USDA-ARS / USWBSI
FY03 Final Performance Report (approx. May 03 – April 04)
July 15, 2004

Cover Page

PI:	**Ann Blechl**
Institution:	**USDA-ARS**
Address:	**Western Regional Research Center** **800 Buchanan Street** **Albany, CA 94710-1105**
E-mail:	**ablechl@pw.usda.gov**
Phone:	**510-559-5716**
Fax:	**510-559-5777**
Year:	**FY2003 (approx. May 03 – April 04)**
FY03 ARS Agreement ID:	**NA**
FY03 ARS Agreement Title:	**Improvements in Wheat's Resistance to Fusarium Infection and Trichothecenes.**
FY03 ARS Award Amount:	**$ 74,912**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Engineering Improved Anti-Fusarium Protein Accumulation in Transgenic Wheat.	$ 74,912
	Total Amount Recommended	**$ 74,912**

7/13/04
Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
VDUN – Variety Development & Uniform Nurseries

Project 1: *Engineering Improved Anti-Fusarium Protein Accumulation in Transgenic Wheat.*

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

The goal of our research is to create transgenic lines of hexaploid and durum wheat carrying novel co-dominant loci with the potential for improving host resistance to Fusarium head blight. We have chosen several potentially anti-*Fusarium* (AF) genes to test differing strategies of FHB control. One set of genes codes proteins that could attack *Fusarium* cell walls, namely an exochitinase (FvExo), an endochitinase (FvEndo), and a glucanase (FvGlu). Experiments in 2003 were aimed at increasing the expression levels of these *Fusarium*-derived genes in transgenic wheat and testing higher expressers for resistance. Another set of genes was chosen to protect wheat cells from DON mycotoxin. Two of these genes are from *Fusarium* and encode a DON acetyltransferase (*TRI101*) and a trichothecene pump (*TRI12*). Another gene, made available to us by collaborator Nilgun Tumer (Rutgers University), encodes the ribosomal protein L3, which is the target of DON inhibition. Over-expression of this protein is expected to protect cells from DON toxicity. A third set of genes is part of a new strategy to enlist the oxidative burst against initial *Fusarium* infection by co-expressing an *Aspergillus* gene encoding the H_2O_2-generating enzyme glucose oxidase (GO) with peroxidase (*Prx*) genes from barley. The barley *Lem1* promoter was chosen to target these proteins involved in enhancing plant structural defenses because our research this year (see below) showed that it is active only in the outer organs of the wheat spikelet at anthesis.

2. What were the most significant accomplishments?

Greenhouse tests by Ruth Dill-Macky (University of Minnesota – St. Paul) showed that the homozygous progeny of our highest expressers of the original FvExo, FvEndo and FvGlu genes possessed no better Type II resistance than the non-transformed parent. Of 20 hexaploid wheat lines transformed with a modified FvGlu containing a wheat-like start codon context, none showed increases in transgene transcript levels. Nine lines carrying another modified FvGlu gene with codons optimized for wheat translation were made and are being analyzed.

Five hexaploid wheat lines containing *TRI101* or *TRI1101* and *TRI12* genes with modified 5' leader sequences showed increased transgene mRNA levels compared to transgenics containing the unmodified *TRI101* construct. Most of these lines also had higher DON acetyltransferase activity than a line carrying the unmodified version of *TRI101*. Homozygous progeny from two of these lines exhibited 40-50% reduction in FHB severity compared to their untransformed parent in two greenhouse tests for Type II resistance. Their field resistance will be evaluated next spring.

Durum cultivars Varano and Appio have been transformed with efficiencies of 1-4%. We identified 20 *TRI101* and/or *TRI12* lines and 10 lines carrying a wheat *tlp-1* gene. Homozygotes are being sought and will be tested for Type II resistance next fall.

Expression analyses of stable wheat transformants revealed that the promoter of a floret-specific barley gene, *Lem1,* is active in the lemma, palea, glume, awns, and rachis at anthesis. Transient assays show that the promoter has a moderate activity compared to the maize *Ubiquitin1* (UBI) promoter used in previous constructs. The organ- and developmental specificity of the *Lem1* promoter makes it very useful for expressing AF genes in wheat.

We constructed transformation vectors fusing *Lem1* to the coding regions of the *GO* and *Prx* genes. Primary transformants and their progeny are being analyzed. We also constructed vectors containing a complete or a C-terminal deleted version of the yeast L3 gene under the control of the UBI or *Lem1* promoters. Transformation experiments with the latter constructs are underway.

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 you grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Presentations:

Janni M, Roberti S, Lin J, Favaron F, Cervone F, De Lorenzo-G, Blechl A and D'Ovidio R. Poster: Production of transgenic wheat expressing a defense gene against phytopathogenic fungi. Tenth International Wheat Genetics Symposium (September 1-6, 2003, Paestum, Italy).

Somleva, M and Blechl AE. Poster: Characterization of organ-specific promoters from maize and barley in transgenic wheat. Annual U.S. Wheat and Barley Scab Initiative Meeting (Dec. 13-15, 2003, Bloomington, MN). Proceedings page 47.