USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY07 Final Performance Report (approx. May 07 – April 08)
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Cover Page

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USDA-ARS Agreement ID:	59-0790-6-067
USDA-ARS Agreement Title:	Engineering Fusarium Head Blight Resistance and Plant Defense Signaling.
FY07 ARS Award Amount:	$ 88,722

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
GET	Engineering Scab Resistance in Wheat with Defense Signaling Genes.	$46,185
HGG	Signaling Mechanism in Host Defense and Susceptibility to Fusarium graminearum.	$ 42,537
	Total Award Amount	**$ 88,722**

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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
IIR – Integrated/Interdisciplinary Research
PGG – Pathogen Genetics & Genomics
VDUN – Variety Development & Uniform Nurseries

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

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

Losses to Fusarium head blight (FHB; also known as Scab) disease of wheat and barley have averaged $200-400 million per annum. *Fusarium graminearum* is the leading agent of FHB in the US. Monogenic resistance against FHB is not known and current control methods utilize a combination of planting partially resistant varieties with fungicide application and crop rotation. Genetic engineering provides an alternative approach for developing wheat and barley germplasms with heightened resistance to FHB. It provides the advantage that novel genes and chimeras that are not currently in the partially resistant germplasms, can be introduced into wheat and barley, thus adding to the repertoire of genes that can be utilized in breeding programs for enhancing FHB resistance. Previously, ectopic expression of the *Arabidopsis thaliana NPR1* (At*NPR1*) gene from the maize ubiquitin promoter was shown to enhance FHB resistance in the partially FHB-resistant cv. Bobwhite under greenhouse conditions and in two field trials conducted in Kansas. *NPR1* controls the activation of salicylic acid-dependent defense responses in plants, which our studies have demonstrated, is important for resistance to *F. graminearum* in *Arabidopsis thaliana*. *PAD4* is another gene from *Arabidopsis thaliana* that enhances resistance to *F. graminearum*. *PAD4* modulates salicylic acid synthesis in pathogen inoculated plants and production of antimicrobial phytoalexins. In addition, it controls other poorly defined defense mechanisms. As part of this USDA-ARS USWBSI-sponsored project we have continued field trials of At*NPR1* expressing cv. Bobwhite and have introduced the At*NPR1*-conferred Scab resistance into two durum cultivars. Furthermore, we have engineered At*PAD4* into 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):

(a) **Accomplishment:** The At*PAD4* gene has been expressed in wheat cv. Bobwhite and in durum cv. Ben. Four independent At*PAD4* cv. Bobwhite lines and three cv. Ben lines have been identified.
Impact: Homozygous progeny derived from these At*PAD4* expressing lines will be evaluated for FHB resistance in the greenhouse.
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?:
These At*PAD4* lines will not only provide germplasms that can be utilized in future breeding programs, but will also provide germplasms that can be utilized to study the involvement of this gene in wheat resistance to aphids, since PAD4 controls aphid resistance, as well.

(b) **Accomplishment**: At*NPR1* has now been expressed in durum cvs. Ben and Maier. Three homozygous lines in cv. Ben and two in cv. Maier are being propagated further. These five lines have shown the best level of enhanced FHB resistance compared to the non-transgenic cv. Ben and Maier plants.
Impact: These germplasms will be made available to the Scab community later this year.
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*:At*NPR1* construct into durum cultivars is expected to provide new resistant germplasms for integration into durum breeding programs.

(c) **Accomplishment:** Unlike two previous field trials in Kansas, in which At*NPR1* expressing cv. Bobwhite plants showed enhanced FHB resistance, no differences in FHB severity between the controls and the AtNPR1 transgenic plants was observed in a field trial conducted in spring 2007 in Minnesota.

Impact: Additional trials are underway to study the conditions under which AtNPR1 conferred expression is most durable. .

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?:

Results from a series of field trials at different locations across the country will determine the conditions under which At*NPR1* expression provides good protection against FHB.

