U.S. Wheat and Barley Scab Initiative
FY01 Final Performance Report (approx. May 01 – April 02)
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Cover Page

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Grant Number:	**59-0790-9-026**
Grant Title:	**Fusarium Head Blight Research**
FY01 ARS Award Amount:	**$ 110,000**

Project

Program Area	Project Title	Requested Amount
Biotech	Enhanced Scab Resistance in Winter Wheat Germplasm by Plant Transformation	$ 70,000
Variety/Uniform	To Enhance Variety Development of Scab Resistant Varieties	$ 50,000
	Total Amount Requested	**$ 120,000**

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Principal Investigator Date

(Form – FPR01)

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 an important and emerging pathogen of wheat not effectively managed chemically or by genetic resistance. We are implementing the tools of biotechnology to enhance wheat germplasm for resistance towards this devastating pathogen. Over the last three years we have introduced into wheat via *Agrobacterium*-mediated gene transfer a number of potential useful anti-fungal transgenes. These include a lytic protein, lactoferrin, and a peptide derivative thereof lactoferricin, a maize leaf ribosomal inactivating protein and a series of novel negative regulators of programmed cell death including, inhibitor of apoptosis (IAP) from a baculovirus, ced-9 from C. elegans, and bcl-xl from chicken. When challenged with the pathogen under greenhouse conditions we have observed significant enhancement in type-II resistance from both the lytic peptide strategy and modulating program cell death approach. For example, we earlier generated nine transgenic wheat lines that carried a synthetic DNA containing a consensus monocot optimized 41 amino acid long lactoferricin-like sequence. A total of 33 progenies of these lines showed high level of resistance (less than 10% infection) when for tested type-II resistance in green house by artificial inoculation. R3 progenies from these lines were obtained and reinoculated in green house condition. 61% of the plants retained high level of resistance. These are being tested for homozygous individuals. To advance this area, the bovine lactoferrin gene was obtained from Dr. Mike Powell and modified for expression in plant by removal of signal peptide and optimization of translational start sequence. Two constructs were made to express the gene by either using the Ubi promoter from maize or the A-16 promoter from a chlorella virus. Bob White cultivar was transformed by *Agrobacterium* methods. Use of bovine gene will allow us to conduct a field evaluation next year.

Currently we are pyramiding subsets of these transgenes via sexual crossing to determine if a combination of these transgenes will display additive resistance. Moreover, our lead lines derived from the respective transformations are being crossed into adapted winter wheat cultivars to combine transgenic and natural tolerance. In addition, a series of additional transformations are planned to introduce into wheat an alternative synthetic lytic peptide and a codon optimized version of a bovine lactoferrin gene (mentioned above).

2. What were the most significant accomplishments?

In order to effectively assess the resistance phenotype and agronomic performance of the novel germplasm produced by our wheat biotechnology team it is essential that field trials be conducted over multiple years and desirably different environments. To this end we have established our first field trials this year in Mead, NE. Wheat lines carrying bcl-xl, ced-9 and IAP transgenes have been incorporated into our scab nursery. Although this growing season has been rather poor for the Nebraska wheat crop due environmental stresses (less than 0.5 cm of rain in June) we are confident the field studies using misting will permit a more realistic evaluation of the scab resistance phenotype than our greenhouse assays. Despite the drought, we obtained and excellent *Fusarium graminearum* infection. We have recorded average scab severity per plot (i.e.: scab index), average scab severity per infected head and average scab intensity per plot. The scab results from the transgenic trial were promising, though due to a low infection of the control not significant. Three of the events possessing transgenes: Bcl X(T), ced 9 and IAP, were three times more scab resistant than Bob White, the control.

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, 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. The specific objectives in our variety development effort are: A) collect FHB resistant germplasm from traditional sources and our transgenic efforts, B) incorporate the resistant germplasm primarily into hard winter wheat germplasm (white and red) by crossing, but for the transgenes, also into hard spring wheats and soft winter wheats, and C) using a modified bulk breeding method to advance the germplasm to elite line status.

As part of this effort, we have worked to refine our *Fusarium* tolerance screens. Great improvements have been made in our field misting system by planting earlier, growing better quality wheat plants, spraying the inoculum on, and having excellent misting cycles (basically the misters are on 2 minutes every half hour during the misting period). While it is too early to summarize our results for this year, more FHB was identified in the nursery than last year, despite this season being extraordinarily hot and dry. In our greenhouse screens, we tried a number of different methods including needle inoculation followed by misting, needle inoculation following by bagging, needle inoculation without bagging or misting (basically just left on the greenhouse benches), and spraying plants with inoculum before misting. While each method has it benefits, spraying followed by misting seems to provide the clearest differences. Needle inoculation with or without bagging also seemed to work well and would be a valuable tool for screening transgenic lines where every tiller is important.

In working with the Dr. Anne McKendry of the germplasm identification effort, we crossed every new line she identified to our adapted material. Our previous crosses to FHB tolerant lines are now being selected for lines development (snapping heads from bulks). Many of the lines appear to have agronomic merit.

2. What were the most significant accomplishments?

We now have lines in the F_1 to F_5 generation that were specifically developed to have FHB tolerance. We continue to improve our screening capabilities, both in the greenhouse and in the field. The improvements in the field were particularly important as we feel it is the most realistic test and the misting system worked (e.g. fostered scab infection) despite the driest June in recorded history in eastern NE. The volume of material screened in the GH was increased 3.5 fold.

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., T. Clemente, X. Ye, M. Dickman, P.S. Baenziger, A. Mitra, J. Schimelfenig, S. Mitra, and J. Watkins. 2001. Evaluation of transgenic wheat lines expressing the baculovirus Op-IAP for tolerance to scab induced by *Fusarium graminearum*. 2001 National Fusarium Head Blight Forum. Cincinnati, OH. p. 24.

Baenziger, P.S., J. Schimelfenig, and J. E. Watkins. 2001 The development of scab (*Fusarium graminearum*) resistant varieties of wheat. 2001 National Fusarium Head Blight Forum. Cincinnati, OH. p. 226.

Peterson, C.J., D. R. Shelton, P.S. Baenziger, D. D. Baltensperger, R. A. Graybosch, W. D. Worrall, L.A. Nelson, D. V. McVey, J. E. Watkins, and J. Krall. 2001. Registration of 'Wesley' Wheat. Crop Sci. 41:260-261.

P. S. Baenziger, B. Moreno-Sevilla, C. J. Peterson, D. R. Shelton, R. W. Elmore, P.T. Nordquist, R. N. Klein, D. D. Baltensperger, L. A. Nelson, D. V. McVey, J. E. Watkins, J. H. Hatchett, and R.A. Graybosch. 2001. Registration of 'Cougar' Wheat. Crop Sci. 41: 1360-1361.

P. S. Baenziger, B. Moreno-Sevilla, C. J. Peterson, D. R. Shelton, R. W. Elmore, P.T. Nordquist, R. N. Klein, D. D. Baltensperger, L. A. Nelson, D. V. McVey, J. E. Watkins, J. H. Hatchett, and G. Hein. 2001. Registration of 'Millennium' Wheat. Crop Sci.411367-1369.