USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY14 Final Performance Report
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Cover Page

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Fiscal Year:	FY14
USDA-ARS Agreement ID:	59-0200-3-003
USDA-ARS Agreement Title:	Engineering Fusarium Head Blight Resistance and Plant Defense Signaling.
FY14 USDA-ARS Award Amount:	$ 43,774

USWBSI Individual Project(s)

USWBSI Research Category*	**Project Title**	**ARS Award Amount**
GDER	Targeting Host Defense Mechanism for Enhancing FHB Resistance in Wheat.	$ 43,774
	FY14 Total ARS Award Amount	**$ 43,774**

6/4/15

Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
GDER – Gene Discovery & Engineering Resistance
PBG – Pathogen Biology & Genetics
EC-HQ – Executive Committee-Headquarters
BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
HWW-CP – Hard Winter Wheat Coordinated Project
WES-CP – Western 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?

Wheat and barley grain yield and quality is adversely impacted by Fusarium head blight (FHB). In addition, mycotoxins that accumulate in infected grain can further limit use of grain for human and animal consumption. This project has utilized genetic engineering to target expression of genes involved in regulating disease resistance, and genes that are targeted by the fungus to promote infection, to develop wheat with heightened resistance to FHB. Three aims were pursued.

(1) RNA interference to silence expression of a wheat 9-lipoxygenase-encoding gene that is targeted by the fungus to facilitate infection, thus depriving the fungus of a host-derived factor that promotes infection.

(2) Targeting defense pathways associated with the activation of Microbe Associated Molecular Pattern (MAMP) signaling to promote non-specific pathogen-triggered immunity (PTI) against *F. graminearum*. As a proof-of-concept effect of this was tested in *Arabidopsis thaliana* and subsequently utilized to engineer FHB resistance in wheat.

(3) Field tests to study the efficacy in promoting FHB resistance and reducing content of mycotoxins by constitutive expression of genes (AtNPR1, AtPAD4 and AtWRKY18) regulating systemic acquired resistance in transgenic wheat.

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: Expression of a wheat *LOX* gene, which encodes a protein with 9-LOX activity, when silenced by RNA interference (RNAi) in transgenic wheat, resulted in enhanced resistance against *F. graminearum*, similar to its effect in Arabidopsis. Silencing of the 9-LOX gene resulted in the faster induction of salicylic acid (SA)-mediated defenses, thus indicating that 9-LOXs suppress the activation of SA signaling. Genetic studies in Arabidopsis have confirmed an important function of SA signaling in the 9-LOX-silencing conferred enhanced disease resistance. This suppression of SA signaling in Arabidopsis is mediated by the requirement of 9-LOXs for the sustained elevation of jasmonic acid (JA) level and JA signaling, which antagonizes the activation of SA signaling. A manuscript resulting describing these results is currently under review in Molecular Plant-Microbe Interaction.

Impact: Preventing the fungus from obtaining host-derived factors/cues that facilitate infection is a novel way to control fungal growth and disease, which is distinct from, but complements other approaches that target plant defenses. Attenuation of 9-LOX expression offers a promising approach. The 9-LOX RNAi lines exhibiting enhanced resistance provide novel germplasms in which availability of a FHB susceptibility factor is limited. Crosses to elite wheat cultivars are ongoing.

(b) <u>Accomplishment</u>: Flg22 peptide, which is derived from bacterial flagellin protein, and an inducer of PTI, when applied to wheat and Arabidopsis enhanced resistance against *F. graminearum*, which was accompanied by upregulation of the WRKY29 transcription factor-encoding gene. Constitutive expression of a chimeric flg22 in Arabidopsis resulted in enhanced resistance against *F. graminearum* that was dependent on the *FLS2* receptor, thus confirming flg22-induced PTI confers enhanced resistance against *F. graminearum*. Several transgenic wheat lines that express flg22 from the maize Ubi promoter have been developed and propagated to yield homozygous progeny. Initial experiments indicate resistance enhancement in some of these lines, which is being investigated further to determine if the level of resistance is dependent on the level of expression.

<u>Impact</u>: Stimulation of PTI provide an alternative strategy that complements existing approaches to control a broad-spectrum of diseases, including FHB, in wheat.

(c) <u>Accomplishment</u>: In an approach that parallels the engineering of flg22 expression in wheat, the flg22-induced ArabidopsisWRKY29 transcription factor encoding gene was constitutively expressed from the maize *Ubi* promoter in transgenic wheat. Three independently-derived Ubi:WRKY29 transgenic lines repeatedly exhibited enhanced resistance to FHB as well as seedling blight. In addition, DON levels were also lower in these lines compared to the non-transgenic plants. Field trials for these lines are underway this spring in Minnesota.

