USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY13 Final Performance Report
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Cover Page

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Fiscal Year:	FY13
USDA-ARS Agreement ID:	59-0200-3-003
USDA-ARS Agreement Title:	Engineering Fusarium Head Blight Resistance and Plant Defense Signaling.
FY13 USDA-ARS Award Amount:	$ 62,314

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Targeting Host Defense Mechanism for Enhancing FHB Resistance in Wheat.	$ 62,314
	FY13 Total ARS Award Amount	**$ 62,314**

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) is an important disease of wheat and barley that affects grain yield and quality. Mycotoxins produced by the fungus further limit the use of grain from infected plants. Genetic engineering is being utilized to develop wheat with heightened resistance to FHB. Three approaches have been undertaken. (1) A gene involved in the regulation of plant defense against *Fusarium graminearum*, which was initially identified and characterized in *Arabidopsis thaliana*, was engineered in wheat to promote resistance against FHB. (2) The feasibility of engineering flg22, a bacterial-derived Microbe Associated Molecular Pattern (MAMP), to promote non-specific pathogen-triggered immunity (PTI) against *F. graminearum* was tested in *Arabidopsis thaliana* and subsequently utilized to engineer FHB resistance in wheat. (3) Expression of a wheat gene that promotes susceptibility to FHB was silenced by RNA-interference to promote resistance against FHB.

Progress:

(1) *WRKY29* encodes a transcription factor that is involved in the regulation of Arabidopsis defenses against pathogens, including basal resistance against *F. graminearum. WRKY29* was expressed from the maize *Ubi* promoter in the wheat cv Bobwhite. Seven independent *Ubi:WRKY29* transgenic lines were generated, evaluated for transgene expression. Furthermore, two lines have been evaluated for their reaction to *F. graminearum*. These two lines exhibited enhanced resistance to head blight as well as seedling blight caused by *F. graminearum* in growth chamber experiments. The other five lines have been propagated to identify homozygous progeny.

(2) The bacterial flg22 peptide, which induces PTI, when applied to Arabidopsis and wheat enhanced resistance against *F. graminearum*. Expression of flg22 was engineered for expression in Arabidopsis and wheat. Two constructs were generated to target the flg22 peptide to the apoplast, where it is expected to bind receptors leading to activation of PTI. A *Ub1:PR1-flg22* construct was designed to express the flg22 peptide as a fusion with the antimicrobial PR1 protein, and a *Ubi:PR1Δ-flg22* construct was designed to express the flg22 peptide fused to the leader (secretory) sequence of PR1. This second construct was generated to overcome any potential toxicity that could result from expression of the full-length PR1 protein in wheat. Transgenic Arabidopsis and wheat plants were generated with these constructs. Both chimeric constructs enhanced resistance against *F. graminearum* in transgenic Arabidopsis that contained the corresponding FLS2 receptor, but not in the *fls2* mutant background, thus confirming the effectiveness of flg22-triggered PTI in enhancing resistance against *F. graminearum*.

Ten independent transgenic wheat *Ubi:PR1Δ-flg22* lines have been identified. Three of these have been propagated further to get homozygous plants. In addition, after some initial trouble we have successfully identified and harvested seeds from a transgenic *Ubi:PR1-flg22* line. Four additional primary transformants of *Ubi:PR1-flg22* are in soil.

(3) 9-LOX (Lipoxygenase) genes are targeted by *F. graminearum* to promote disease. Silencing of two 9-LOX genes in Arabidopsis resulted in enhanced resistance against FHB. Similarly, expression of a wheat *LOX* gene, which encodes a protein with 9-LOX activity, when silenced by RNAi in transgenic wheat, resulted in enhanced resistance against *F. graminearum*, thus confirming that the corresponding *LOX* gene contributes to wheat susceptibility to *F. graminearum*. The enhanced FHB resistance phenotype of these transgenic lines has been confirmed in subsequent generations. Homozygous silenced lines have now been identified. Silencing of the 9-LOX gene results in the faster induction of salicylic acid-mediated defenses.

