USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report
Due date: July 31, 2018

Cover Page

Principle Investigator (PI):	Jyoti Shah
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Fiscal Year:	2017
USDA-ARS Agreement ID:	59-0206-7-006
USDA-ARS Agreement Title:	Developing Resistance to Fusarium Head Blight in Wheat.
FY17 USDA-ARS Award Amount:	$ 46,757
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:	GF10501
Project/Grant Reporting Period:	7/10/17 - 7/9/18
Reporting Period End Date:	07/09/18

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	RNA-Interference Targeting of Fungal Genes for Enhancing FHB Resistance.	$ 24,955
GDER	Wheat Variants Deficient in a FHB Susceptibility Factor	$ 21,802
	FY17 Total ARS Award Amount	$ 46,757

July 15, 2018
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: *RNA-Interference Targeting of Fungal Genes for Enhancing FHB Resistance.*

1. What are the major goals and objectives of the project?

The goal of this project is to determine whether the expression of effector-encoding genes in *Fusarium graminearum* can be silenced by host-induced gene silencing (HIGS) to adversely impact fungal pathogenicity and thus promote resistance to *F. graminearum* in Arabidopsis and wheat. *FGL1* and *FgNahG* are the two *F. graminearum* genes that encode secreted proteins, which are the target of this project. The specific objectives are:

(i) Host-induced silencing of *F. graminearum FGL1* effector gene to enhance disease resistance.
(ii) Fungal *FgNahG* gene as a target for host-induced silencing to engineer resistance to *F. graminearum.*

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

1) Major activities

(i) Both the inverted repeat containing RNAi constructs targeting *FGL1* and *FgNahG* were made for expression in Arabidopsis and wheat. Transgenic lines were obtained and initial characterization conducted.

(ii) Training opportunities were provided to a graduate student and an undergraduate student.

2) Specific objectives

(i) The inverted repeat containing *FGL1*-RNAi and *FgNahG*-RNAi chimera were cloned for expression of the RNAi from the *Cauliflower mosaic virus 35S* promoter in *Arabidopsis thaliana*. The same RNAi chimera were also cloned for expression from the maize *Ubi* gene promoter in wheat.

(ii) Both the *35S:FLG1*-RNAi and *35S:FgNahG*-RNAi constructs were transformed into Arabidopsis and multiple transgenic lines obtained and propagated, and initial experiments to study the impact of the transgene on Arabidopsis resistance to *F. graminearum* conducted.

(iii) The *Ubi:FGL1*-RNAi and *Ubi:FgNahG*-RNAi constructs were transformed into wheat embryos by the plant transformation lab at Kansas State University. Several transgenic wheat plants are at different stages of growth. First round of seed are expected in summer 2018, with seeds to additional lines expected by end of 2018.

3) Significant results

Initial analysis of three independent Arabidopsis transgenic lines for *35S:FGL1*-RNAi and *35S:FgNahG*-RNAi indicate that presence of the RNAi construct increases resistance to *F. graminearum* in Arabidopsis leaf assays.

4) Key outcomes or other achievements

Preliminary experiments indicate that HIGS-mediated silencing of *FGL1* and *FgNahG* provide good targets for controlling *F. graminearum* infection.

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

Training: A graduate student associated with this project received training on multiple fronts including molecular biology and plant pathology. The graduate student has worked on developing the recombinant constructs for HIGS in wheat and Arabidopsis. During the course of this project, the graduate student received one-on-one training from 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, the graduate student was provided training in developing scientific writing and presentation skills. The graduate student was enrolled in dissertation hours under the PI and participated in teaching, as well.

An undergraduate student and a technician worked part-time on this project under the direct mentorship of the graduate student. They received training in molecular biology, plant biology and pathology.

Professional Development: This project has further contributed to the professional development of the graduate student. The graduate student presented her work at a local meeting. In addition, a poster arising out of her work was presented at the 2017 FHB forum. She also coauthored two manuscripts arising out of previous years funding from the USWBSI/USDA-ARS. Both these manuscripts are undergoing peer-review. The PI has worked individually with the graduate student towards achieving the student's long-term professional goal in academia.

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

Results associated with this project were disseminated to the wheat and barley scab community via a poster at the 2017 Annual USWBSI Forum in Milwaukee, WI. Results were disseminated to the local community through a talk presented by the graduate student, and by the PI Shah in his Biology classes as part of lectures on sustainability and improving plant health.

