USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY09 Final Performance Report
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Cover Page

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Fiscal Year:	2009
USDA-ARS Agreement ID:	59-0790-8-060
USDA-ARS Agreement Title:	Engineering Fusarium Head Blight Resistance and Plant Defense Signaling.
FY09- USDA-ARS Award Amount:	$ 47,528

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
GDER	Targeting Host Defense Mechanism for Enhancing FHB Resistance in Wheat.	$ 47,528
	Total Award Amount	**$ 47,528**

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Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Winter Wheat Region
SWW – Southern Sinter Wheat Region

Project 1: *Targeting Host Defense Mechanism for Enhancing FHB Resistance in Wheat.*

1. What major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Genetic engineering provides an alternative approach for developing wheat and barley germplasms with heightened resistance to Fusarium head blight (FHB; also known as Scab), a devastating disease of small grains. Losses due to FHB 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 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* and *WRKY18* are two other genes that when overexpressed in *Arabidopsis thaliana* enhance resistance to *F. graminearum*. *PAD4* modulates salicylic acid synthesis and *WRKY18* is a transcription factor functioning in the SA signaling pathway. In addition, 9-lipoxygenases contribute to Arabidopsis susceptibility to *F. graminearum*, presumably by providing lipid signals that are required for the fungus for pathogenicity. As part of this USDA-ARS USWBSI-sponsored project we have engineered At*PAD4* and At*WRKY18* into wheat, and generated transgenic wheat expressing RNAi constructs to silence expression of three wheat lipoxygenase encoding genes. In addition, we have evaluated the impact of a newly identified plant defense-inducing diterpenoid in promoting resistance against *Fusarium graminearum* and generated transgenic NahG plants to further evaluate the role of salicylic acid in wheat resistance to *F. graminearum*.

2. List the most important accomplishment and its impact (i.e. how is it being used) to minimize the threat of Fusarium head blight or to reduce mycotoxins. Complete both sections (repeat sections for each major accomplishment):

(a) **<u>Accomplishment</u>:** Two At*PAD4* expressing wheat cv. Bobwhite have been propagated to the T4 generation. Preliminary studies indicate that one of these lines exhibits enhanced resistance to *F. graminearum*. Additional repeat experiments are underway to confirm these results.

<u>Impact</u>: Transgenic lines repeatedly exhibiting enhanced FHB resistance will be bulked for future field trials in Minnesota. In addition, At*PAD4* will be coexpressed with At*NPR1* in wheat to determine if the combined expression of these two genes can further enhance FHB resistance.

<u>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?:</u>

These At*PAD4* lines will not only provide germplasms that potentially 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**: Three independent transgenic wheat lines (cv. Bobwhite) expressing At*WRKY18* have been identified and propagated to the T2 generation.
Impact: Homozygous plants will be identified for each of these lines for evaluating the impact of this recombinant construct on FHB resistance and DON content.
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 *AtWRKY18* construct into wheat is expected to provide new, potentially FHB resistant germplasms.

(c) **Accomplishment:** Three homozygous wheat lines (derived from one transformation event) that express the bacterial *nahG* gene, which encodes salicylate hydroxylase that degrades salicylic acid have been characterized for SA content and preliminary evaluation of FHB resistance. Expression of *nahG* compromised the basal content of SA in these transgenic plants and also resulted in enhanced disease susceptibility in preliminary experiments.
Impact: The NahG transgenic line will be used to study the role of salicylic acid in wheat defense against *F. graminearum* and to evaluate the impact of other defense inducers on activation of SA signaling.
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 NahG wheat line will provide an excellent genetic tool for the community to study plant defense against other pathogens, as well. In addition, these lines will be useful for studying the mechanism of action of other inducers of FHB resistance.

(d) **Accomplishment**: Several transgenic lines containing three RNAi constructs for silencing three different LOX genes in wheat (cv. Bobwhite) have been identified and are currently being propagated.
Impact: Homozygous lines (once identified) will be used to determine the extent of gene silencing and to study the impact of silencing these LOXs on FHB resistance/susceptibility. In corn, different LOXs have opposite impacts on resistance to fungal pathogens, some promoting resistance and others contributing to susceptibility. It is likely that one or more of these LOXs when silenced in wheat may enhance FHB 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?:
Transgenic LOX-silenced lines might alter resistance not only to FHB but also to other fungal pathogens. Lines exhibiting enhanced resistance will provide novel germplasms for breeding FHB resistance.

(e) **Accomplishment**: We have identified a novel plant derived diterpenoid that enhances systemic resistance against a variety of pathogens, including *Fusarium graminearum* in Arabidopsis by systemically activating SA signaling.
Impact: This diterpenoid defense activator could provide a new approach to promote host resistance that could complement current approaches to control FHB.
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?:
This diterpenoid , which is effective in the picomolar levels in enhancing disease resistance in dicots, if effective in stimulating defenses in wheat would provide an alternative strategy that complements existing approaches to control a broad-spectrum of diseases in monocots, including wheat.

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 (peer reviewed):

- Makandar, R., Nalam, V., Chaturvedi, R., Jeannotte, R., Sparks, A.A., and Shah, J. (2010) Involvement of salicylate and jasmonate signaling pathways in Arabidopsis interaction with *Fusarium graminearum*. Mol. Plant-Microbe Interact. 23:861-870.
- Shah, J. (2009) Plants under attack: systemic signals in defence. Curr. Opin. Plant Biol. 12:459–464.
- Chaturvedi, R., Venables, B., Petros, R., Nalam, V., Li, M., Wang, X., Takemoto, L.J., and Shah, J. (2010) Dehydroabietinal signaling in systemic acquired resistance. Science (under review).

Proceedings:

- Dahleen, L., Dill-Macky, R., Shah, J., Muehlbauer, G., Skadsen, R., Manoharan, M., Abebe,T., and Jurgenson, J. (2009) Transgenic field trials for FHB resistance and related research in wheat and barley. In: Ouellet, T and Leger, D. (eds.). Proceedings of the 6th Canadian Workshop on Fusarium Head Blight. Nov. 1-4, 2009. Ottawa, ON, Canada. Page 38.
- Nalam, V., Makandar, R., McAfee, D., Essig, J., Lee, H., Trick, H., and Shah, J. (2009) 'Host factors contributing to resistance and susceptibility to *Fusarium graminearum*- role of lipoxygenases'. In: S. Canty, A. Clark, J. Mundell, E. Walton, D. Ellis and D. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2009 Dec 7-9; Orlando, FL. Lexington, KY: University of Kentucky. pp. 165. (http://www.scabusa.org).

Presentations:

- Title: Transgenic solutions to wheat biotic stresses
  Conference: Joint Congress of Hard Winter Wheat Workers Workshop and the National Wheat Genomics Workshop, March 7-10, 2010. University of Nebraska-Lincoln.
  Authors: H.N. Trick, J.P. Fellers, M.S. Chen, J. Shah, L. Cruz, X. Liu and V. Nalam
  (Invited *Oral Presentation* by H. N. Trick)

- Title: Systemic acquired resistance in *Arabidopsis thaliana*: a terpenoid is involved in long-distance signaling.
  Conference: International Molecular Plant-Microbe Interaction Congress, Quebec, Canada- July 2009
  Authors: J. Shah, R. Chaturvedi and B. Venables
  (*Poster Presentation*)

- Title: Role of Lipoxygenases in Plant defense response.
  Conference: International Molecular Plant-Microbe Interaction Congress, Quebec, Canada- July 2009
  Authors: V. Nalam, D. Maier, J. Louis, R. Chaturvedi and J. Shah
  (*Poster Presentation*)