USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY15 Final Performance Report
Due date: July 15, 2016

Cover Page

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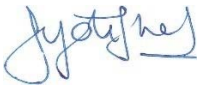
Fiscal Year:	2015
USDA-ARS Agreement ID:	59-0200-3-003
USDA-ARS Agreement Title:	Engineering Fusarium Head Blight Resistance and Plant Defense Signaling.
FY15 USDA-ARS Award Amount:	$ 43,732
Recipient Organization:	University of North Texas 1155 Union Clrele #305250 Denton, Texas 76203-5017
DUNS Number:	614168995
EIN:	756002149
Recipient Identifying Number or Account Number:	GF0501
Project/Grant Reporting Period:	05/09/15-05/08/16
Reporting Period End Date:	05/08/16

USWBSI Individual Project(s)

USWBSI Research Category*	**Project Title**	**ARS Award Amount**
GDER	Targeting Host Defense Mechanism for Enhancing FHB Resistance in Wheat.	$ 43,732
	FY15 Total ARS Award Amount	**$ 43,732**



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Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
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
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 are the major goals and objectives of the project?

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 engineered the expression of plant genes to develop wheat with heightened resistance to FHB. Genes involved in regulating disease resistance and genes that are targeted by the fungus to promote infection were the focus of this study.

The specific objectives were to:

(i) Assess the impact of silencing wheat 9-LOX on FHB resistance and mycotoxin accumulation.

(ii) Target defense pathway associated with the activation of Microbe Associated Molecular Pattern (MAMP) signaling to promote non-specific pathogen-triggered immunity (PTI) against *F. graminearum*.

(iii) Assess the impact of constitutive expression of a calcium-binding protein associated with systemic acquired resistance on FHB and mycotoxin accumulation in wheat.

2. What was accomplished under these goals?

1) Major activities

(i) FHB disease severity and mycotoxin level were evaluated in wheat lines that expressed an RNA-interference construct designed to silence expression of a 9-LOX susceptibility factor gene, and in lines constitutively expressing the defense regulatory genes *WRKY29*, *WRKY18*. Transgenic wheat plants expressing a defense associated calcium-binding protein (CaBP) were generated.

(ii) Training opportunities were provided to an undergraduate and graduate student and a postdoctoral fellow. A part-time technician was also trained in analyzing and working with transgenic wheat. Professional development of the graduate student and postdoc was facilitated through one-on-one interaction with the PI and participation and presentations at conferences,

2) Specific objectives

(i) FHB disease severity and mycotoxin accumulation were evaluated in three independently-derived wheat lines in which expression of *WhLpx1*, which encodes a 9-LOX was reduced as a result of RNAi.

(ii) FHB disease severity and mycotoxin accumulation were evaluated in three independently-derived *WRKY18* and *WRKY29* expressing lines. FHB severity was also evaluated in flg22 peptide expressing lines.

(iii) Transgenic wheat lines expressing a calcium binding protein that is involved in promoting systemic acquired resistance were generated.

3) Significant results

(i) Resistance against FHB was enhanced and mycotoxin accumulation reduced in wheat plants in which expression of the *WhLpx1* gene was silenced by RNAi. 9-LOX silencing

resulted in stronger induction of defenses. A manuscript describing these results as well as that involving parallel studies in *Arabidopsis thaliana* was published (Nalam et al. 2015. Molecular Plant-Microbe Interact. 28, 1142-1152).

(ii) FHB resistance was enhanced and accompanied by reduced mycotoxin accumulation in transgenic wheat constitutively expressing the *WRKY18* and *WRKY29* transcription factors. These transcription factors are involved in regulating defenses associated with MAMP-triggered resistance. In contrast, expression of flg22 fused to the secretory PR1 protein although was capable of enhancing resistance against *F. graminearum* in Arabidopsis, was not effective in enhancing FHB resistance in wheat. Expression in Arabidopsis utilized the strong *35S* promoter. In contrast, the maize *Ubi* promoter was used to express this construct in wheat. Differences in the relative strengths of these promoters could likely explain the differences in impact of flg22 expression on resistance against *F. graminearum* in Arabidopsis and wheat.

