USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY19 Final Performance Progress Report
Due date: August 31, 2021

Cover Page

Principle Investigator (PI):	Jyoti Shah
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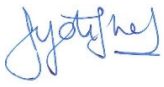
Phone:	940-565-4494
Fiscal Year:	2019
USDA-ARS Agreement ID:	59-0206-7-006
USDA-ARS Agreement Title:	Developing Resistance to Fusarium Head Blight in Wheat
FY19 USDA-ARS Award Amount:	$ 63,650
Recipient Organization:	University of North Texas 1155 Union Clrele #305250 Denton, Texas 76203-5017
DUNS Number:	614168995
EIN:	756002149
Recipient Identifying Number or Account Number:	GF10501
Project/Grant Reporting Period:	7/8/19 - 7/9/21
Reporting Period End Date:	7/9/2021

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**



08/05/2021

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R – Research
S – Service (DON Testing Lab)
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 research project?**
   The goal of this project is to transiently silence expression of pathogenicity genes in *Fusarium graminearum* (*Fg*) to mitigate FHB. Host-induced gene silencing (HIGS) is the approach that was used to silence expression of pathogenicity genes in the fungus while infecting plants. Fungal pathogenicity genes encoding secretory proteins were targeted by HIGS in Arabidopsis and wheat.

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

   a) What were the major activities?
   - Transgenic Arabidopsis and wheat TILLING lines containing the HIGS constructs were evaluated for transgene expression and disease severity. DON levels were also tested in wheat.
   - At different times during the course of this project, training and professional development opportunities were provided to an undergraduate and two graduate students, a research scientist, and a postdoc. One of the graduate students successfully completed her PhD degree.

   b) What were the significant results?
   Multiple Arabidopsis and wheat lines expressed the HIGS constructs. In comparison to the control non-transgenic lines, presence of the HIGS construct targeting the *Fg FGL1* and *Fg NahG* gene, which encode a putative lipase and salicylate hydroxylase, respectively, resulted in reduced severity of disease by *Fg* in Arabidopsis leaf and floral tissues. In the wheat *FGL1*-HIGS lines, reduced severity of FHB was observed in the early generations, which was associated with reduced accumulation of fungal DNA, thus confirming reduced growth of the fungus on these HIGS lines. In subsequent generations there was a general loss of resistance in the *FGL1*-HIGS wheat lines. We are currently evaluating if this is associated with silencing of the transgene in subsequent generations. Experiments are ongoing to test the impact on mycotoxin accumulation. The effect on FHB severity in wheat *Fg NahG*-HIGS lines is currently under evaluation.

   c) List key outcomes or other achievements.
   This study provides proof-of-concept that silencing of fungal pathogenicity genes that encode secretory enzymes provides a strategy for controlling *Fg* growth in planta.

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

For a 2.5 month period between March and May 2020, the University of North Texas campus was shut down to research. Several experiments had to be prematurely terminated and plants discarded due to the shutdown. During this period, students and staff associated with this project worked 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. Added to this was the increase in cost of supplies, especially PPE, disposable plasticware and reagents required for quantitative gene expression work.

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

Training: The undergraduate and graduate students received training in plant molecular biology, pathology and physiology. They learnt first-hand on how to plan experiments, collect, record, analyze and interpret data. They also developed their writing and presentation skills. The graduate students were enrolled in Dissertation hours and Individual Research under the PI. The undergraduate was registered in Special Problems under the PI. A research scientist who worked part-time on this project, received training in plant physiology and pathology. The PI mentored the postdoc to develop his mentoring skills by working with the students on planning experiments, troubleshooting, and providing hands-on-training.

Professional Development: This project contributed to the professional development of the students who participated in the weekly group meetings, department seminars, the BioDiscovery Institute research talks, the FHB forum, and other conferences, where they presented their work as posters and/or talks. The PI has worked individually with the graduate students, the research scientist and postdoc towards achieving their long-term professional goal. Upon receiving her PhD, the graduate student was employed as an instructor at a local university. She will be a full- time instructor/supervisor for undergraduate Microbiology labs at the University of North Texas. The postdoc was engaged by the PI in planning and developing projects and grant submissions.

