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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FY02 ARS Award Amount:	**$ 69,680**

Project

Program Area	Project Title	USWBSI Recommended Amount
BIO	Engineering Improved Fusarium Resistance in Hexaploid and Durum Wheat.	$71000
	Total Amount Recommended	**$71,000**

July 11, 2003
Principal Investigator Date

(Form – FPR02)

Project 1: Engineering Improved Fusarium Resistance in Hexaploid and Durum Wheat.

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

Host plant resistance is the most efficient and cost-effective way to protect the wheat crop from Fusarium Head Blight (FHB). In order to generate novel germplasm with scab resistance encoded by one or a few genes, we used genetic transformation to introduce candidate anti-*Fusarium* (AF) genes into hexaploid and durum wheat. We have identified 23 independent lines of the hexaploid cultivar Bobwhite containing either a wheat *tlp1* (thaumatin-like protein) gene or *Fusarium*-derived coding regions for an endochitinase (FvEndo), exochitinase (FvExo), glucanase (FvGlu) or DON acetyltransferase (*TRI101*), each under control of the maize *Ubi1* promoter. Two of these – *TRI101* line AB6-156 and FvGlu line AB9-59 - showed improved Type II resistance. Our specific objectives for 2002 were to 1) test homozygotes of the highest expressers of the FvGlu, FvEndo and FvExo transgenes for Type II resistance; 2) cross the highest expressers of FvGlu, *tlp1*, FvExo, FvEndo and *TRI101* in pairwise combinations and identify double homozygotes; 3) characterize new transgenic lines containing *TRI101* + *TRI12* (a *Fusarium* gene encoding a membrane pump for DON) or FvGlu constructs modified for improved wheat expression; 4) introduce the *tlp1*, FvGlu and *TRI101* + *TRI12* constructs into durum wheat; and 5) characterize the expression pattern of a new maize glutamine synthase promoter in transgenic wheat.

2. What were the most significant accomplishments?

Greenhouse resistance tests showed that the homozygous progeny of the highest expressers of *Fusarium venanatum* exochitinase, endochitinase and glucanase genes and of a wheat *tlp1* gene possessed no better Type II resistance than the non-transformed parent.

Seeds from all pairwise combinations of our AF transgenes were obtained from genetic crosses. So far, doubly homozygous F_2 plants have been identified for wheat *tlp-1*x *TRI101*and FvGlu x *tlp-1*. These will be tested for Type II resistance next fall.

New hexaploid wheat transgenic lines were produced carrying two *Fusarium*-derived genes - *TRI101* and *TRI12*. The genes had been modified to have 5'untranslated regions similar to highly expressed wheat genes. Among 6 homozygous plants identified so far, one had higher *TRI101* expression than the line AB6-156 that contains the unmodified version of the gene.

We identified 20 new hexaploid wheat transformants containing a version of the *Fusarium venenatum* glucanase gene with a modified start codon context. Transgene expression was measured in 10 lines. All showed very low mRNA accumulation in the T_1 generation. Based on this result, we will not use this construct for durum transformation (without further modifications).

We have transformed two durum cultivars, Appio and Varano. Despite the relatively low regeneration frequency (approximately 25% of the calluses formed plantlets), the transformation efficiency was 1-4%. So far, we have identified 12 transgenic lines containing the modified *TRI101* gene and another 10 lines carrying the wheat *tlp-1* gene. Homozygotes are being sought and will be tested for Type II resistance.

A maize glutamine synthase promoter fused to the GUS reporter gene coding region is expressed in the pericarp and in the scutellum of mature embryos in stable hexaploid wheat transformants. Unfortunately, neither of these tissues is a target for *Fusarium* infection. Therefore, this promoter is not suitable for expression of anti-*Fusarium* genes.

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.

Peer-reviewed publications:

Okubara PA, Blechl AE, McCormick SP, Alexander NJ, Dill-Macky R and Hohn TM. 2002. Engineering deoxynivalenol metabolism in wheat through the expression of a fungal trichothecene acetyltransferase gene. **Theor Appl Genet** 106: 74-83.

Other publications:

Somleva MN, Okubara PA and Blechl AE. 2002. Transgene expression in spring wheat (*Triticum aestivum* L.) transformed with candidate anti-*Fusarium* genes. **Proceedings of the 2002 National Fusarium Head Blight Forum** pp. 42-45**.** Eds: SM Canty, J Lewis, L. Siler, and RW Ward, Kinko's, Okemos, MI 48864 (Dec. 7-9, 2002, Cincinnati, OH).

Presentations:

Blechl, A. Talk: "Wheat Genetic Engineering" at the 2002 ARS/UC Davis Wheat Genome Center meeting, October 9, 2002, in Albany, CA.

Blechl, A. Talk: "Engineering wheat resistance to scab by introducing genes for anti-*Fusarium* proteins" at the tour of the ARS Western Regional Research Center in conjunction with annual meeting of the North American Millers Association, October 22, 2002, in Albany, CA.

Somleva MN, Okubara PA and Blechl AE. Poster: Transgene expression in spring wheat (*Triticum aestivum* L.) transformed with candidate anti-*Fusarium* genes. Annual U. S. Wheat & Barley Scab Initiative Meeting (Dec. 7-9, 2002, Cincinnati, OH).

Blechl, A. Talk: "Application of Biotechnology to Improve Bread-Making Potential and Fusarium Resistance of Wheat", Pacific Northwest Wheat Quality meeting, January 22, 2003, in Oakland, CA.