

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY06 Final Performance Report (approx. May 06 – April 07)
July 16, 2007**

Cover Page

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Fiscal Year:	2006
USDA-ARS Agreement ID:	59-0790-6-067
USDA-ARS Agreement Title:	Engineering Fusarium Head Blight Resistance and Plant Defense Signaling.
FY06 ARS Award Amount:	\$ 122,966

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Award Amount
GET	Engineering Scab Resistance in Wheat with Plant Defense Signaling Genes.	\$ 65,595
HGG	Signaling Mechanism Associated with Host Defense against Fusarium graminearum.	\$ 57,371
	Total Award Amount	\$ 122,966

7-3-07

Principal Investigator

Date

* CBCC – Chemical, Biological & Cultural Control
 EEDF – Etiology, Epidemiology & Disease Forecasting
 FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
 GET – Genetic Engineering & Transformation
 HGR – Host Genetics Resources
 HGG – Host Genetics & Genomics
 PGG – Pathogen Genetics & Genomics
 VDUN – Variety Development & Uniform Nurseries

Project 1: *Engineering Scab Resistance in Wheat with Plant Defense Signaling Genes.*

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

Annual losses due to Scab (*Fusarium* head blight) disease of wheat in the US have averaged \$ 200-400 million. *Fusarium graminearum* is the principal causative agent of wheat scab in the US. Development of Scab resistant wheat varieties has been limited by the absence of any known source of monogenic resistance against *F. graminearum*. Genetic engineering provides an alternative approach for developing Scab resistant wheat. Previously, it was shown that ectopic expression of the *Arabidopsis thaliana NPR1* (*AtNPR1*) gene from the maize ubiquitin promoter in transgenic wheat cv. Bobwhite enhanced Scab resistance under greenhouse conditions. *NPR1* is a key regulator of salicylic acid-dependent defense responses in plants and ectopic expression of *AtNPR1* in wheat promotes faster activation of defenses in transgenic wheat. As part of this USDA-ARS USWBSI-sponsored project we have further studied *Ubi1:AtNPR1*-conferred Scab resistance under field conditions. Furthermore, we have engineered *AtNPR1* expression in Durum wheat.

**2. List the most important accomplishment and its impact (how is it being used?).
Complete all three sections (repeat sections for each major accomplishment):**

Accomplishment:

In a field trial conducted in spring 2005 in Kansas, we had observed that *Ubi1:AtNPR1* construct enhanced Scab resistance in the hexaploid wheat cultivar Bobwhite. A second field trial conducted in Manhattan during spring 2006 confirmed the *Ubi1:AtNPR1*-conferred enhancement of resistance against *F. graminearum*. An independent field trial was conducted in spring 2006 in Minnesota. However, due to dry conditions in Minnesota during spring 2006, disease prevalence was low in transgenic and non-transgenic controls.

We have developed several independent *Ubi1:AtNPR1* transformants for the Durum cultivars Ben and Maier. In preliminary studies with *Ubi1:AtNPR1* transgenic cultivar Ben plants, we have observed enhanced resistance to *F. graminearum* in greenhouse studies. Additional transformation experiments in Durum and hexaploid wheat are in progress. The *Ubi1:AtNPR1* construct was provided to Dr. Lynn Dahleen at USDA-ARS (Fargo, North Dakota) who will test the efficacy of this construct in transgenic barley. Since *Ubi1:AtNPR1* also promotes resistance against powdery mildew, this transgene provides the added advantage of promoting resistance against a broad-spectrum of pathogens.

Impact: Our studies demonstrate that the *AtNPR1* regulated pathway is an excellent target for engineering Scab resistance in Durum and hexaploid wheat. The introduction of *AtNPR1* into elite cultivars will expedite the availability of resistant germplasm to Scab breeding programs.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?

The introduction of the *Ubi1:AtNPR1* construct into elite cultivars is expected to expedite the availability of Scab resistant genetic material for integration into wheat breeding programs. These transgenic plants will also provide important genetic material for understanding wheat defense mechanisms against this devastating pathogen.

Project 2: Signaling Mechanism Associated with Host Defense against *Fusarium graminearum*.

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

Fusarium graminearum is an important pathogen of small grains. In wheat and barley, *F. graminearum* is the principal cause of *Fusarium* head blight (Scab) disease. Monogenic gene-for-gene resistance has not been identified for this pathogen, thus limiting the development of Scab resistant wheat and barley. The development of strategies to control this pathogen has been further hampered by the lack of knowledge on host defense mechanism against the pathogen. We have utilized the model plant *Arabidopsis thaliana*, which is also a host for this pathogen, to characterize plant mechanisms that contribute to defense and susceptibility to this pathogen. We have utilized a combination of genetic, molecular and biochemical approaches to identify phytohormones involved in resistance and susceptibility. In the future, these could be targeted for controlling Scab in wheat and barley.

