USDA-ARS / USWBSI
FY04 Final Performance Report
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Cover Page

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Year:	**FY2004**
FY04 ARS Agreement ID:	**NA**
FY04 ARS Agreement Title:	**Engineering Wheat's Resistance to Fusarium Infection and Trichothecenes.**
FY04 ARS Award Amount:	**$ 62,439**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Engineering Scab-Resistant Wheat by Employing Oxidative Defense Responses.	$ 62,439
	Total ARS Award Amount	**$ 62,439**

7/8/2005
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

(Form – FPR04)

Project 1: ***Engineering Scab-Resistant Wheat by Employing Oxidative Defense Responses.***

1. What major problem or issue is being resolved and how are you resolving it?
Our ultimate goal is to develop germplasm of hexaploid and durum wheat with improved resistance to Fusarium head blight (FHB) by transformation-mediated combinations of antifungal (AF) genes. Our specific objectives for 2004 were to: 1) introduce the *Aspergillus* glucose oxidase (*GO*) gene and barley peroxidase genes (*Prx7* and *Prx8)* under the control of the *Lem1* promoter into hexaploid wheat; 2) analyze the expression of the *GO* and *Prx* genes at the mRNA level; 3) measure GO and peroxidase enzyme activities and protein levels in transformed tissues and plants; 4) directly test some of the gene products for anti-*Fusarium* activity in an *in vitro* assay; 5) generate stably transformed lines of hexaploid wheat carrying two versions of the yeast ribosomal protein L3 driven by the constitutive promoter UBI; 6) identify homozygotes and measure mRNA levels of *TRI101* and *tlp-1* in durum wheat lines; and 7) analyze trichothecene 3-O-acetyltransferase activity encoded by *TRI101* in durum wheat lines.

2. What were the most significant accomplishments?
One hundred independent transformation events carrying the *GO* and *Prx* genes alone or in different combinations have been identified by PCR. Genetic and expression analyses of the progeny of 50 T_0 plants are in progress. Using *in situ* methods we have localized the recombinant proteins in the chlorenchyma of the organs surrounding the developing floret at anthesis. As expected, GO and Prx8 are targeted to the apoplast of the lemma and palea, while Prx7 is detectable within the cells. We have determined the activities of the recombinant enzymes in protein extracts from lemmas and paleas of T_2 and T_3 plants at anthesis. GO activities in different transformed plants differed as much as three-fold and resulted in accumulation of sublethal levels of H_2O_2, a compound with direct anti-microbial activity. Four substrates specific for various isoforms were used for analyses of the peroxidase activity. Plants co-expressing the *GO* and *Prx8* genes showed the highest enzyme activity. The lignin content was significantly increased (up to 350%) in most of the analyzed transgenic plants. We found that the activities of the transgene-encoded enzymes did not cause metabolic changes leading to damage of the membranes of the plant cells. Transgene transcripts and products are not present in the endosperm and embryo of developing seeds as shown by RT-PCR and *in situ* techniques.
Impact: A novel strategy for improving host resistance to initial *Fusarium* infection by engineering the metabolism of reactive oxygen species has been designed and implemented. The newly created anti-*Fusarium* gene expression cassettes and germplasm with improved disease resistance are tools that will be useful to scab researchers, wheat pathologists, and wheat breeders. A provisional patent has been filed on this strategy and these materials, which will facilitate technology transfer to wheat seed producers. The lack of recombinant proteins in the grain will minimize concerns about the safety of foods derived from these plants and facilitate their approval by regulators and acceptance by consumers.

We have identified a homozygous durum line carrying two *Fusarium sporotrichioides* genes: *TRI101* encoding a trichothecene 3-O-acetyltransferase and *TRI12* encoding a trichothecene efflux transporter. We have previously shown that transgenic lines of hexaploid wheat co-expressing these genes had 40-50% reductions in FHB severity in greenhouse tests.
Impact: This is the first report on the application of this approach for detoxifying *Fusarium* mycotoxins in durum wheat. Expression analyses of these plants will provide more information on the interaction of the *TRI*-encoded proteins in making wheat cells resistant to the trichothecenes produced by *Fusarium* as virulence factors during infection.

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.

Peer-reviewed publications:

Somleva M.N. and A. Blechl. 2005. The barley *Lem* 1 gene promoter drives expression specifically in outer floret organs at anthesis in transgenic wheat. Cereal Research Communications. Accepted for publication 3/10/2005.

Other publications:

Somleva M.N. and A. Blechl. 2004. Characterization of organ specific promoters in transgenic wheat. Proceedings of the 2nd International Symposium on Fusarium Head Blight pp. 263-267**,** Dec. 11-15, 2004, Orlando, FL.

Patent (provisional application):

Somleva M.N. and A. Blechl. 2005. A method for improvement of plant resistance to *Fusarium* spp. and other pathogens" (Patent Serial No. 60/686,225).

Presentations:

Somleva M.N. and A. Blechl. 2004. A novel strategy for transgenic control of Fusarium head blight in wheat. Proceedings of the 2nd International Symposium on Fusarium Head Blight p. 262**,** Dec. 11-15, 2004, Orlando, FL. (*invited talk*)

Somleva M.N. and A. Blechl. 2004. Combined expression of candidate anti-*Fusarium* genes in wheat spikelets. Proceedings of the 2nd International Symposium on Fusarium Head Blight p. 268**,** Dec. 11-15, 2004, Orlando, FL. *(poster)*

Somleva M.N. and A. Blechl. 2004. Organ-specific expression of putative anti-*Fusarium* genes in transgenic wheat. The 2004 World Congress on In Vitro Biology, May 22-26, San Francisco, CA, USA. Abstract No P2025. *(poster)*