U.S. Wheat and Barley Scab Initiative
FY02 Final Performance Report (approx. May 02 – April 03)
July 15, 2003

Cover Page

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Year:	**FY2002 (approx. May 02– April 03)**
Grant Number:	**59-0790-9-026**
Grant Title:	**Fusarium Head Blight Research**
FY02 ARS Award Amount:	**$ 100,819**

Project

Program Area	Project Title	USWBSI Recommended Amount
BIO	Enhanced Scab Resistance in winter Wheat Germplasm by Plant Transformation.	$54,500
VDUN	To Ehance Variety Development of Scab Resistant Varieties.	$48,839
	Total Amount Recommended	**$103,339**

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Principal Investigator Date

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?
   Fusarium graminearum is not effectively controlled chemically or by genetic resistance anywhere in the world. To augment the germplasm identification, utilization, and cultivar development efforts, we are incorporating known and novel anti-fungal genes into transgenic wheat. Small scale field plantings of our lead transgenic wheat lines carrying the negative regulators of programmed cell death genes, ced-9, bcl-xl and IAP, along with several lines carrying a potential antifungal ribosomal inactivating protein, RIP, are ongoing. Many of these lines were sent to Dr. Yue Jin in March to confirm our greenhouse results. We have successfully created seven events containing the bovine lactoferrin gene (blf, a gene that we have received approval for field testing). All lines are nptII positive (ELISA) and southern positive, but two lines are western negative (tested twice). We should have additional western data shortly. With limited amounts of seed for testing, we opted to plant seed of the blf events in hills in the field. Our remnant seed is sufficient to increase seed next fall and to do a greenhouse screen. An additional negative regulator of programmed cell death gene from an insect, sf-IAP, was assembled into a binary plasmid under the control of the maize ubiquitin promoter coupled with its first intron. In addition, a synthetic 19 mer lytic peptide was obtained from Demegen Corp. designated D4. This peptide was previously shown to possess antifungal activity towards a variety of plant pathogens (Demegen personnel communications). We fused the D4 peptide to the tobacco etch translation enhancer element. The TEV leader-D4 19 mer was subsequently cloned downstream of the maize ubiquitin promoter coupled with its first intron. Wheat transformations with both genes were begun in early March 2003. Our wheat transformation group completed a genotype survey of 30 spring wheat genotypes for enhanced *Agrobacterium*-mediated transformation frequency. An elite spring wheat genotype was identified in which transformation frequencies ranged from 4% to 5% on an immature embryo per transformed line in soil basis. In comparison Bobwhite typically results in transformation frequencies of 1% to 3%. We are currently bulking the seed of this genotype for future transformations.
   We have recently demonstrated that wheat scab disease involves a programmed cell death response in the host. When wheat heads are infected, hallmark criteria associated with mammalian apoptosis is observed, including DNA fragmentation, DNA laddering and TUNEL positive nuclei. This is of particular interest as it provides mechanistic insight into the disease; it is not merely a necrosis tissue death, but rather an orderly dismantling of the cell triggered by the fungus. Importantly, transgenic wheat harboring animal anti-apoptotic genes, are tolerant/resistant consistent with the idea that a programmed cell death process is occurring. Thus, the ability to control scab by modulating cell death has been reinforced. We are currently evaluating whether DON alone can mimic these observations.

2. What were the most significant accomplishments?
   We showed that the scab disease involves programmed cell death in the host. We successfully undertook our first field test of transgenic wheat in 2002 and planted our second field test in early April, 2003. We also obtained a movement permit to ship our transgenic seed to Dr. Yue Jin for cooperative testing to confirm our greenhouse tests.

Project 2: To Ehance Variety Development of Scab Resistant Varieties.

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

   The long-term goals of this project are to: 1. develop elite winter wheat varieties that are resistant to Fusarium head blight (FHB, scab) using breeding (**part of the Scab Initiative's effort on plant breeding and variety development**), 2. determine the level of FHB and need for FHB resistant varieties in dryland and irrigated wheat production, and 3. to screen experimental lines in hard winter wheat regional nurseries to identify the level of FHB resistance within the existing elite winter germplasm of the Great Plains (**part of the Scab Initiative's effort on plant breeding and variety development and also part of the germplasm introduction and enhancement efforts)**. The specific objectives in our conventional breeding and variety development effort are: A) collect FHB resistant germplasm, B) incorporate the resistant germplasm (including transgenic sources) into hard winter wheat germplasm (white and red) by crossing, and C) using a modified bulk breeding or backcrossing method to advance the germplasm to elite line status.

   We continue to collect FHB resistant germplasm, specifically from the FHB germplasm identification programs of the Scab Initiative. We have concentrated on crossing with the winter germplasm identified by Dr. McKendry's program. Previous crosses were advanced using the modified bulk method and our populations specifically made for FHB tolerance are currently in the F_1 to F_6 generation. Relatively few lines have made it to the later generations, however, the current germplasm base appears to be agronomically superior and it should be easier to select good lines. Crosses with winter FHB tolerant lines from Purdue University (e.g. 'Goldfield') look particularly promising. We continue to modify our breeding strategy and will select more for FHB tolerance using molecular markers and to sample more broadly within our FHB populations. Our goal is to insure we have the tolerance in backgrounds that may not be commercial, but are closer to our commercial standards than the original germplasm. Using soft wheat parents will require close monitoring of end-use quality for bread and noodle making.

2. What were the most significant accomplishments?

   Probably the most significant accomplishment in this year is that 'Wesley' wheat, a recently released line from our program appears to have a better than average level of FHB tolerance and a low DON level in our field tests which confirms our earlier greenhouse tests. Wesley is adapted to the primary regions where FHB is most common. 'Goodstreak' also appears to have a good level of FHB tolerance and a low level of DON, but this line is a tall wheat that was released in 2002 for production in the drought prone areas of western Nebraska and eastern Wyoming. While it will be a good parent, Goodstreak is not adapted to the primary FHB prone areas.

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.

Haliloglu, K., and P. S. Baenziger. 2003. Agrobacterium tumefaciens mediated wheat transformation. Cereal Res. Comm.31: 9-16.