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

Cover Page

PI:	**Stephen Baenziger**
Institution:	**University of Nebraska - Lincoln**
Address:	**Department of Agronomy** **P.O. Box 830915** **Lincoln, NE 68583-0915**
E-mail:	**pbaenziger1@unl.edu**
Phone:	**402-472-1538**
Fax:	**402-472-7904**
Year:	**FY2003 (approx. May 03 – April 04)**
FY03 ARS Agreement ID:	**59-0790-9-026**
FY03 ARS Agreement Title:	**Enhanced Scab Resistance in Wheat Germplasm by Plant Transformation.**
FY03 ARS Award Amount:	**$ 112,929**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Enhanced Scab Resistance in Winter Wheat Germplasm by Plant Transformation.	$ 67,620
VDUN	To Enhance Variety Development of Scab Resistant Varieties.	$ 45,308
	Total Amount Recommended	**$ 112,929**

_____

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: ***Enhanced Scab Resistance in Winter Wheat Germplasm by Plant Transformation.***

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

We are trying to broaden the sources of resistance in wheat to Fusarium head blight (FHB) by incorporating transgenes that have antifungal properties. A total of 28 transgenic wheat lines carrying the negative regulators of programmed cell death, IAP, ced9, or Bcl-xl along with a maize ribosomal inactivating protein, were evaluated under field conditions in Lincoln, and Mead, Nebraska in the spring of 2004. The transgenics were in spring wheat backgrounds, Bobwhite, Sakha 206 or Yang Mai 10. The Mead site was lost to an unexpected infection of wheat streak mosaic virus (extremely unusual in eastern NE and not visible until the temperature warmed up, long after spring planting). The Lincoln site was productive (albeit the number of lines was significantly smaller than our Mead location due to seed quantity constraints). Data was collected on days to anthesis, vigor and plant height, in addition to FHB severity and incidence. Analyses on the data sets are currently ongoing, but interestingly four lines, designated 102-01-01-01; 102-01-01-03, 102-01-01-04, and 102-01-01-05 all derived from one event that carries the negative regulator of programmed cell death, bcl-xl, displayed similar disease incidence ratings to Alsen and ND2710. Disease severity is currently being tabulated. However, two events previously determined to display enhanced tolerance to FHB under greenhouse conditions, designated 34-03-01-02 and 38-11-06-01, carrying the negative regulator of programmed cell death IAP, displayed similar FHB incidence to the control, Bobwhite. We are actively pursuing getting the lead transgenics in to suitable winter wheat backgrounds to permit us to conduct reliable field tests in Nebraska. Four winter wheat F_2 transgenic populations were grown in 2004. Moreover, we are actively pursuing outside collaborations with groups who are willing to conduct additional greenhouse and field-testing of the novel spring wheat transgenic material our group has generated.

A total of 21 bovine lactoferrin lines have characterized for expression level, number of transgenic loci and in vitro antifungal activity. The lactoferrin expression levels varied between 1.47 and 0.22% of the total soluble protein as determined by an indirect ELISA assay. Total protein extracts from all the lines exhibited statistically significant anti *F. graminearum* activity on potato dextrose agar plates. Seven of these lines are confirmed homozygous. Additional transgenic wheat lines carry other negative regulators of programmed cell death have recently been established in the greenhouse. Moreover, transgenic lines carrying a synthetic lytic peptide have also been generated. T_1 seed from these events will be handed-off to our breeding program within the next month and half. To monitor the interaction of potential antifungal transgene(s) with endogenous FHB tolerance gene(s) a series of crosses have been generated with our lead transgenic wheat lines to Alsen and to known susceptible wheat genotypes. These crosses permit the direct testing of the additive effect of the trangene(s) in genetic backgrounds that display varying degrees of tolerance to FHB pathogenesis.

2. What were the most significant accomplishments?

Our wheat biotechnology team completed a survey of 30 spring wheat genotypes for enhanced transformation frequencies. From this work, a hard white genotype Xin chun 9 was identified that displayed improved transformability over Bobwhite. Current efforts are focused on evaluating a series of novel *Agrobacterium tumefaciens* strains in a comparative study with both Bobwhite and Xin chun 9 to improve transformation efficiency.

Project 2: ***To Enhance Variety Development of Scab Resistant Varieties.***

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

Our goals in this research are threefold. Specifically we are: 1. breeding new cultivars that have heightened tolerance to Fusairum head blight, 2. determining the key areas within Nebraska that are most prone to Fusairum head blight infection, and 3. identifying hard winter wheat lines with above average tolerance to Fusarium head blight by testing lines in Regional Germplasm Observation Nursery (RGON, a nursery that has lines from every hard winter wheat breeding program in the Great Plains). In our breeding project, we have continued to make crosses between lines identified with Fusarium head blight tolerance in the germplasm identification program. In this reporting period, 34 new crosses using this germplasm. A major change in incorporating this germplasm into adapted wheat germplasm was that we only used adapted winter wheat parents that have an above average level of Fusarium head blight tolerance. Previous crosses involving diverse sources of tolerance and Sumai 3 derivatives are advancing in the breeding program (41 F_2 populations, 34 F_3 populations, 1000 head rows, 34 F_5 lines, and 2 F_6 lines). All F_5 lines that involved a Fusarium head blight tolerant parent and were selected for agronomic performance were submitted to the USDA Genotyping Center to determine if they contained the Sumai 3 allele. Unfortunately none of the lines did which was not totally unexpected as we have had difficulties deriving good lines form the winter by spring single crosses involving Sumai 3 derivatives. We currently have a number of three way (winter by (winter by spring)) crosses that are in our head row nurseries. For our second objective, after a number of drought years (Nebraska is in the fifth year of a drought), we have adequate rains in eastern Nebraska to identify naturally occurring Fusarium head blight. So far every field looked at from Omaha to Clay Center (approximately 160 miles to the west) has a low level of Fusarium head blight in incidence. Only fields of 2137, a known Fusarium head blight susceptible line have commercial losses. In the peak year for Fusarium head blight (the flood years of the late 1980s and early 1990s, Fusarium head blight was found from Omaha to McCook (about 250 miles west)). For our third objective, we screened the RGON nursery and are summarizing the results. Preliminary data indicates 66% of the RGON nursery had a head severity <20%, This year's screen, perhaps aided by naturally conditions, was exceptional and we had very good levels of infection 34% of field trial had greater than 25% incidence. In addition, we completely rebuilt our pump and water supply to increase reliability.

2. What were the most significant accomplishments?

Our most important accomplishment in 2003-2004 was the active promotion of Wesley as the cultivar to be grown in eastern Nebraska in regions where Fusarium head blight could be a problem. Wesley's acreage continues to increase and we believe those growers who switched form 2137 to Wesley avoided serious yield and economic losses by making this switch. The identification of Wesley as having better Fusarium head blight tolerance than other cultivars is a direct result of the National Scab Initiative Funding.

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

Langston, B., Z.E. Vaghchhipawala, T. Clemente, S. Baenziger, J. Schimelfenig and M.B. Dickman. 2003. Expression of Anti-Apoptotic Genes in Spring Wheat Confer Resistance to Necrotropic Pathogens (*Fusarium graminearum)* by Inhibiting Host-Cell Death. 2003 National Fusarium Head Blight Forum. Pg 20.

Schimelfenig, J., P.S. Baenziger, and J.E. Watkins. 2003. The Development of Fusarium Head Blight Tolerant Varieties of Wheat in Nebraska from 2001 to 2003. 2003 National Fusarium Head Blight Forum. Pg 275.