Project 2: *Wheat Variants Deficient in a FHB Susceptibility Factor*

1. What are the major goals and objectives of the project?

The major goal of this project is to utilize TILLING as a non-GMO (non-genetically modified organism) approach to target the activity of wheat genes that contribute to susceptibility to *F. graminearum*. It is expected that reduction in activity of these wheat genes will confer resistance to *F. graminearum* and thus provide genetic material that can be utilized in wheat breeding programs. Wheat *Lpx3*, which is located on chromosome 4, is the target of this study. While work funded by this project in the Shah lab at UNT is being conducted on the hexaploid wheat Cadenza, parallel work being conducted by collaborator Rawat at the University of Maryland is with the tetraploid wheat Kronos. The specific objectives are:

1. Isolate homozygous *Lpx3* mutant lines from the three sub-genomes, and backcross them to clear background mutations.
2. Characterize the response of *Lpx3* mutants to *F. graminearum*.
3. Develop wheat lines containing combinations of *Lpx3* mutant alleles at the homeologous chromosomes.

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

1) Major activities

(i) TILLING lines containing mutations in the *Lpx3* homeologs on chromosomes 4A, 4B and 4D in the hexaploid wheat Cadenza were identified and preliminary response of these liens to *F. graminearum* was studied at the University of North Texas.

A parallel approach at University of Maryland was carried out with the tetraploid wheat Kronos.

(ii) Training opportunities were provided to a graduate student and an undergraduate student.

2) Specific objectives

(i) Seven TILLING lines containing mutations in the Lpx3 homeologs on chromosomes 4A, 4B, and 4D in the hexaploid wheat variety Cadenza were identified. Three of these lines contain mutations in the homeolog on chromosome 4A with two bearing nonsense mutations and one containing a missense mutation. Two lines contained nonsense mutations in the homeolog on chromosome 4B, and two contained nonsense mutations in the homeolog on chromosome 4D.

(ii) The reaction to Fusarium graminearum was characterized in these lines and the parental variety Cadenza.

In a parallel undertaking TILLING mutants in the tetraploid Kronos were evaluated at the University of Maryland.

3) Significant results

(i) Compared to the wheat variety Bobwhite, the variety Cadenza exhibits higher level of resistance to *F. graminearum* infection.

(ii) Preliminary experiments with two TILLING lines that contained nonsense mutations in the *Lpx3* homeolog on chromosome 4A showed heightened resistance compared to the parental Cadenza. In contrast, a single line with a missense mutation at the Lpx3 locus on chromosome 4A exhibited extremely high susceptibility to *F. graminearum*. We postulate that the missense allele encodes a hyperactive allele.

(iii) Similarly, collaborator Rawat at the University of Maryland observed higher resistance to *F. graminearum* in two TILLING lines that contained nonsense mutations in the *Lpx3* homeolog on chromosome 4A.

4) Key outcomes or other achievements

Although very preliminary, these results suggest that mutants with reduced function of the *Lpx3* homeolog on chromosome 4A will provide a good source of resistance to FHB.

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

Training: A graduate student associated with this project received training on multiple fronts, including molecular biology and plant pathology. The graduate student has worked on developing the recombinant constructs for HIGS in wheat and Arabidopsis. During the course of this project, the graduate student received one-on-one training from 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, the graduate student was provided training in developing scientific writing and presentation skills. The graduate student was enrolled in dissertation hours under the PI and participated in teaching, as well.

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

Results were disseminated to the local community through a talk presented by the graduate student, and to the student community in lectures on sustainability and plant health developed by the PI Shah for his undergraduate Biology class and his graduate class. Results associated

with this project were also disseminated to the wheat and barley scab community via a poster at the 2017 Annual USWBSI Forum in Milwaukee, WI.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17 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 FY17 award period?**

   If yes, how many? Nothing to report

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY17 award period?**

   If yes, how many? Nothing to report

3. **Have any post docs who worked for you during the FY17 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**

   If yes, how many? Nothing to report

4. **Have any post docs who worked for you during the FY17 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**

   If yes, how many? Nothing to report

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 FY17 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

Instructions: Refer to the FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17 grant. Only include citations for publications submitted or presentations given during your award period (7/10/17 - 7/9/18). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each reference/citation, you must indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in publication/ presentation.

Journal publications.

Nothing to report

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

Nothing to report

Other publications, conference papers and presentations.

Shah J., Alam, S., and Rawat, N. 2017. Targeting Wheat Genes Associated with Susceptibility to *Fusarium graminearum* for Enhancing FHB Resistance. In: Proceedings of the 2017 National Fusarium Head Blight Forum. Milwaukee, WI.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), YES (abstract)

Alam, S., and Shah, J. 2018. Novel approaches for enhancing resistance to Fusarium head blight in wheat. 15th Annual Graduate Student Research Day, Department of Biological Sciences, University of North Texas, Denton, TX.
Status: Talk Presented by graduate student Alam.
Acknowledgement of Federal Support: YES (talk)