**2. List the most important accomplishment and its impact (how is it being used?).
Complete all three sections (repeat sections for each major accomplishment):**

Accomplishment:

Our studies indicate that salicylic acid (SA) biosynthesis and signaling are essential for basal resistance to *F. graminearum* in leaves and flowers of *Arabidopsis thaliana*. Mutations in *Arabidopsis* genes that block SA synthesis and signaling resulted in enhanced susceptibility to the fungus. In contrast, *Arabidopsis* mutants in which SA signaling is hyperactivated exhibited heightened resistance to *F. graminearum*. The application of SA or benzothiadiazole (BTH), a synthetic functional analog of SA, to *Arabidopsis* and a variety of wheat cultivars enhanced resistance against *F. graminearum*, confirming the involvement of SA signaling in host defense against *F. graminearum*.

In contrast to SA, prior exposure to methyljasmonate enhanced susceptibility to *F. graminearum* in wheat and *Arabidopsis*, suggesting that jasmonic acid (JA) may contribute to susceptibility. In agreement with a role of JA in susceptibility to this fungus, the JA-insensitive *jar1* mutant of *Arabidopsis* exhibited heightened resistance to *F. graminearum*. Similar to JA, ethylene signaling also contributes to susceptibility to this fungus.

Impact: Since SA is an important signaling molecule in host defense against *F. graminearum*, SA synthesis and signaling can now be targeted for promoting resistance against Scab. For example, we have demonstrated that ectopic expression of *AtNPR1*, a key regulator of SA signaling in *Arabidopsis*, can be utilized for enhancing Scab resistance in wheat. Similarly, compounds that efficiently activate SA signaling in wheat could be designed to promote Scab resistance.

As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?

The identification of salicylic acid as a critical mediator of plant defense against *F. graminearum* and jasmonic acid and ethylene as susceptibility factors allows for these pathways to be targeted for controlling Scab in wheat and possibly barley.

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.

Publications:

Chaturvedi, R., and Shah, J. (2006). Salicylic acid in plant disease resistance. In "Salicylic Acid-A Plant Hormone" ed. S. Hayat and A. Ahmad, pp 335-370, Springer, Dordrecht, The Netherlands.

Makandar, R. and Shah, J. Salicylic acid promotes while jasmonic acid antagonizes *Arabidopsis* defense against *F. graminearum*. Manuscript in preparation for submission to Mol. Plant-Microbe Interact.

Presentations:

Invited Lecture: Oklahoma State University, Stillwater, OK. April 13, 2007
Title: Plant Response to Biotic Stress.
Presenter: Jyoti Shah

Invited Lecture: Kansas State University Functional Genomics Consortium Workshop. April 5, 2007.
Title: Lipids in plant defense against pathogens.
Presenter: Jyoti Shah

Invited Lecture: College of Life Sciences, Jawaharlal Nehru University, New Delhi, India. June 15 2006.
Title: Lipids in plant defense
Presenter: Jyoti Shah

Meeting: Great Plains Plant Lipidomics Consortium Retreat, Danforth Plant Sciences Center, St. Louis; May 2, 2006
Title: Lipid biosynthesis and metabolizing enzymes in plant defense response
Presenter: Jyoti Shah

Posters:

Conference: Proceedings of the 2006 National Fusarium Head Blight Forum, Raleigh, NC. December 2006.
Title: Engineering Scab Resistance in Wheat with Plant Defense Signaling Genes.
Authors: R. Makandar, V. Nalam, M. Schapaugh, W. Bockus, R. Dill-Macky and H.N. Trick

Conference: Proceedings of the 2006 National Fusarium Head Blight Forum, Raleigh, NC. December 2006.
Title: Signaling Mechanisms involved in plant defense against *Fusarium graminearum*.
Authors: R. Makandar, V. Nalam, D. Maier and H.N. Trick

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Conference: 17th International Conference on Arabidopsis Research, Madison, Wisconsin.
June-July 2006,

Title: *Arabidopsis* as a model system to study plant defense against *Fusarium graminearum*, the causative agent of Scab in wheat and barley

Authors: Vamsi Nalam, Ragiba Makandar, Darcy Maier, Harold Trick and Jyoti Shah

Meeting: KSU Division of Biology Undergraduate Research Scholar's Forum, Kansas State University, Manhattan, Kansas; April 2006

Title: *Fusarium graminearum* co-opts the *Arabidopsis thaliana* *LOX1* gene for pathogenicity

Authors: Darcy Maier, Ragiba Makandar, Vamsi Nalam, Jyoti Shah