U.S. Wheat and Barley Scab Initiative
FY01 Final Performance Report (approx. May 01 – April 02)
July 15, 2002

Cover Page

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Year:	**FY2001 (approx. May 01 – April 02)**
Grant Number:	**N/A**
Grant Title:	**Fusarium Head Blight Research**
FY01 ARS Award Amount:	**$ 86,638**

Project

Program Area	Project Title	Requested Amount
Biotech	Engineering Improved Anti-Fusarium Gene Expression in Wheat	$ 101,767
	Total Amount Requested	**$ 101,767**

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Principal Investigator Date

(Form – FPR01)

Project 1: Engineering Improved Anti-Fusarium Gene Expression in Wheat

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

Host plant resistance is most efficient and cost-effective way to protect the wheat crop from Fusarium Head Blight (FHB). Yet, there are few natural sources of dominant resistance genes that provide significant levels of protection. In order to generate novel germplasm with scab resistance encoded by one or a few marked genes, Patricia Okubara and I have used genetic transformation to introduce new candidate anti-*Fusarium* (AF) genes into wheat. These genes encode proteins that target either fungal cell walls or the DON mycotoxin. The genes - with their encoded proteins in parentheses - are: FvGlu (glucanase), FvEndo (endochitinase), FvExo (exochitinase) and *TRI101* (DON acetyltransferase) from *Fusarium*, and *tlp-1* (thaumatin like protein) from wheat. Each gene was fused to the maize *Ubi1* promoter/first intron for expression throughout wheat plants. For each construct, we have generated 3-4 independent transgenic lines. We've identified homozygous progeny for each line and measured gene expression levels by semi-quantitative RT-PCR. On average, plants containing AF constructs with coding regions derived from Fusarium exhibited 10-fold lower transgene mRNA levels than plants containing the wheat tlp-1 construct. Our primary objectives for this grant were 1) to improve the expression levels of the AF constructs containing *Fusarium*-derived coding regions; 2) to test homozygous transgenic lines for *Fusarium* resistance; and 3) to make transformants with two new candidate anti-toxin genes, *TRI12* and *DONPEP.2*.

2. What were the most significant accomplishments?

We developed a transient assay system to quickly compare the mRNA accumulation of different AF constructs in embryogenic barley callus cells. This assay showed the same approximately 10-fold difference in mRNA levels between the FvEndo and *tlp-1* constructs that was found between expression levels in plants stably transformed with these constructs.

Semi-quantitative RT-PCR on mRNA prepared from different organs of our transgenic plants revealed construct- and organ-specific differences in transgene mRNA stability.

The FvGlu and *TRI101* genes were modified by changing the nucleotides immediately proceeding their start codons to sequences more typical of highly expressed wheat genes.

Homozygous plants for some of our transgenes were tested 3 times for type II *Fusarium* resistance in the greenhouse and once for overall *Fusarium* resistance in a field trial. All tests were conducted by Ruth Dill-Macky at the University of Minnesota – St. Paul. A low expressor of FvGlu and a medium expresser of *TRI101* showed more Type II resistance than their non-transformed parent in the greenhouse. However, none of 5 transgenics (including these 2) showed any resistance in the field test. We will begin tests of higher expresser lines next fall.

We made constructs fusing the UBI promoter to two new candidate AF genes modified for expression in wheat. One construct contains the coding region from *TRI12*, a *Fusarium* gene that encodes a DON exporter protein that, in yeast, increased the detoxifying action of the DON acetyltransferase encoded by the *TRI101* gene. The other construct, *DONPEP.2*, encodes a fusion between the green fluorescence protein (GFP) and a peptide that interferes with DON's inhibition of ribosomes. The *TRI12* construct has been co-transformed into wheat along with the modified *TRI101* construct. We have obtained 7 transformants so far. These are being characterized for gene expression levels. Thus far, no improvement has been noted in expression levels of the *TRI101* gene with the new start codon context compared to the original version.

Based on Patrick Hart's results that the DONPEP.2:GFP fusion protein does not protect Arabidopsis ribosomes from DON, we have set aside the DONPEP.2 construct for now.

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: none (one accepted for publication)

Other publications: none

Presentations:

Okubara seminars “Molecular Approaches to Enhancing Host Resistance to Fungal Pathogens” for the Washington State University Plant Pathology and Plant Physiology seminar series in Pullman, WA, Sept. 24, 2001 and Feb. 15, 2002.

Blechl AE invited seminar "Engineering changes in wheat bread-making quality and Fusarium resistance via genetic transformation" for the Interdepartmental Plant Molecular Biology group, Texas A & M University, College Station, TX, 10/25/01

Blechl AE et al. poster: "Expression of two different candidate anti-*Fusarium* protein genes affords partial protection against the spread of *Fusiarum graminearum*" at the annual U.S. Wheat and Barley Scab Initiative meeting in Erlanger, KY, Dec. 9-11, 2001.

Blechl AE talk “Update on scab biotechnology research” at the annual joint ARS - American Bakers’ Association meeting in Beltsville, MD, April 16, 2002.