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

Results were disseminated in multiple forms.

- As talks by the PI and the graduate students, and multiple posters at the Annual USWBSI Forum in 2019 and 2020 and other national/international conferences.
- Results were disseminated to the local community via the dissertation talk of a graduate student, which was open to the general public. Another talk was given by the PI to a broad audience at a BioDiscovery Institute seminar at UNT in spring 2020.
- The PI also disseminated outcomes of this work to undergraduate students as part of a lecture on 'Plant Interaction with the Environment' in an introductory biology course offered by the PI. In addition, the PI communicated about FHB and this work to graduate students enrolled in a course entitled 'Signaling Mechanism in Plants'.

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 mitigate FHB by knockdown of the wheat lipoxygenase *Lpx3* locus. TILLING was utilized to knock down activity of *Lpx3*, with the purpose of providing non-GMO genetic resource that can be integrated into wheat breeding programs. This project involved work 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 these three items below.)*

a) What were the major activities?

- Molecular makers were developed to distinguish between wild type and mutant alleles at the *Lpx3* locus on each o chromosome 4A, 5B, and 4D. These have been utilized to identify plants that are homozygous for the mutant *Lpx3* alleles.
- FHB disease severity was characterized for TILLING lines that contain mutations in the *Lpx3* homeologs on chromosomes 4A, 4B and 4D.
- Crosses were conducted to generate plants with mutations at two homeologous *Lpx3* loci.
- At different times during the course of this project, training and professional development opportunities were provided to two graduate students and a postdoc. One graduate student successfully completed her PhD degree.

b) What were the significant results?

Lines containing mutations on the *Lpx3* homeolog on Chromosome 4A exhibited the strongest FHB resistance phenotype. Mutations at the *Lpx3* loci in Chromosome 4B also resulted in reduced FHB severity.

c) List key outcomes or other achievements.

Knockdown of L*px3* function, in particular the *Lpx3* on chromosome 4A and 4B provides a good source of resistance to Fusarium Head Blight. The TILLING lines with these mutations offer a non-GMO germplasm for breeders.

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

For a 2.5 month period between March and May 2020, the University of North Texas campus was shut down to research. Several experiments had to be prematurely terminated and plants discarded due to the shutdown. During this period, students and staff associated with this project worked 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. Added to this was the increase in cost of supplies, especially PPE, disposable plasticware and reagents required for quantitative gene expression work.

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

Training: Two graduate students received training in plant molecular biology, pathology and physiology. They learnt first-hand on how to plan experiments, collect, record, analyze and interpret data. They also developed their writing and presentation skills. These graduate students were enrolled in Dissertation and/or Individual research under the PI. The PI mentored the postdoc to develop his mentoring skills by working with the students on planning experiments, troubleshooting, and providing hands-on-training.

Professional Development: This project contributed to the professional development of the students who participated in the weekly group meetings, department seminars, the BioDiscovery Institute research talks, the FHB forum, and other conferences, where they presented their work as posters and/or talks. The PI has worked individually with the graduate students and the postdoc towards achieving their long-term professional goal. Upon receiving her PhD, the graduate student was employed as an instructor at a local university. She will be a full- time instructor/supervisor for undergraduate Microbiology labs at the University of North Texas. The postdoc was engaged by the PI in planning and developing projects and grant submissions.

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

Results were disseminated in multiple forms.

- As talks by the PI and the graduate students, and multiple posters at the Annual USWBSI Forum in 2019 and 2020 and other national/international conferences.
- Results were disseminated to the local community via the dissertation talk of a graduate student, which was open to the general public, and a talk by the other graduate student at the annual graduate research day in spring 2021 at UNT. Another talk was given by the PI to a broad audience at a BioDiscovery Institute seminar at UNT in spring 2020.
- The PI also disseminated outcomes of this work to undergraduate students as part of a lecture on 'Plant Interaction with the Environment' in an introductory biology course offered by the PI. In addition, the PI communicated about FHB and this work to graduate students enrolled in a course entitled 'Signaling Mechanism in Plants'.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the **FY19 award period (7/8/19 - 7/9/21)**. 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?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Click to enter number here.

