

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY16 Final Performance Report
Due date: July 28, 2017**

Cover Page

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Fiscal Year:	2016
USDA-ARS Agreement ID:	59-0200-3-003
USDA-ARS Agreement Title:	Engineering Fusarium Head Blight Resistance and Plant Defense Signaling.
FY16 USDA-ARS Award Amount:	\$ 25,004
Recipient Organization:	University of North Texas 1155 Union Circle #305250 Denton, Texas 76203-5017
DUNS Number:	614168995
EIN:	756002149
Recipient Identifying Number or Account Number:	GF0501
Project/Grant Reporting Period:	5/9/16 - 5/8/17
Reporting Period End Date:	05/08/17

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	RNA-Interference Targeting of Fungal Genes for Enhancing FHB Resistance.	\$ 25,004
	FY16 Total ARS Award Amount	\$ 25,004



7-25-17

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?

This is a new project that will test the hypothesis that host-induced gene silencing (HIGS) of effector-encoding genes in *Fusarium graminearum* will adversely impact pathogenicity and thus promote resistance against *F. graminearum* in wheat and Arabidopsis. FGL1 and NahG are *F. graminearum* proteins that are predicted to be secreted in the host during infection, thus suggesting that they act in the host to promote susceptibility.

The specific objectives are:

- (i) Host-induced silencing of *F. graminearum* *FGL1* effector gene to enhance disease resistance.
- (ii) Fungal *NahG* 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) Recombinant constructs were generated to test the effector function in planta and the impact of host-induced gene silencing of FGL1 and NahG on resistance to *F. graminearum*.
- (ii) Training opportunities were provided to a graduate student and a postdoctoral fellow. Professional development of the graduate student and postdoc was facilitated.

2) Specific objectives

- (i) *F. graminearum* *NahG* and *FGL1* coding sequences were cloned in a Gateway system compatible vector, and subsequently used to generate recombinant constructs in which the coding sequence of these two genes were cloned behind the *Cauliflower mosaic virus* 35S promoter and transformed into *Arabidopsis thaliana* to generate transgenic Arabidopsis and to determine the effect of these fungal proteins when expressed in planta to confer susceptibility.
- (ii) A 250 bp region of the *FGL1* coding sequence was used to generate an inverted repeat containing RNAi construct in which the *FGL1* inverted repeated is flanked by a linker derived from the bacterial *UidA* gene. The *Cauliflower mosaic virus* 35S promoter is being used to express this recombinant RNAi construct in Arabidopsis, and the maize Ubiquitin gene promoter and 5' intron is being utilized for expression in wheat.
- (iii) The initial approach to make the *NahG* RNAi construct were unsuccessful, presumably due to instability of the inverted repeat construct. Subsequently, the inverted repeat DNA sequence was successfully synthesized and prepped for cloning in vectors for expression in Arabidopsis and wheat.

FY16 Final Performance Report
PI: Shah, Jyoti
USDA-ARS Agreement #: 59-0200-3-003
Reporting Period: 5/9/16 - 5/8/17

3) Significant results

After initial difficulties, presumably due to instability of the inverted repeat constructs in bacteria, all inverted-repeat RNAi constructs have been successfully generated and cloned for expression in Arabidopsis and wheat.

4) Key outcomes or other achievements

Nothing to report

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

The postdoc and graduate student associated with this project received training on multiple fronts. The postdoc who began this work, received training in molecular biology and plant pathology. Subsequently, the project was passed down to a graduate student who has worked on developing additional recombinant constructs for HIGS in wheat and Arabidopsis. During the course of this project, the postdoc and the graduate student 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 in developing scientific writing skills. The graduate student was enrolled in dissertation hours under the PI and also in the GSTEP program for students interested in a teaching career.

This project has further contributed to the professional development of the graduate student and the postdoc. The PI has worked individually with them to achieve their long-term professional goals. The PI worked with the postdoctoral fellow on developing his resume and preparing him for job interviews. The postdoc successfully landed a job in the plant biotech industry. The graduate student who is interested in a STEM teaching career successfully participated in the two semester long GSTEP program, which has prepared her for a teaching role.

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

Details of the protocol developed for *Fusarium graminearum* infection of leaf and floral tissues of *Arabidopsis thaliana* was disseminated in the form of a freely downloadable bio-protocols that is available at <http://www.bio-protocol.org/e1877>. This protocol should facilitate further utilization of this Arabidopsis-*F. graminearum* pathosystem by the scientific community for rapid testing of genes in plant defense and susceptibility to *F. graminearum*. This protocol will also be useful in undergraduate and graduate labs to train students in plant pathology.

FY16 Final Performance Report
PI: Shah, Jyoti
USDA-ARS Agreement #: 59-0200-3-003
Reporting Period: 5/9/16 - 5/8/17

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY16 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 FY16 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 FY16 award period? No**

If yes, how many?

- 3. Have any post docs who worked for you during the FY16 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 FY16 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? One. The postdoc who worked on this project took up a position in Botanical Genetics, a subsidiary of the 22nd Century Group.

FY16 Final Performance Report
 PI: Shah, Jyoti
 USDA-ARS Agreement #: 59-0200-3-003
 Reporting Period: 5/9/16 - 5/8/17

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 FY16 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

FY16 Final Performance Report
PI: Shah, Jyoti
USDA-ARS Agreement #: 59-0200-3-003
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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY16-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY16 grant. Only include citations for publications submitted or presentations given during your award period (5/9/16 - 5/8/17). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

Nalam, V. J., Sarowar, S. and Shah, J. (2016). Establishment of a *Fusarium graminearum* Infection Model in *Arabidopsis* Leaves and Floral Tissues. *Bio-protocols* 6(14): e1877.
<http://www.bio-protocol.org/e1877>

Status: Paper published

Acknowledgement of Federal Support: YES

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

Other publications, conference papers and presentations.