Project 2: *Signaling Mechanism in Host Defense and Susceptibility to Fusarium graminearum.*

1. **What major problem or issue is being resolved and how are you resolving it?**
   Fusarium graminearum is an important pathogen of wheat and barley, and other small grains. 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* 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. These studies have indicated that salicylic acid and jasmonic acid signaling influence Arabidopsis defense against *F. graminearum*. We are simultaneously testing the role of these mechanisms in controlling FHB resistance in wheat, and using the Arabidopsis-*F. graminearum* interaction to identify additional genes important for host defense and susceptibility to this fungus.

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

   (a) **Accomplishment:** We have developed several transgenic wheat plants that express the bacterial *NahG* gene, which encodes salicylate hydroxylase that degrades salicylic acid. In addition, we have identified Arabidopsis *WRKY18* as another gene that is a good candidate for enhancing FHB resistance in wheat and barley. In addition, we have demonstrated that a systemic acquired resistance (SAR) inducing activity purified from Arabidopsis also induces FHB resistance in wheat. Finally, our studies indicate that salicylic acid-regulated defenses target fungal spore germination.
   Impact: The NahG transgenic plants are being propagated further. Once homozygous lines are identified we will use these to study the role of salicylic acid in wheat defense against *F. graminearum*. A construct for expressing WRKY18 in wheat is being developed and this will be used to transform hexaploid and durum wheat to determine if it enhances FHB resistance in wheat. Our results indicate that studies in Arabidopsis can expedite the identification of plant genes and factors that can promote FHB resistance in wheat.
   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?:
   NahG wheat lines have never been developed. These lines will provide an excellent genetic tool for the community to study plant defense against other pathogens, as well.

   (b) **Accomplishment:** We have uncovered a dual role for jasmonic acid in plant (Arabidopsis and wheat) interaction with *F. graminearum*. During the early stages of infection in wheat (first two days), jasmonic acid contributes to susceptibility by slowing down the activation of salicylic acid dependent defenses. In contrast, at later time points (4 dpi onwards), jasmonic acid enhances resistance, presumably by preventing spread of fungus.

Impact: These results indicate that for high level FHB resistance in wheat, both salicylic acid and jasmonic acid signaling need to be targeted. We are working towards strategies to target both these mechanisms.

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?:

These studies have uncovered a complex interaction between defense signaling mechanisms in wheat interaction with *F. graminearum*. Furthermore, these results indicate that in susceptible varieties the pathogen hijacks the plants jasmonic acid machinery to suppress the timely activation of defenses. Controlling this process in the host or the pathogens ability to do this, is key for developing good resistance to FHB.

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., Krothapalli, K., Makandar, R., Nandi, A., Sparks, A., Roth, M., Welti, R. and Shah, J. (2008) Plastid ω-3 desaturase-dependent accumulation of a systemic acquired resistance inducing activity in petiole exudates of *Arabidopsis thaliana* is independent of jasmonic acid. Plant J. 54: 106-117.

Makandar, R., Nalam, V., Essig, J.S., Schapaugh, M.A., Trick, H., Dill-Macky, R., and Shah, J. (2007) Enhancing Fusarium head blight resistance in wheat by manipulating mechanisms contributing to host resistance and susceptibility. In: Proceedings of the 2007 National Fusarium Head Blight Forum; Dec 2-4 (http://www.scabusa.org).

Makandar, R., Chaturvedi, R., Sparks, A., and Shah, J. *Fusarium graminearum* macroconidia germination is inhibited by a salicylic acid and *NPR1* (*NONEXPRESSER OF PR GENES 1*)-dependent mechanism that is constrained by jasmonate signaling in *Arabidopsis thaliana*. (submitted).

Makandar, R., Nalam, V., Trick, H., and Shah, J. A complex interaction between salicylic acid and jasmonic acid controls wheat interaction with *Fusarium graminearum*, the causative agent of Fusarium head blight. (submitted).

Presentations:

1. Title: *Engineering Scab Resistance with Plant Defense Signaling Genes*, at the National Fusarium Head Blight Forum, Kansas City, Missouri; December 2007 Presenter: Jyoti Shah
2. Title: *Targeting Scab with Defense Regulatory Genes*, at the 5th Canadian Workshop on Fusarium Head Blight, Winnipeg, Canada; November 2007; Presenter: Jyoti Shah
3. Title: *Plants at War*, Departmental of Biological Sciences Seminar, University of North Texas, Nov 16 2007; Presenter: Jyoti Shah