<u>Impact</u>: These AtWRKY29 wheat lines along with the PR1:flg22 lines will provide germplasms that potentially can be utilized in future FHB breeding programs. Crosses to elite wheat cultivars are ongoing.

Besides the above accomplishments, homozygous lines for two AtNPR1, two AtPAD4, and three AtWRKY18 lines, that previously had shown enhanced FHB resistance in greenhouse studies were propagated for bulking seeds for field trials, which are currently underway in Minnesota. Results describing the effect of AtPAD4 expression on FHB resistance in greenhouse studies was accepted for publication in Molecular Plant-Microbe Interaction (Makandar et al. 2015).

We have continued engineering vectors into Bobwhite as well as elite cultivars such as RB07, Rollag, SD4313 and SD4338. We have performed over 14 independent transformation experiments and have identified three events expressing WRKY29 and over 18 events transformed with the PCB gene, another gene that regulates SAR. Additional experiments are still in tissue culture, however the majority of these lines have been harvested or are close to maturity.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY14 award period. The term "support" below includes any level of benefit to the student, ranging from full stipend plus tuition to the situation where the student's stipend was paid from other funds, but who learned how to rate scab in a misted nursery paid for by the USWBSI, and anything in between.

1. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their MS degree during the FY14 award period?** No

   If yes, how many?

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY14 award period?** No

   If yes, how many?

3. **Have any post docs who worked for you during the FY14 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?** No

   If yes, how many?

4. **Have any post docs who worked for you during the FY14 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?** No

   If yes, how many?

Include below a list of all germplasm or cultivars released with full or partial support of the USWBSI during the FY14 award period. List the release notice or publication. Briefly describe the level of FHB resistance. *If not applicable because your grant did NOT include any VDHR-related projects, enter N/A below.*

N/A

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 FY14 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

<u>**Peer-reviewed publications**</u>

Makandar, R., Nalam, V.J., Chowdhury, Z., Sarowar, S., Klossner, G., Lee, H., Burdan, D., Trick, H.N., Gobbato, E., Parker, J. and Shah, J. (2015). The combined action of *ENHANCED DISEASE SUSCEPTIBILITY1*, *PHYTOALEXIN DEFICIENT4* and *SENESCENCE-ASSOCIATED101* promotes salicylic acid-mediated defenses to limit *Fusarium graminearum* infection in *Arabidopsis thaliana*. *Mol. Plant-Microbe Interact*. Epub ahead of print April 27; http://apsjournals.apsnet.org/doi/pdf/10.1094/MPMI-04-15-0079-R

Nalam, V. J., Alam, S., Keereetaweep, J., Venables, B., Burdan, D., Lee, H., Trick, H.N., Sarowar, S., Makandar, R., and Shah, J. (2015). 9-lipoxygeases facilitate *Fusarium graminearum* infection in *Arabidopsis* and Wheat. *Mol. Plant-Microbe Interact*. (in review)

<u>**Forum/Conference Proceedings**</u>

Sarowar, S., Alam, S., Silvaraman, V., Lee, H., Tyagi, N., Trick, H., and Shah, J. (2014). Engineering resistance against *Fusarium graminearum*. *In*: Canty, S., Clark, A., Turcott N., and Van Sanford, D (Eds.). *Proceedings of the 2014 National Fusarium Head Blight Forum* (pp. 61). East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative.

<u>**Oral and Poster Presentations**</u>

Alam, S., Nalam, V., Keereetaweep, J., Burdan, D., Lee, H., Trick, H.N., Makandar, R., and Shah, J. *Fusarium graminearum* hijacks plant 9-lipoxygenases to facilitate infection in Arabidopsis and wheat**.** Biology Graduate Student Association Graduate Day, University of North Texas, April 17, 2015. Oral Presentation by S. Alam (Graduate student supported by funding from this project).

Sarowar, S., Louis, J., Lorenc-Kukula, K., Keereetaweep, J., Welti, R., and Shah, J. The *MYZUS PERSICAE-INDUCED LIPASE1* gene functions in oxylipin metabolism and plant response to biotic stress. Plant Biology 2014, American Society of Plant Biologists annual meeting, Portland, OR; July 12-16, 2014. Poster Presentation by S. Sarowar (Postdoctoral fellow supported by funding from this project).