U.S. Wheat and Barley Scab Initiative
Annual Progress Report
September 18, 2000

Cover Page

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Year:	**FY2000**
Grant Number:	
Grant Title:	**Fusarium Head Blight Research**
Amount Granted:	**$80,000.00**

Project

Program Area	Objective	Requested Amount
Biotechnology	Provide novel alternatiive sourcews of co-dominat scab resistance in cv. Bobwhite, which can readily be crossed to tolerant hard red spring and soft red winter wheat varieties grown in regions of the Midwest most severely affected by scab.	$100,620.00
	Requested Total	$100,620.00[1]

_____ 9/8/00 _____
Principal Investigator Date

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

1. The major problem and how we are resolving it: Thus far, no effective *Fusarium*-resistance genes have been identified in wheat. Yet, host resistance would be the safest and most cost-effective way to protect the crop from scab. To generate novel germplasm with scab resistance encoded by one or a few genes, Patricia Okubara and I have used genetic transformation to introduce six new genes into wheat that target either fungal cell walls or membranes or the DON mycotoxin. The genes - with their encoded proteins in parentheses - are: FvGlu (glucanase), FvEndo (endochitinase), FvExo (exochitinase) and *Tri101* (DON acetylase) from *Fusarium*, *tlp-1* (thaumatin like protein) from wheat, and *PDR5* (multi-drug efflux transporter) from bakers' yeast. Each gene was fused to the maize *Ubi1* promoter/first intron for expression throughout wheat plants. We have generated 3 to 11 independent transgenic plants for each construct.
2. Comparison of the actual accomplishments with the objectives established: Our objectives this year were to **A**) measure transgene expression levels in the endosperm and glumes of our various transgenic lines; **B**) examine codon usage in cereal genes known to be expressed in florets and leaves; and **C**) combine different transgenes by genetic crosses. **A**) Using Northern blots and RT-PCR, we showed that transgene expression levels in endosperm ranged from undetected to high. The abundance of transgene mRNA averaged over each set of lines decreased for the various constructs in the following order: wheat *tlp-1* > FvEndo, FvExo > *TRI101* > *PDR5* > FvGlu . A percentage of the *TRI101* and *PDR5* endosperm transcripts were incompletely spliced, and the *PDR5* mRNA appeared to be truncated at the 3' end. The relative abundance of transgene mRNAs was very similar in both endosperm and glume tissues in all five lines tested so far. Collaborator Susan McCormick (ARS-Peoria) measured moderate DON acetylase activity in one of the four *Tri101* transgenics, low activity in another line, and none in the remaining two. Ten different homozygous transgenics were tested by collaborator Ruth Dill-Macky (University of Minnesota - St. Paul) for resistance to spread of *Fusarium* in inoculated wheat heads. Two (see question 4 below) showed moderate resistance that was intermediate between those of the susceptible (including the non-transformed parent) and resistant checks. **(B)** We compared codon usage of the fungal genes to that of monocot pollen genes, *Triticum aestivum* leaf genes, and *T. aestivum* endosperm genes. For each class of plant genes, a minimum of ~3000 codons were compiled from 10 to 17 non-redundant GenBank entries. Differences between the Fusarium-derived and monocot genes were observed in the usage of codons for certain amino acids - notably phe, lys, val, ala, his, glu, gln, asp and gly. (**C**) Six homozygous parental lines representing 5 of our 6 transgenes have been crossed so far. PCR analysis identified F_1 progeny that had received the transgene from the male parent. F_2 progeny analysis is currently underway. Two of the parental lines were later shown to be low mRNA expressers. New lines expressing FvEndo, FvExo, or *tlp-1* mRNAs are currently being analyzed for homozygosity, and will be used in subsequent crosses.
3. Reasons objectives were not met: All the objectives were met. However, our expression data show that, on average, the transgenes of fungal origin do not support levels of mRNA accumulation as high as those supported by wheat *tlp* transgenes with the same promoter. We believe this is due to features of the RNA sequence and are designing modified versions of the genes. We are also generating more transformants to be characterized.
4. Most significant accomplishment: Identification of two different transgenics that have moderate levels of resistance to the spread of scab in two different greenhouse trials conducted by Ruth Dill-Macky. One transgenic contains high levels of the wheat *tlp* mRNA in (at least) glume and endosperm tissue. The other is our highest accumulator of DON acetylase activity.

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.

Dahleen LS, Okubara PA, Blechl AE. Transgenic approaches to combat Fusarium head blight in wheat and barley. Accepted by Crop Sci

Okubara P, Hohn T, Berka R, Anderson O, Blechl A (2000). Expression of candidate anti-*Fusarium* protein genes in hexaploid wheat. Poster for American Phytopathological Society meetings, New Orleans, LA

Okubara P, Beamish C, Lin J, Montejo C, Anderson A, Blechl A (1999). Expression of candidate anti-*Fusarium* protein genes in hexaploid wheat. Poster for 1999 National Fusarium Head Blight Forum, Sioux Falls, SD