U.S. Wheat and Barley Scab Initiative
FY00 Final Performance Report (approx. May 00 – April 01)
July 30, 2001

Cover Page

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Year:	**FY2000 (approx. May 00 – April 01)**
Grant Number:	
Grant Title:	**Fusarium Head Blight Research**
2000 ARS Award Amount:	**$78,049**

Project

Program Area	Project Title	Requested Amount
Biotechnology	Expression of Candidate Anti-Fusarium Genes in Wheat.	$100,620.00
	Requested Total	$100,620.00[1]

_____ 7/25/01 _____
Principal Investigator Date

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

Project 1: Expression of Candidate Anti-Fusarium Genes in Wheat.

1. What major problem or issue is being resolved and how are you 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. For each construct, we have generated 3 to 11 independent transgenic plants. We have or will identify homozygous progeny for each line and then test them for gene expression levels and type II *Fusarium* resistance.

2. What were the most significant accomplishments?

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 average abundance of transgene mRNA in the first set of lines decreased for the various constructs in the following order: wheat *tlp-1* > FvEndo, FvExo > *TRI101* > *PDR5* > FvGlu . Some of the *TRI101* and *PDR5* endosperm transcripts were incompletely spliced, and the *PDR5* mRNA appeared to be truncated at the 3' end. In response to these findings, we initiated new bombardments and identified higher expressor lines for FvEndo, FvExo, and FvGlu. Still, none of the transgenes derived from fungal sources are as well-expressed as the wheat *tlp* transgene with the same promoter. In five lines tested, the relative abundance of transgene mRNAs was very similar in endosperm and glumes.

Collaborator Susan McCormick (ARS-Peoria) measured moderate DON acetylase activity in one of the four *Tri101* transgenics. Ten different homozygous transgenics were tested twice by collaborator Ruth Dill-Macky (University of Minnesota - St. Paul) for resistance to spread of *Fusarium* in inoculated wheat heads. The test was repeated a third time for five of the lines. Two showed moderate resistance in all three tests that was intermediate between those of the susceptible (including the non-transformed parent) and resistant checks. One partially resistant transgenic contains moderate levels of FvGlu mRNA. The other is the *TRI101* transformant shown to accumulate DON acetylase activity.

(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. Homozygous progeny have been identified for the cross between the highest expressor lines containing wheat *tlp-1* and FvEndo transgenes.

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 publication:
Dahleen LS, Okubara PA and Blechl, AE (2001) Transgenic Approaches to Combat Fusarium Head Blight in Wheat and Barley, **Crop Science** 41:628-637.

Patent filed:
Okubara PA, Blechl AE, Hohn TM and Berka RM (8/30/2000) "Nucleic Acid Sequences Encoding Cell Wall-Degrading Enzymes and Use to Engineer Fusarium Resistance"

Other publications:

Okubara PA, Hohn TM, Berka RM, Alexander NA, Wang Z, Hart LP and Blechl AE (2000) Optimizing the expression of candidate anti-*Fusarium* protein genes in hexaploid wheat. **Proceedings of the 2000 National Fusarium Head Blight Forum** (Cincinnati, OH, Dec. 10-12, 2000), eds. R.W. Ward, S.M. Canty, J. Lewis and L. Siler, Michigan State University, East Lansing, pp. 39-42.

Anderson OD and Blechl AE (2000) Transgenic Wheat - Challenges and Opportunities. **Transgenic Cereals**, eds. L. O'Brien and R.J. Henry, American Association of Cereal Chemists, St. Paul, MN, pp. 1-27.

Presentations:

Blechl AE talk: "Expression of candidate anti-*Fusarium* protein genes in hexaploid wheat" at the annual U.S. Wheat and Barley Scab Initiative meeting in Cincinnati, OH, Dec. 11, 2000.

Okubara PA poster: "Expression of candidate anti-*Fusarium* protein genes in hexaploid wheat" for the American Phytopathology Society meeting, in New Orleans, LA, Aug. 13-17, 2000.