

**U.S. Wheat and Barley Scab Initiative
Annual Progress Report
September 15, 1999**

Cover Page

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Year:	FY1999

Project

Program Area	Objective	Requested Amount
Biotechnology	Develop transgenic germplasms of <i>Triticum aestivum</i> expressing genes encoding candidate anti-fungal proteins and new promoters for use in wheat and barley.	\$50,000
	Requested Total	\$70,000 ¹

Principle Investigator

Date

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

Project 1: Develop transgenic germplasms of *Triticum aestivum* expressing genes encoding candidate anti-fungal proteins and new promoters for use in wheat and barley.

1. What major problem or issue is being resolved and how are you resolving it? No effective host plant resistance to scab has yet been identified in wheat, barley or their relatives. This project uses biotechnology to generate novel germplasms of wheat that express candidate antifungal protein genes. Such transgenes would provide single-locus, co-dominant traits that can be added either singly or in combination by breeders to existing multigenic traits for scab tolerance. Seeds from scab-resistant varieties will contain less DON mycotoxin and thus be safer for human and monogastric animal consumption. We selected six candidate genes that encode proteins having two different modes of action: for reduction of DON in host tissues, 1) *Fusarium* DON acetyltransferase and 2) the yeast PDR5 multidrug transporter (both from the ARS in Peoria, IL); and for disruption of the pathogen cell wall and plasma membrane, 3) a wheat thaumatin-like protein (*tlp-1*), 4) a *Fusarium* glucanase, 5) a *Fusarium* endochitinase, and 6) a *Fusarium* exochitinase. Each of the transgene constructs have now been stably integrated in wheat cultivar Bobwhite. Plants showing the highest levels of mRNA expression are currently being identified.

2. Please provide a comparison of the actual accomplishments with the objectives established. At present, a total of 31 independent lines of wheat carrying one of the six transgenes has been identified; 18 lines are now homozygous for the transgene, and at least three of these tested so far show accumulation of transgene mRNA in northern blot analyses.

<u>Encoded Protein</u>	<u>No. of Lines</u>	<u>Homozygotes</u>	<u>Expressing Lines</u>
DON acetyltransferase	4	4	at least 1
PDR5 transporter	11	6	
<i>tlp-1</i>	3	1	1
Glucanase	3	1	
Endochitinase	7	5	at least 1
Exochitinase	3	1	

3. What were the reasons established objectives were not met? We have met the major objective for 1999, the development of transgenic germplasms carrying each of the above six transgenes. We have identified plants that are homozygous for the transgenes and that express transgene mRNA, and can now evaluate of the activity of the transgenes *in planta*. We had requested money for a half-time technician, but since the award was not sufficient to cover that, we did not progress beyond the discussion and planning stages for the proposed work on promoter characterization. We plan to use facilities and protocols for gene discovery that are being established in Olin Anderson's laboratory for development of new promoters in the next funding year.

4. What were the most significant accomplishments this past year? The most significant accomplishments were the demonstration that the transgenes are producing messenger RNA in the wheat plants, and the production of wheat seed homozygous for each of the six of the transgene constructs. The latter will now be tested for resistance to inoculation and spread of FHB.

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.

Okubara P, Beamish C, Lin J, Montejo C, Anderson O, Blechl A (1999) Transgenic lines of wheat (*Triticum aestivum*) carrying genes for candidate anti-*Fusarium* proteins. Poster for American Society of Plant Physiologists Conference, Baltimore, MD

Okubara PA, Blechl AE, Hohn TM, Berka RM (1999) Provisional patent entitled "Genes Encoding Cell Wall-Degrading Enzymes and Their Use to Engineer *Fusarium* Resistance"

Blechl A, (1999) Talk entitled "Anti-fungal Proteins" for the Research Forum of National Association of Wheat Growers Meeting, Nashville, TN