USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Performance Report
Due date: September 30, 2020

Cover Page

Principle Investigator (PI):	Jyoti Shah
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Fiscal Year:	2019
USDA-ARS Agreement ID:	59-0206-7-006
USDA-ARS Agreement Title:	Developing Resistance to Fusarium Head Blight in Wheat
FY19 USDA-ARS Award Amount:	$ 63,650
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:	7/8/19 - 7/9/20
Reporting Period End Date:	7/9/2020

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	RNA-Interference Targeting of Fungal Genes for Enhancing FHB Resistance	$ 35,486
GDER	Wheat Variants Deficient in a FHB Susceptibility Factor	$ 28,164
	FY19 Total ARS Award Amount	**$ 63,650**

September 23, 2020

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

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Project 1: *RNA-Interference Targeting of Fungal Genes for Enhancing FHB Resistance*

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

   The goal of this project is to utilize host-induced gene silencing (HIGS) as a tool to silence expression of pathogenicity genes in *Fusarium graminearum* (*Fg*) when the fungus infects a plant. It is anticipated that this will attenuate fungal pathogenicity to promote plant resistance to *Fg*. Fungal genes encoding secretory proteins are being targeted by HIGS in Arabidopsis and wheat.

2. **What was accomplished under these goals or objectives?** *(For each major goal/objective, address items a-b) below.)*

   a) What were the major activities?
   - Several transgenic Arabidopsis and wheat lines containing the HIGS constructs that were generated in previous years were propagated, genotyped, evaluated for transgene expression and response to *Fg*.
   - Training and Professional opportunities were provided to a Research Associate, an undergraduate, and three graduate students. One graduate student successfully completed her PhD degree.

   b) What were the significant results?
   - The HIGS construct was expressed in Arabidopsis and wheat. In comparison to the control non-transgenic lines, presence of the HIGS construct resulted in reduced severity of disease by *Fg* in Arabidopsis leaf and floral tissues, and reduced severity of FHB in wheat. This reduction in disease severity was associated with reduced accumulation of fungal DNA, thus confirming reduced growth of the fungus on these HIGS lines.

   c) List key outcomes or other achievements.
   - HIGS-mediated silencing of fungal pathogenicity genes that encode secretory enzymes provides a strategy for controlling growth of *Fg* in planta thereby conferring resistance against this fungus.

3. **Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.**

   The University of North Texas campus was shut down to research for 2.5 months during March-May 2020, after which it reopened during summer with reduced occupancy that involved social distancing, use of PPE and staggered scheduling. The research shut down resulted in premature termination of several experiments, including loss of plant material. During this period, students and staff associated with this project spend their time working

remotely on literature review, data analysis, writing and planning of experiments. When campus reopened for research in late summer, the difficulty and delay in ordering and procuring supplies resulted in further interruptions. Together, these have put back the research on this project by five months. A no-cost extension is in place.

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

Training: Three graduate students (a senior student who graduated and two new students) and the undergraduate student who were associated with this project received training in plant physiology and pathology and molecular biology. Besides training in the application of molecular methods for studying *Fg* infection and disease control, they also received training on experiment planning, and on data collection, recording, analysis and interpretation. In addition, all trainees received training in developing writing and presentation skills. The graduate students were enrolled in Individual Research or Dissertation hours under the PI, and two of them participated in teaching during fall 2019. The undergraduate was registered in Special Problems class under the PI.

A Research Associate who worked part-time on this project under the mentorship of the PI, received training in plant physiology and pathology.

Professional Development: This project has contributed to the professional development of the graduate students. The graduate students participated in the weekly group meetings, department seminars, and the BioDiscovery Institute research talks. In addition, a poster arising out of their work associated with this project was presented at the 2019 FHB forum. The PI worked with one of the graduate students on a manuscript arising out of her research that was supported by previous funding from the USWBSI/USDA-ARS. This manuscript is currently under peer review. The PI also worked with the undergraduate student on the submission of his research report that was required as part of his participation in the Special Problems course. The PI has worked individually with the graduate students and the Research Associate towards achieving their long-term professional goal. One of the graduate students is now an Adjunct faculty at a local university.

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

Results associated with this project were disseminated in multiple forms to the community.

- Results were disseminated to the wheat and barley scab community via a talk by the PI and a poster presented at the 2019 Annual USWBSI Forum in Milwaukee.
- Results were disseminated to the local community via a graduate student (Syeda Alam) dissertation talk in fall 2019, which was open to the public, and another talk by the PI at a BioDiscovery Institute seminar at the University of North Texas in spring 2020.

- Results were disseminated to undergraduate students at the University of North Texas who were enrolled in an introductory biology class taught by the PI as part of a lecture on 'Plant Interaction with the Environment.