(iii) Five CaBP expressing transgenic wheat plants were identified and propagated further with the goal of obtaining homozygous lines that stably express CaBP. The impact of CaBP expression on FHB resistance will be evaluated once homozygous lines are available. Work on previously generated transgenic plants expressing *PAD4* which modulates SA content and promotes FHB resistance under greenhouse conditions was completed and published (Makandar et al. 2015. Molecular Plant-Microbe Interact. 28, 943-953).

4) Key outcomes or other achievements

FHB resistance is strong in the *WhLpx1*-silenced as well as the *WRKY29* expressing wheat lines. Mycotoxin accumulation was also reduced in these lines. These results have been reproduced over three years of experiments on successive generation of lines, thus indicating that these traits are stable. These lines have been bulked so that field trials can be conducted in subsequent years.

3. What opportunities for training and professional development has the project provided?

A graduate and an undergraduate student, a postdoctoral fellow, and a part-time technical staff were provided training opportunities as part of this project. They received one-on-one training with the PI on the application of molecular methods for studying *Fusarium* infection and disease control, in planning of experiments, data collection and recording, and data analysis and interpretation. In addition, they were provided training by the PI in developing scientific writing skills. The graduate student was enrolled in dissertation hours under the PI. She successfully completed her PhD qualifying exams. The graduate student and postdoctoral fellow participated and presented their work at conferences and seminars. Further, the mentor worked with the postdoctoral fellow on developing his resume and preparing him for job interviews.

4. How have the results been disseminated to communities of interest?

Information was disseminated via submissions to the Fusarium Focus (2015, Volume 15 Issue 2), the online newsletter published periodically by the U.S. Wheat & Barley Scab Initiative.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY15 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 FY15 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 FY15 award period?** No

   If yes, how many?

3. **Have any post docs who worked for you during the FY15 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 FY15 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?

Release of Germplasm/Cultivars

Instructions: In the table below, list all germplasm and/or cultivars released with full or partial support through the USWBSI during the FY15 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *Leave blank if you have nothing to report or if your grant did NOT include any VDHR-related projects.*

Name of Germplasm/Cultivar	**Grain Class**	**FHB Resistance** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**

Add rows if needed.

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

Publications, Conference Papers, and Presentations
Journal publications.

1. Nalam, V. J., Alam, S., Keereetaweep, J., Venables, B., Burdan, D., Lee, H., Trick, H.N., Sarowar, S., Makandar, R., and Shah, J. (2015). Facilitation of *Fusarium graminearum* infection by 9-lipoxygenases in *Arabidopsis* and wheat. Mol. Plant-Microbe Interact. 28, 1142-1152.
   Status: Published
   Acknowledgement of Federal Support: YES

2. Makandar R, Nalam VJ, Chowdhury Z, Sarowar S, Klossner G, Lee H, Burdan D, Trick HN, 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. 28, 943-953.
   Status: Published
   Acknowledgement of Federal Support: YES

Books or other non-periodical, one-time publications.

1. Shah, J., Sarowar, S., Alam, S., Shulaev, E., Behera, S., Lee, H., Tyagi, N., and Trick, H.N. (2015) Engineering resistance against *Fusarium graminearum* in wheat. In: S. Canty, A. Clark, S. Vukasovich and D. Van Sanford (Eds.), *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 50.
   Status: Abstract Published and poster presented
   Acknowledgement of Federal Support: YES

2. Dill-Macky, R., Elakkad, A.M., Shah, J., Trick, H.N., Sarowar, S., Alam, S., Dahleen, L.S., Skadsen, R.W., and Bregitzer, P.P. (2015). Testing transgenic spring wheat and barley lines for reaction to Fusarium head blight: 2015 Field Nursery Report. In: S. Canty, Clark, S. Vukasovich and D. Van Sanford (Eds.), *Proceedings of the 2015 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p.45.
   Status: Abstract Published and poster presented
   Acknowledgement of Federal Support: YES

Other publications, conference papers and presentations.

1. Sarowar, S., Chowdhury, Z., Louis, J., Mondal, H., Keereetaweep, J., Behera, S., and Shah, J. Role of the *MYZUS PERSICAE-INDUCED LIPASE 1* (*MPL1*) gene in plant biotic stress. Southern Section of American Society of Plant Biologists annual meeting, Denton, TX; April 2-4, 2015.
   Status: Oral Presentation by S. Sarowar (Post doc supported by this project).
   Acknowledgement of Federal Support: YES