

**U.S. Wheat and Barley Scab Initiative
 FY00 Final Performance Report (approx. May 00 – April 01)
 July 30, 2001**

Cover Page

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Grant Number:	59-0790-9-026
Grant Title:	Fusarium Head Blight Research
2000 ARS Award Amount:	\$80,976

Project

Program Area	Project Title	Requested Amount
Biotechnology	Enhance scab resistance in winter wheat germplasm by plant transformation.	\$70,000.00
Variety Development & Uniform Nurseries	To enhance variety development of scab resistant varieties.	\$70,000.00
	Requested Total	\$140,000.00¹

Principal Investigator

Date

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

Project 1: Enhance scab resistance in winter wheat germplasm by plant transformation.

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

To date, little genetic resistance to *Fusarium spp.* has been identified within wheat germplasm. The tools of biotechnology provide an avenue to introduce into elite wheat germplasm novel genes to complement an integrated program to manage *F. graminearum* pathogenesis. Therefore, the goal of this project is to evaluate transgenes with antimicrobial activity and transgenes involved in inhibition of apoptosis in wheat for durable field resistance towards *F. graminearum*.

2. What were the most significant accomplishments?

There are three main accomplishments in this research. First, we continue to test and advance our initial set of transgenes (Inhibitor of apoptosis [IAP] and lactoferricin-like) and they continue to show promise in our greenhouse screen for providing tolerance to *F. graminearum*. We have now tested the IAP transgene from a number of events over three generations and continue to find a level of tolerance (disease rating of 10%) similar to that of ND2710, our scab tolerant check. Wheaton and Bobwhite (susceptible checks) generally have disease ratings of 100%, but greenhouse variability is present and a few plants have lower values. Based on the continued low disease ratings, the IAP transgenic lines are being increased for field-testing in our scab nursery in 2002 and will be crossed into elite cultivars. For the lactoferricin-like transgene, nine western positive plants were obtained and 8-15 progeny of 3 transgenic lines were tested for type-II tolerance in green house. The plants were either 100% susceptible or had a high level of tolerance (disease rating 10). The tolerance correlated with the expression of the lactoferricin-like protein. In line 1, out of 15 plants, 6 were resistant (disease rating 10%) and 9 were susceptible (disease rating 100%); in line 2, out of 14 plants, 9 were resistant (disease rating 10%) and 5 were susceptible (disease rating 100%); and in line 3, out of 8 plants, 5 were resistant (disease rating 10%) and 3 were susceptible (disease rating 100%). Some PCR positive plants were susceptible, however, no protein could be detected in these plants. We are currently assembling binary vectors in which multiple transgenes are being pyramided to potentially enhance the disease tolerance phenotype observed in single gene events. Vector combinations of Bcl-xl, bovine lactoferrin and maize RIP are being generated.

The second accomplishment was the development of additional lines with antiapoptotic and antifungal/microbial genes. Specifically, we are characterizing more than 10 events of antiapoptotic genes, Bcl-xl and ced-9. Transformations are currently being conducted with a maize ribosomal inactivating protein. We are also building lactoferricin-like constructs based on non-mammalian sources for future transformation experiments to avoid possible consumer concerns, as well as, proprietary conflicts for gene ownership.

The third accomplishment is that we have developed an efficient wheat transformation system using *Agrobacterium tumefaciens*. We have shared and will continue to share our transformation protocols and *A. tumefaciens* strains with numerous transformation efforts within the U.S. Wheat & Barley Scab Initiative.

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?

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 conventional 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 tolerance 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. The specific objectives of our efforts to determine the level and need for FHB tolerance in dryland and irrigated wheat production are: A) to survey dryland and irrigated wheat production fields throughout Nebraska for the level of FHB infection, and B) determine the level of FHB tolerance in the varieties grown under these diverse conditions.

2. What were the most significant accomplishments?

There are two major accomplishments in our research. The first was the development of an irrigated misting nursery which lead to the field screening of 60 advanced lines, 60 intermediate lines, and 300 early generation lines from the Nebraska breeding program, 49 lines in the Regional Fusarium Headblight Nursery, and 396 lines from the Regional Germplasm Observation Nursery (RGON, which includes advanced lines from every breeding program in the hard winter wheat region). The abbreviated protocol was: Strips of agar colonized with *Fusarium graminearum* were added to autoclaved corn. The corn in the pans was stirred in a laminar flow hood with a metal spoon every 6 days, to keep the kernels from clumping. The inoculum was incubated for at least 2 weeks prior to field inoculation. Four applications of 50g of corn inoculum/1m² were broadcast onto the 10 000 ft² wheat field. The applications were made on a weekly basis from 5/22, to 6/18. A misting system, with 50psi on 10 ft risers, kept the inoculum moist and allowed *F. graminearum* germination. The irrigation system was turned off on 6/26. The severity of the scab infection was evaluated, on 6/29 by visually estimating the rate of spread on infected heads. Partially resistant lines had infections of less than 40%, and susceptible lines reactions had higher percentages. As expected the level of tolerance in the hard wheat region was low. Of the early generation lines, only 6 lines (out of 300) had scores of 10% or lower and another 14 appeared to be segregating for tolerance. Of the intermediate and advanced Nebraska lines, only 2 lines (out of 120) had scores of 10% or less. In the RGON, only 3 lines (out of 396) had scores of 10% or less. Further testing will be needed to insure these lines are not random escapes. The

second accomplishment was development of new F₁ to F₅ populations using known scab tolerant parent lines for our breeding program.

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.

Sato, S. J., J. A. Schimelfenig, S. Mitra, T.E. Clemente, A. Mitra, M. Dickman, J.E. Watkins, and P.S. Baenziger. 2000. Preliminary Characterization of Wheat Events Harboring Novel Transgenes for Scab Resistance. 2000 National Fusarium Head Blight Forum. Holiday Inn Cincinnati-Airport Erlanger, KY December 10-12, 2000. pg. 45

Baenziger, P. S. R.A. Graybosch, J.E. Watkins, J.A. Schimelfenig, and D. Baltensperger. 2000. RGON: A Regional Strategy for Fusarium Head Blight Improvement. 2000 National Fusarium Head Blight Forum. Holiday Inn Cincinnati-Airport Erlanger, KY December 10-12, 2000
U.S. Wheat & Barley Scab Initiative Forum, Cincinnati, OH. pg. 199.