(4) On the suggestion of the EC we have begun efforts to transform elite wheat varieties that we have received from the community with some of these promising chimeric constructs. Although embryogenic calli formation in the wheat varieties RB07, SD4313, Rollag (contains FHB1) and SD4338 (contains FHB1) cultivars was 30-50% lower than in Bobwhite, they were still sufficient for transformation purposes. We have initiated experiments for the biolistic transformation of these wheat varieties with the *Ubi:WRKY29*, *Ubi:PR1Δ-flg22* and *Ubi:PR1-flg22* chimeric constructs.

(5) Homozygous lines for two AtNPR1, two PAD4, and three AtWRKY18 lines have been propagated for bulking seeds for field trials in 2015 and 2016.

2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

(a) Accomplishment: WRKY29 expression was engineered to enhance resistance against head blight and seedling blight caused by *F. graminearum*.

Impact: These WRKY29 wheat lines will provide germplasms that potentially can be utilized in future FHB breeding programs. Alternatively, the *Ubi:WRKY29* construct is being transformed into elite wheat cultivars to promote FHB resistance.

(b) Accomplishment: Expression of the flg22 peptide has been engineered for expression in wheat to promote PTI, which based on experiments in Arabidopsis is expected to enhance resistance to FHB.

Impact: Stimulation of PTI by expression of flg22 could provide an alternative strategy that complements existing approaches to control a broad-spectrum of diseases, including FHB, in wheat.

(c) Accomplishment: Silencing of a 9-LOX gene that is targeted by the fungus to promote disease resulted in enhanced FHB resistance, which was accompanied by the faster induction of defense mechanisms.

Impact: Lines exhibiting enhanced resistance will provide novel germplasms for breeding FHB resistance by limiting availability of a host-derived factor that is required by the fungus for pathogenicity.

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 FY13 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Peer Reviewed:

Nalam, V. J., Alam, S., Keereetaweep, J., Burdan, D., Lee, H., Trick, H.N., Makandar, R., and Shah, J. Facilitation of *Fusarium graminearum* infection by 9-lipoxygenases in Arabidopsis and wheat. Mol. Plant-Microbe Interact (under revision for resubmission; First submitted for review in March 2014).

Makandar, R., Nalam, V.J., Sarowar, S., Klossner, G., Chowdhury, Z., Lee, H., McAfee, D., Trick, H.N., Gobbato, E., Parker, J. and Shah, J. The combined action of *ENHANCED DISEASE SUSCEPTIBILITY 1* and *PHYTOAELXIN DEFICIENT 4* regulates salicylic acid-mediated resistance against *Fusarium graminearum*. Mol. Plant-Microbe Interact (under revision for resubmission; First submitted February 2014).

Proceedings:

Sarowar, S., Alam, S., Lee, H., Burdan, D., Trick, H., and Shah, J. (2013). Engineering microbial elicitors of defense to promote resistance against *Fusarium graminearum*. In: S. Canty, A. Clark, Y. Salat and D. Van Sanford (Eds.), *Proceedings of the 2013 National Fusarium Head Blight Forum* (pp.77). East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.

Oral Presentations:

Title: Understanding mechanisms underlying plant-pest interaction for the development of sustainable agriculture
Invited presentation by J. Shah, December 17, 2013; Agricen Sciences, Pilot Point, TX

Title: Engineering microbial elicitors of defense to promote resistance against *Fusarium graminearum.*
Authors: Sarowar, S., Alam, S., Lee, H., Burdan, D., Trick, H.N., and Shah, J.
Oral presentation by post doctoral fellow (S. Sarowar), December 4, 2013; Fusarium Head Blight Forum, Milwaukee, WI (Dec 3-5, 2013).

Title: Targeting Host Defense and Susceptibility Mechanisms for Engineering FHB Resistance
Authors: Shah, J., Nalam, V., Sarowar, S., Alam, S., Behera, S., Lee, H., and Trick, H.N.
Invited presentation by J. Shah, December 4, 2013; Fusarium Head Blight Forum, Milwaukee, WI (Dec 3-5, 2013).