002. In addition, we have developed BC_4 material for both the chromosome 2 and 6 regions.

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:

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.

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.

F.L. Kolb, 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.

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.* (Accepted 7/01).

Smith, K., Schwarz, P., and Barr, J. 2001. Impact of Fusarium Head Blight disease management on malting barley. *Brewers Digest* 76(3):41.

Abstracts:

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

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, San Diego, CA.

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, San Diego, CA.

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 Abstracts,* p. 38, Erlanger, KY.

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

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. *American Society of Agronomy Abstracts,* p. 105, Minneapolis, MN.

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. *American Society of Agronomy Abstracts,* p. 185, Minneapolis, MN.

Smith, L., M. Wyckoff, G. Baldridge, R. Zeyen and G.J. Muehlbauer. 2000. Antifungal protein gene expression in transgenic wheat. *American Society of Agronomy Abstracts,* p. 186, Minneapolis, MN.

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

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*. Brookings, SD.

F.L. Kolb, 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. *American Society of Agronomy Abstracts*, p. 83.

Muehlbauer, G.J. and L. Smith. 1999. Developing transgenic barley carrying antifungal protein genes. Barley Workers Meeting, Idaho Falls, ID.

Invited Talks:

Muehlbauer, G.J. Barley Transformation. 2001 Barley Improvement Conference. San Antonio, TX.

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

Smith, K. P. Breeding FHB Resistant Malting Barley in Minnesota. 2001 Barley Improvement Conference. San Antonio, TX.