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 ☐No ☐ Not Applicable
   If yes, how many? 1 - Syeda Alam successfully defended her dissertation in fall 2019 and was awarded her PhD degree in spring 2020.

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?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Click to enter number here.

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?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Click to enter number here.

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 (7/8/19 - 7/9/21)**. 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	FHB Rating (0-9)	Year Released
Not applicable to this project.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

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

Publications, Conference Papers, and Presentations

Instructions: Refer to the 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 - 7/9/21**) 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:

> Z.J. Winn, R. Acharya, J. Lyerly, G. Brown-Guedira, C. Cowger, C. Griffey, J. Fitzgerald, R.E. Mason and J.P. Murphy. 2020. "Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat." In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum (*p. 12.), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
> 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 (Talk and Dissertation)

Other publications, conference papers and presentations.

Mittal, I., Alam, S., Chabra, B., Shulaev, E., Mohan, V., Rawat, N., Shah, J. 2021. 9-lipoxygenase as a susceptibility factor in Arabidopsis and wheat interaction with *Fusarium graminearum*. 31st International Conference on *Arabidopsis* Research-ICAR 2021 Abstract book. (p. 112). Virtual; June 21-25. Online: http://icar2020.arabidopsisresearch.org/uploads/1/1/7/5/117554241/icar_2021_abstract_book.pdf
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Poster)

Shah, J. 2021. Mitigating FHB by knockdown of plant susceptibility and fungal virulence genes. US Wheat and Barely Scab Initiative Gene Discovery and Engineering Resistance Research Area Mid-Year Meeting. Virtual: May 27.
Status: Talk Presented
Acknowledgement of Federal Support: YES (Talk)

Mittal, I., Alam, S., Chabra, B., Shulaev, E., Mohan, V., Rawat, N., Shah, J. 2021. Targeting Wheat Genes Associated with Susceptibility to *Fusarium graminearum* for Enhancing FHB Resistance. 2021. 82nd Meeting of the Southern Section of the American Society of Plant Biologists. (p. 36). Virtual; April 16-18. Online: https://southern.aspb.org/wp-content/uploads/2021/04/Abstract-book-2021-SS-ASPB-meeting.pdf
Status: Abstract Published and Talk Presented
Acknowledgement of Federal Support: YES (Talk)

Mittal, I., Alam, S., Chabra, B., Shulaev, E., Mohan, V., Rawat, N., Shah, J. 2021. Targeting Wheat Genes Associated with Susceptibility to *Fusarium graminearum* for Enhancing FHB Resistance. 17th Annual Biology Graduate Student Association Research Symposium. Virtual; April 7-8. Online: https://untbgsa.weebly.com/schedule--program.html
Status: Abstract Published and Talk Presented
Acknowledgement of Federal Support: YES (Talk)

Mohan, V., Alam, S.T., Shulaev, E., Lee, H., Trick, H. N., Shah, J. 2020. Enhancing wheat resistance to *Fusarium graminearum* via host-induced gene silencing (HIGS) of the fungal virulence gene FGL1. In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum (*p. 70.), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Mittal, I., Alam, S., Chabra, B., Shulaev, E., Mohan, V., Rawat, N., Shah, J. 2020. Targeting Wheat Genes Associated with Susceptibility to *Fusarium graminearum* for Enhancing FHB Resistance. In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum (*p. 71.), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

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

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*, Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY. p. 55. https://scabusa.org/pdfs/NFHBF19_Proceedings_Web.pdf

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*, Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY. p. 57. https://scabusa.org/pdfs/NFHBF19_Proceedings_Web.pdf

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*, Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY. p. 46. https://scabusa.org/pdfs/NFHBF19_Proceedings_Web.pdf

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*, Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY. p. 56. https://scabusa.org/pdfs/NFHBF19_Proceedings_Web.pdf

Status: Abstract Published and Talk Presented
Acknowledgement of Federal Support: YES (Abstract and Talk)

Alam, S. 2019. Novel approaches for enhancing resistance to *Fusarium graminearum* in *Arabidopsis* and wheat by targeting defense and pathogenicity factors. PhD Defense Seminar. December 5, Denton, TX

Status: Talk Presented
Acknowledgement of Federal Support: YES (Talk)