USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY18 Performance Report
Due date: July 12, 2019

Cover Page

Principle Investigator (PI):	Jyoti Shah
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Fiscal Year:	2018
USDA-ARS Agreement ID:	59-0206-7-006
USDA-ARS Agreement Title:	Developing Resistance to Fusarium Head Blight in Wheat.
FY18 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:	7/10/18 - 7/9/19
Reporting Period End Date:	07/09/19

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
	FY18 Total ARS Award Amount	$ 63,650

Jyoti Shah Digitally signed by Jyoti Shah
Date: 2019.06.25 16:06:28 -05'00'

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

FY18 Performance Report
PI: Shah, Jyoti
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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 project?

The goal of this project is to determine if host-induced gene silencing (HIGS) can be used to silence expression of effector-encoding genes in *Fusarium graminearum* (*Fg*), thus causing a reduction in fungal pathogenicity *in planta* and thereby promoting plant resistance to *Fg*. Two *Fg* pathogenicity genes, *FgNahG* and *FGL1*, which encode secretory proteins encoding a salicylate hydroxylase and a lipase, respectively, will the targeted by HIGS in Arabidopsis and wheat.

2. What was accomplished under these goals?

1) Major activities

(i) Several transgenic Arabidopsis and wheat lines containing the *FGL1*-RNAi and *FgNahg*-RNAi have been generated, genotyped, evaluated for transgene expression and response to *Fg*.

(ii) Training opportunities were provided to two graduate students and two undergraduate students.

2) Specific objectives

The specific research objectives of this project are:

(i) Host-induced silencing of *F. graminearum* FGL1 effector gene to enhance disease resistance in both Arabidopsis and wheat.

(ii) *FgNahG* gene as a target for host-induced silencing to engineer resistance to *Fg* in Arabidopsis and wheat.

3) Significant results

(i) Several transgenic Arabidopsis *FGL1*-RNAi and *FgNahG*-RNAi lines which express inverted repeat with hair-pin loop containing *FGL1* and *NahG* RNAi chimeras, respectively, for silencing expression of *Fg FGL1* and *NahG* genes, have been identified. The *Cauliflower mosaic virus 35S* gene promoter was used to express these chimeric RNAi constructs in Arabidopsis.

(ii) Analysis of three independent Arabidopsis transgenic lines for *FGL1*-RNAi and three independent *FgNahG*-RNAi transgenic lines showed that presence of the RNAi construct resulted in reduced severity of disease by *Fg* in both leaf and inflorescence assays.

(iii) In agreement with reduced disease severity, fungal DNA was also present at lower amounts in the *FGL1*-RNAi and *FgNahG*-RNAi transgenic lines compared to the non-transgenic lines, thus indicating reduced fungal growth.

(iv) Callose deposition, a hallmark of defense activation, was induced to higher level in the *FGL1*-RNAi and *FgNahG*-RNAi transgenic lines compared to the non-transgenic lines, thus indicating that these transgenic lines respond to *Fg* infection with more robust defense activation.

(v) Initial *Fg* disease assays conducted on wheat inflorescence showed reduced disease severity in the *FGL1*-RNAi and *FgNahG*-RNAi transgenic lines, in which the chimeric constructs are expressed from the maize *Ubiquitin* (*Ubi*) gene promoter.

4) Key outcomes or other achievements

Experimental evidence indicates that HIGS-mediated silencing of *FGL1* and *FgNahG* provides a good strategy for controlling *Fg* infection in Arabidopsis leaf and floral tissues. Preliminary experiments indicate that this strategy also enhances resistance against *Fg* in wheat.

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

Training: Two graduate students (a senior student who is close to graduating and a new student) who are associated with this project received training in molecular biology and plant pathology. During the course of this project, the graduate students received 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 students received training in developing scientific writing and presentation skills. The graduate students were enrolled in dissertation/research hours under the PI and participated in teaching, as well.

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

Professional Development: This project has contributed to the professional development of the graduate students. The senior graduate student presented her work at a local meeting. In addition, a posters arising out of her work was presented at the 2018 FHB forum. She also coauthored two manuscripts arising out of previous years funding from the USWBSI/USDA-ARS. One of the manuscripts was published in 2019 in Molecular Plant Pathology and a second manuscript that was submitted in summer 2018 is undergoing revision for resubmission. 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 2018 Annual USWBSI Forum and also to local community via on campus poster presentations by the graduate student.

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

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

The goal of the project is to utilize a non-GMO approach, TILLING, in order to target wheat genes that contribute to susceptibility to *F. graminearum* (*Fg*). The reduction in activity of susceptibility genes is expected to enhance resistance to *Fg* and therefore provide an approach to utilize the mutant genetic material in wheat breeding programs. Wheat lipoxygenase *Lpx3*, located on chromosome 4, is the target of this project. Part of the project has been conducted on hexaploid wheat Cadenza in the Shah lab at UNT whereas part of it is being conducted by collaborator Rawat at the University of Maryland is on the tetraploid wheat Kronos.

2. What was accomplished under these goals?

1) Major activities

(i) PCR based co-dominant markers were developed to distinguish between wild type and mutant alleles. These markers were used to identify homozygous mutant lines.