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

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

The goal of this project is to enhance wheat resistance to FHB by targeting activity of wheat lipoxygenase locus, which encodes a factor that contributes to susceptibility to *Fusarium graminearum* (*Fg*). TILLING, a non-GMO approach is being utilized to knock down the activity of this locus, which is expected to enhance FHB resistance and thus provide non-GMO genetic material that can be integrated into wheat breeding programs. This project is being conducted with hexaploid cv Cadenza in the Shah lab at UNT and with tetraploid cv Kronos by collaborator Rawat at the University of Maryland.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address items a-b) below.)*

a) What were the major activities?
- Co-dominant markers that can rapidly determine the genotype at the *Lpx3* locus were developed to distinguish between wild type and mutant alleles. These have been utilized to identify lines that are homozygous for the mutant alleles.
- FHB disease severity was characterized for TILLING lines that contain mutations in the *Lpx3* homeologs on chromosomes 4A, 4B and 4D.
- Training opportunities were provided to graduate students and a Research Associate.

b) What were the significant results?
- Homozygous lines were identified for the mutants for all three homeologs. Backcrosses have been completed for most mutant lines, which have also been crossed to each other to obtain plants that contain mutant alleles at more than one homeologous chromosome. Lines containing mutations on the *Lpx3* homeolog on Chromosme 4A exhibited the strongest FHB resistance phenotype.

c) List key outcomes or other achievements.
- These results suggest that mutants with reduced function of the *Lpx3* homeolog on chromosome 4A will provide a good source of resistance to Fusarium Head Blight. Breeders at USDA in North Dakota are working towards integrating some of these mutant alleles in their wheat breeding program.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

The University of North Texas campus was shut down to research for 2.5 months during March-May 2020, after which it reopened during summer with reduced occupancy that involved social distancing, use of PPE and staggered scheduling. The research shut down resulted in premature termination of several experiments, including loss of plant material.

During this period, students and staff associated with this project spend their time working remotely on literature review, data analysis, writing and planning of experiments. When the campus reopened to research in late summer, the difficulty and delay in ordering and procuring supplies resulted in further interruptions. Together, these have put back the research on this project by five months.

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

Training: Two graduate students (a senior student who graduated and a new student) associated with this project received training in plant physiology and pathology, and molecular biology. Besides training in the application of molecular methods for studying *Fg* infection and disease control, they also received training on experiment planning, and on data collection, recording, analysis and interpretation. In addition, all trainees received training in developing writing and presentation skills. The graduate students were enrolled in Individual Research or Dissertation hours under the PI, and one of them participated in teaching during fall 2019.

A Research Associate who worked part-time on this project under the mentorship of the PI, received training in plant physiology and pathology.

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Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (7/8/19 - 7/9/20). 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 FY19 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 FY19 award period?**
   Yes
   If yes, how many? 1. Syeda Alam successfully defended her dissertation in fall 2019 and was awarded her PhD degree in spring 2020. She is currently an Adjunct Faculty at Texas Women's University, Denton, TX.

3. **Have any post docs who worked for you during the FY19 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 FY19 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 FY19 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

NOTE: 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 FY19-FPR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY19 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (7/8/19 - 7/9/20**) should be included. If you did not publish/submit or present anything, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each citation, you **must** indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in the publication/ presentation. See example below for a poster presentation with an abstract:

De Wolf, E., D. Shah, P. Paul, L. Madden, S. Crawford, D. Hane, S. Canty, R. Dill-Macky, D. Van Sanford, K. Imhoff and D. Miller. 2019. "Impact of Prediction Tools for Fusarium Head Blight in the US, 2009-2019." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum*, Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY. p. 12.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Nothing to Report

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

Alam, S. 2020. Novel approaches for enhancing resistance to *Fusarium graminearum* in *Arabidopsis* and wheat by targeting defense and pathogenicity factors. PhD Dissertation, May 2020. https://discover.library.unt.edu/catalog/b6731253
Status: PhD Dissertation Published
Acknowledgement of Federal Support: YES

Other publications, conference papers and presentations.

Shah, J., Alam, S.T., Mohan, V, Shulaev, E., Nagarajan, A., Gill, J., Tyagi, N., Lee, H., and H. N. Trick. 2019. Targeting fungal virulence genes via host-induced gene silencing (HIGS) for enhancing plant resistance to *Fusarium graminearum*. In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 55). Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Shah, J., Alam, S.T., Chabra, B., Mohan, V, Shulaev, E., Nagarajan, A., Gill, J., Rawat, N. (2019). Targeting wheat genes associated with susceptibility to *Fusarium graminearum* for enhancing FHB resistance. In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 57). Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Dill-Macky, R., Curland, R.D., Zargaran, B., Muehlbauer, G.J., Bethke, G., Funnell-Harris, D., Shah, J., McLaughlin, J., and Tumer, N. (2019). Testing Transgenic Spring Wheat and Barley Lines for Reaction to Fusarium Head Blight: 2019 Field Nursery Report. In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 46). Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Shah, J., Alam, S., Chabra, B., Mohan, V., Shulaev, E., Nagarajan, A., Gill, J., Rawat, N., Tyagi, N., Lee, H., and H. N. Trick. Targeting Pathogenicity Mechanisms to Promote FHB-Resistance in Wheat. In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 56). Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Talk Presented
Acknowledgement of Federal Support: YES (Abstract and Talk)

Shah, J. Developing plant resistance to disease - breaking susceptibility. Invited Talk: BioDiscovery Institute Seminars, University of North Texas. March 3, 2020.
Status: Talk Presented
Acknowledgement of Federal Support: YES (Talk)