USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
July 15, 2011

Cover Page

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Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0790-8-060
USDA-ARS Agreement Title:	Engineering Fusarium Head Blight Resistance and Plant Defense Signaling.
FY10 USDA-ARS Award Amount:	$ 47,528

USWBSI Individual Project(s)

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

June 30, 2011

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 Soft Winter Wheat Region
SWW – Southern Soft Red Winter 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?

Fusarium head blight (FHB; also known as Scab) is a devastating disease of small grains, including wheat and barley. In the absence of monogenic resistance against FHB, 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* 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 blocking SA accumulation in transgenic NahG wheat to further characterize the role of salicylic acid in wheat resistance to *F. graminearum*, and have identified two potential activators of resistance against *F. graminearum*. One of these is a plant-derived terpenoid and the second is flg22, which is a bacterial peptide that elicits non-host defenses.

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>Accomplishments</u>

- Two At*PAD4* expressing wheat cv. Bobwhite have been studied in green house experiments. Both these lines exhibits enhanced resistance to *F. graminearum*.
- AtPAD4 expressing wheat has been crossed with an At*NPR1* expressing wheat with the ultimate aim of developing wheat plants that constitutively express both, At*PAD4* and At*NPR1*.

<u>Impact</u>

- Seeds from transgenic At*PAD4* expressing lines exhibiting enhanced FHB resistance have been submitted for mycotoxin testing to determine if At*PAD4*-determined reduction of FHB severity is accompanied by reduced mycotoxin accumulation.
- At*PAD4* wheat are currently undergoing field trials in Minnesota to determine the effectiveness of At*PAD4* in enhancing FHB resistance under field conditions.

- Plants coexpressing At*NPR1* and At*PAD4* will provide germplasms in which both SA synthesis and signaling are promoted and hence potentially offer stronger protection against 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?:
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) that are homozygous for the At*WRKY18* chimera have been evaluated. Two of these lines exhibit enhanced FHB resistance in green house experiments.

Impact

- Seeds from transgenic At*WRKY18* expressing lines exhibiting enhanced FHB resistance have been submitted for mycotoxin testing to determine if At*WRKY18* -determined reduction of FHB severity is accompanied by reduced mycotoxin accumulation.
- At*WRKY18* wheat are currently undergoing field trials in Minnesota to determine the effectiveness of At*WRKY18* in enhancing FHB resistance under field conditions.

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 FHB resistant germplasms.

(c) Accomplishment:

- Transgenic NahG wheat expressing a SA degrading salicylate hydroxylase consistently accumulated less SA and showed higher severity of FHB compared to non-transgenic controls.
- NahG wheat was crossed with At*NPR1* wheat to generate At*NPR1* NahG wheat in which both chimera are expressed. Expression of NahG compromised At*NPR1*-determined resistance, indicating that SA is required for At*NPR1*-determined FHB resistance.

Impact:

- Seeds from At*NPR1* NahG plants have been submitted for mycotoxin testing to determine if expression of NahG also enhances mycotoxin accumulation in these plants, compared to the At*NPR1* plants.

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) **<u>Accomplishment</u>**: Several transgenic lines containing three RNAi constructs for silencing three different LOX genes in wheat (cv. Bobwhite) have been identified and propagated.
In preliminary studies, silencing expression of one gene resulted in enhanced FHB resistance, and silencing expression of another LOX resulted in enhanced FHB susceptibility, suggesting that like in maize different LOXs have opposite impacts on resistance to fungal pathogens, some promoting resistance and others contributing to susceptibility.
Impact: These lines will be used to further characterize the impact of these genes/processes on FHB resistance and their impact on mycotoxin content.
<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>
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) **<u>Accomplishment</u>**: The conserved flg22 peptide from gram negative bacteria, which elicits non-host resistance in dicotyledonous plants, also enhances resistance against *F. graminearum* in Arabidopsis and in wheat spikes. A chimeric construct in which the flg22 peptide has been fused to a plant protein to target it to the extracellular space has been developed. Wheat embryos have been bombarded with this construct to develop wheat plants that express the flg22 peptide in the apoplast.
<u>Impact</u>: Stimulation of wheat defenses by flg22 could provide a new approach to promote non-host resistance that could complement current approaches to control FHB.
<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>
If flg22 expression is effective in stimulating defenses in wheat, it would provide an alternative strategy that complements existing approaches to control a broad-spectrum of diseases in wheat. This would also lay the ground work for similar approaches in other crop plants.

(f) **<u>Accomplishment</u>**: The At*WRKY29* gene, which is required for flg22-elicited non-host resistance in Arabidopsis and resistance against *F. graminearum* in Arabidopsis, is being engineered in wheat. The At*WRKY29* chimeric construct has been bombarded into wheat embryos and transformed calli are currently at the regeneration stage.
<u>Impact</u>: At*WRKY29* expression may bypass the need for flg22 to activate non-host resistance mechanisms in wheat and could complement the flg22 expression approach. Furthermore, these lines in the future could be used to generate plants that simultaneously express flg22 and At*WRKY29* and thus provide stronger FHB resistance in wheat.
<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>
If At*WRKY29* expression is effective in stimulating defenses in wheat, it would provide an additional set of FHB resistant germplasms that could be combined with other genes for strong FHB resistance.

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 and manuscripts submitted:

- Nalam, V., Makandar, R., McAfee, D., Essig, J., Lee, H., Trick, H.N., Shah, J. (2010) Engineering defense regulatory genes and host susceptibility factors for enhancing FHB resistance. In: S. Canty, A. Clark, A. Anderson-Scully, D. Ellis and D. Van Sanford (Eds.), Proceedings of the National Fusarium Head Blight Forum; 2010 Dec 7-9; Milwaukee, WI. Lexington, KY: University of Kentucky. pp. 26.

- Makandar, R., Nalam, V., Klossner, G., Jeannotte, R., Sparks, A.A., Trick, H.N. and Shah, J. Complex interaction between salicylic and jasmonic acid signaling modulates *NPR1* overexpression-conferred enhanced *Fusarium* head blight resistance in wheat. Mol. Plant-Microbe Interact (submitted).

- Chaturvedi, R, Venables, B., Petros, R.A., Nalam, V., Li, M., Wang, X., Takemoto, L.J., and Shah, J. An abietane diterpenoid is a potent activator of systemic acquired resistance. Plant J (submitted).

Oral Presentations:

- Title: Identification of a diterpenoid as a vasculature translocated signal associated with the activation of systemic acquired resistance.
  Conference: Plant Lipids: Structure, Metabolism and Function, Galveston TX, Jan 30-Feb 4, 2011
  Authors: Chaturvedi, R., Nalam, V., Venables, B., Petros, R., and Shah, J.
  Oral Presentation: Jyoti Shah

- Title: Identification of a diterpenoid as a vasculature translocated signal associated with the activation of systemic acquired resistance
  Conference: Plant Vascular Biology 2010, Columbus, Ohio, July 24-28, 2010
  Authors: Chaturvedi, R., Nalam, V., Venables, B., Petros, R., and Shah, J.
  Oral Presentation: Ratnesh Chaturvedi