(ii) Preliminary response of the TILLING lines containing mutations in the *Lpx3* homeologs on chromosomes 4A, 4B and 4D in the hexaploid wheat Cadenza to *F. graminearum* was studied.

(iii) Training opportunities were provided to graduate and undergraduate students.

2) Specific objectives

The specific research objectives of this project are:

(i) Isolate homozygous *Lpx3* mutant lines from the three sub-genomes, and backcross them to clear background mutations.

(ii) Characterize the response of *Lpx3* mutants to *F. graminearum*.

(iii) Develop wheat lines containing combinations of *Lpx3* mutant alleles at the homeologous chromosomes.

3) Significant results

(i) Eight TILLING lines containing mutations in the *Lpx3* homeologs on chromosomes 4A, 4B, and 4D in the hexaploid wheat variety Cadenza were identified. PCR based co-dominant markers were used to identify plants that are homozygous for the *Lpx3* mutant alleles. Three of these lines contain mutations in the *Lpx3* homeolog on chromosome 4A with two bearing nonsense mutations and one containing a missense mutation. Three lines contained nonsense mutations in the *Lpx3* homeolog on chromosome 4B, and two contain nonsense mutations in the *Lpx3* homeolog on chromosome 4D.

(ii) Homozygous *Lpx3* mutant lines for the three sub-genomes have been identified.

(iii) Some of the mutant lines have been backcrossed to clear background mutations and additional crosses have been conducted involving different combinations of TILLING lines, with the goal of identifying lines containing non-sense mutations at more than one *Lpx3* homeolog.

(iv) In preliminary experiments with segregating populations, two TILLING lines containing

(v) Nonsnse mutations in the *Lpx3* homeolog on chromosome 4A showed enhanced resistance compared to the parental Cadenza. In contrast, a line containing missense mutation at the *Lpx3* locus on chromosome 4A showed high susceptibility to *F.*

graminearum. We postulate that the missense allele encodes a hyperactive *Lpx3* allele, or alternatively contains a second mutation at another locus.

(vi) Seedling blight assays performed with *Fg* indicate that both the parental Cadenza and mutant lines show enhanced resistance to *F. graminearum* infection compared to the wheat variety Bobwhite.

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 Fusarium Head Blight. However, since these lines contain additional mutations elsewhere in the genome, at this stage we cannot rule out impact of other mutations on the observed results.

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

The two graduate students (a senior student who is closed to graduating and a new student) who are associated with this project received training in molecular plant pathology. During the course of this project, the graduate student received 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 students were provided training in developing scientific writing and presentation skills. The graduate students were enrolled in dissertation/research hours under the PI and participated in teaching, as well. A technician, who worked part-time on this project, received training in molecular biology, plant biology and pathology. More recently, a post-doc has begun working part-time on this project and is receiving training in plant pathology.

Professional Development: This project has contributed to the professional development of the graduate students. The senior graduate student presented her work at a local meeting. In addition, a posters arising out of her work was presented at the 2018 FHB forum. She also coauthored two manuscripts arising out of previous years funding from the USWBSI/USDA-ARS. One of the manuscripts was published in 2019 in Molecular Plant Pathology and a second manuscript that was submitted in summer 2018 is undergoing revision for resubmission. 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 2018 Annual USWBSI Forum and also to local community through poster presentations by the graduate student.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY18 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 FY18 award period?** Nothing to report

   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 FY18 award period?** Nothing to report

   If yes, how many?

3. **Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?** Nothing to report

   If yes, how many?

4. **Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?** Nothing to report

   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 FY18 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 FY18-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY18 grant. Only include citations for publications submitted or presentations given during your award period (7/10/18 - 7/9/19). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.
Nothing to report

Books or other non-periodical, one-time publications.
Nothing to report

Other publications, conference papers and presentations.

Alam, S., Tyagi, N., Trick, H.N. and J. Shah. 2018. Host-induced gene silencing (HIGS) for enhancing resistance to *Fusarium graminearum*. In: Proceedings of the 2018 National Fusarium Head Blight Forum.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), YES (abstract)

Alam, S., Chhabra, B., Rawat, N. and J. Shah. 2018. Targeting wheat genes associated with susceptibility to *Fusarium graminearum* for enhancing FHB resistance. In: Proceedings of the 2018 National Fusarium Head Blight Forum.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), YES (abstract)

Alam, S., Tyagi, N., Trick, H. N. and J. Shah. 2019. Host-induced gene silencing (HIGS) for enhancing resistance to *Fusarium graminearum*. In: BDI Retreat, 2019, Department of Biological Sciences, University of North Texas, Denton, TX.
Status: Poster Presented
Acknowledgement of Federal Support: YES (poster)

Alam, S., Chhabra, B., Rawat, N. and J. Shah. 2019. Targeting wheat genes associated with susceptibility to *Fusarium graminearum* for enhancing FHB resistance. In: BDI Retreat, 2019, Department of Biological Sciences, University of North Texas, Denton, TX.
Status: Poster Presented
Acknowledgement of Federal Support: YES (poster)

Chhabra, B., Singh, L., Shah, J., Tiwari, V. and N. Rawat. 2019. Using the mediators for winning the fight against Fusarium head blight. College of Agriculture and Natural Resources Symposium, University of Maryland, College Park, MD.
Status: Poster Presented
Acknowledgement of Federal Support: YES (poster)