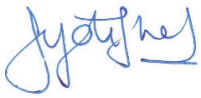
USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Final Performance Progress Report
Due date: August 31, 2021

Cover Page

Principle Investigator (PI):	Jyoti Shah
Institution:	University of North Texas
E-mail:	jyoti.shah@unt.edu
Phone:	940-565-4494
Fiscal Year:	2020
USDA-ARS Agreement ID:	59-0206-0-186
USDA-ARS Agreement Title:	Developing Resistance to Fusarium Head Blight in Wheat
FY20 USDA-ARS Award Amount:	$ 80,256
Recipient Organization:	University of North Texas 1155 Union Clrcle #305250 Denton, Texas 76203-5017
DUNS Number:	614168995
EIN:	756002149
Recipient Identifying Number or Account Number:	GF10504
Project/Grant Reporting Period:	7/10/20 - 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	$ 41,117
GDER	Wheat Variants Deficient in a FHB Susceptibility Factor	$ 39,139
	FY20 Total ARS Award Amount	**$ 80,256**



08/05/2021

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R- Research
S – Service (DON Testing Labs)
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 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 wheat lines containing the HIGS constructs were evaluated for transgene expression, disease severity and DON accumulation.
   - At different times during the course of this project, training and professional development opportunities were provided to a graduate student, a research scientist and a postdoc.

   b) **What were the significant results?**
   Although in earlier generations the wheat *FGL1*-HIGS lines exhibited reduced severity of FHB, in subsequent generations this was not found to be a stable phenotype and there was a decline in level of resistance to FHB observed in these lines. We are currently evaluating if this is associated with silencing of the transgene in wheat. The effect on FHB severity in the wheat *FgNahG*-HIGS 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 growth of *Fg* 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.**

   UNT campus reopened for research in summer 2020 with reduced occupancy and social distancing and masking. This has required staggering the times when individuals can work in the lab, resulting in general slowdown of research activity. An additional impact of the pandemic has been the difficulty and delay in ordering and procuring supplies. Added to this was the increase in cost of supplies, especially PPE, disposable plasticware and reagents required for quantitative gene expression work. Together, these have at times

interrupted experiments and/or caused delays in initiating and/or completing experiments.

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

Training: A research scientist who worked on this project, received training in plant physiology and pathology. The postdoc was mentored by the PI to develop his mentoring skills by working with the research scientist on planning experiments, troubleshooting, and providing hands-on-training.

Professional Development: This project contributed to the professional development of the research scientist and postdoc who participated in the weekly group meetings, department seminars, the BioDiscovery Institute research talks and the FHB forum. Posters/talks arising out of their work were presented at the 2019 and 2020 FHB forum as well as the mid-year GDER meeting that was held virtually in May 2021. The PI has worked individually with the research scientist and postdoc towards achieving their long-term professional goal and developing their scientific writing and presentation skills.

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

Results were disseminated in multiple forms.

- As multiple posters at the USWBSI Forum in 2020, and a talk by the PI at the mid-year GDER research area meeting in May 2021, and other conferences in 2021.
- The PI disseminated outcomes of this work to undergraduate enrolled in an introductory biology class taught by the PI in Spring 2021, as part of a lecture on 'Plant Interaction with the Environment. In addition, the PI communicated about FHB and this work to graduate students enrolled in a course entitled 'Signaling Mechanism in Plants' that was taught in Fall 2020.

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?**
   - FHB disease severity was characterized in multiple generations of TILLING lines that contain mutations in the *Lpx3* homeologs on chromosomes 4A, 4B and 4D.
   - Double mutants that contained mutant *Lpx3* alleles at more than one homeologous chromosomes were generated.
   - This project provided training and professional development opportunities to a graduate student and a postdoc.

   b) **What were the significant results?**
   TILLING Lines containing mutations on the *Lpx3* homeolog on Chromosome 4A and 4B have over multiple generations shown strong FHB resistance, which was accompanied by reduced DON levels.

   c) **List key outcomes or other achievements.**
   Knockdown of individual *Lpx3* genes on chromosome 4A and 4B provide a good source of resistance to Fusarium Head Blight that also results in reduced DON accumulation. 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.**

   UNT campus reopened for research in summer 2020 with reduced occupancy and social distancing and masking. This has required staggering the times when individuals can work in the lab, resulting in general slowdown of research activity. An additional impact of the pandemic has been the difficulty and delay in ordering and procuring supplies. Added to this was the increase in cost of supplies, especially PPE, disposable plasticware and reagents required for quantitative gene expression work. Together, these have at times

interrupted experiments and/or caused delays in initiating and/or completing experiments.

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

Training: The graduate student received training in plant molecular biology, pathology and physiology, on planning experiments, collecting, recording, analyzing and interpreting data. She also developed her writing and presentation skills. The graduate student was enrolled in Individual research under the PI. The postdoc was mentored by the PI to develop his mentoring skills by working with the graduate student on planning experiments, troubleshooting, and providing hands-on-training.

Professional Development: This project contributed to the professional development of the graduate student and the postdoc who participated in the weekly group meetings, department seminars, the BioDiscovery Institute research talks, the FHB forum and other national/international conferences. The PI has worked individually with the graduate student and the postdoc towards achieving their long-term professional goal and developing their scientific writing and presentation skills.

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

Results were disseminated in multiple forms.

- As multiple posters at the USWBSI Forum in 2020, a talk by the PI at the mid-year GDER research area meeting in May 2021, and other conferences in 2021.
- Results were disseminated to a broader audience via a talk by the graduate student at the annual graduate research day in April 2021 at UNT. The PI also disseminated outcomes of this work to undergraduate enrolled in an introductory biology class taught by the PI in Spring 2021, as part of a lecture on 'Plant Interaction with the Environment. In addition, the PI communicated about FHB and this work to graduate students enrolled in a course entitled 'Signaling Mechanism in Plants' that was taught in Fall 2020.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (7/10/20 - 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 FY20 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 FY20 award period?**
   ☐Yes ☒No ☐ Not Applicable
   If yes, how many? Click to enter number here.

3. **Have any post docs who worked for you during the FY20 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 FY20 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 FY20 award period (7/10/20 - 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 PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (7/10/20 - 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.

Nothing to Report

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)