

USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report
Due date: October 2, 2018

Cover Page

Principle Investigator (PI):	Nidhi Rawat
Institution:	University of Maryland
E-mail:	nidhirwt@umd.edu
Phone:	301 405 6244
Fiscal Year:	2017
USDA-ARS Agreement ID:	59-0200-6-018
USDA-ARS Agreement Title:	New Sources of Resistance to FHB and DON.
FY17 USDA-ARS Award Amount:	\$ 70,252
Recipient Organization:	University of Maryland Office of the Comptroller Contract and Grant Accounting RM 4101, Chesapeake Bldg College Park, MD 20742-3141
DUNS Number:	790934285
EIN:	52-6002033
Recipient Identifying Number or Account Number:	KFS 5258230
Project/Grant Reporting Period:	9/6/17 - 9/5/18
Reporting Period End Date:	09/05/18

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Over-expression and Allele Mining for Fhb1 in Wheat.	\$ 48,450
GDER	Wheat Variants Deficient in a FHB Susceptibility Factor	\$ 21,802
	FY17 Total ARS Award Amount	\$ 70,252



10/30/2018

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: *Over-expression and Allele Mining for Fhb1 in Wheat.*

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

Overall project goals:

Fhb1 is the most consistently reported source of Type 2 resistance to the devastating Fusarium Head Blight (FHB) disease of wheat. The Fhb1 QTL has been recently cloned in our Lab and validated using TILLING and RNAi. The overall goal of the proposed research is to over-express the Fhb1 QTL from Sumai3 in wheat. The long term applications will be to deploy *Fhb1* in popular wheat and barley cultivars to minimize losses due to FHB.

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

1) Major activities: Transgenic lines in Bobwhite and Fielder background over-expressing PFT were developed.

2) Specific objectives:

- a. Constructs of *Fhb1* in pAHC17 vector were generated.
- b. Transformed wheat cultivars Bobwhite and Fielder over-expressing *Fhb1* were generated.
- c. Seeds of T0 generation were grown to produce T1 plants.
- d. The T1 generation of cultivars with over-expression of *Fhb1* were tested for scab resistance. These plants showed FHB resistance/ significant delay in FHB spread as compared to wild type plants.

3) Significant results: Transgenic lines over-expressing PFT were found to have either reduced FHB severity or significantly slower progress of disease as compared to wild type.

4) Key outcomes or other achievements:

Establishing that PFT plays a major role in FHB resistance in wheat.

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

A graduate student was trained in inoculation techniques, FHB scoring, and data analysis for Fusarium Head Blight in wheat. In addition: DNA and RNA extraction to validate the expression of the transformed gene was included in the training. Several undergraduate students involved in various semester had hands on training in growing wheat plants, inoculating them and scoring for FHB.

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

Oral presentation of results was made in USWBSI 2017 meeting. In addition to the publication of preliminary results of over-expression of gene (Rawat et al. 2016), The results were presented at International Wheat Genetics Symposium 2017, in Tulln, Austria in front of an international audience. The audience included scientists specializing in genetics and pathology of wheat, barley and other grain crops.

Project 2: Wheat Variants Deficient in a FHB Susceptibility Factor

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

The major goal of this project is to utilize TILLING as a non-GMO (non-genetically modified organism) approach to target the activity of wheat genes that contribute to susceptibility to *Fusarium graminearum*. It is expected that reduction in activity of these wheat genes will confer resistance to *F. graminearum* and thus provide genetic material that can be utilized in wheat breeding programs. Wheat *Lpx3* gene, which is located on chromosome 4, is the target of this study. Work funded by this project was done with tetraploid wheat Kronos in the Rawat Lab at University of Maryland in coordination with Dr. Jyoti Shah at UNT with hexaploid wheat Cadenza. The specific objectives are:

- a. Isolate homozygous *Lpx3* mutant lines from the three sub-genomes, and backcross them to clear background mutations.
- b. Characterize the response of *Lpx3* mutants to *F. graminearum*.
- c. Develop wheat lines containing combinations of *Lpx3* mutant alleles at the homeologous chromosomes.

2. What was accomplished under these goals? Address items 1-4) below for each goal or objective.

1) major activities

- a. Knock-out mutants were identified for *Lpx3* in tetraploid wheat variety Kronos for both A and B genomes
- b. Seeds were procured and grown in greenhouse
- c. Genome-specific primers were designed to confirm the mutations in the plants
- d. Plants were grown, DNA isolated and sequencing confirmed the knock-out mutations expected in the plants
- e. Plants were phenotyped for Fusarium Head Blight by point inoculations in the greenhouse.

2) specific objectives

- a. Identify TILLING lines containing mutations in the *Lpx3* homeologs on chromosomes 4A, 4B and 4D in the hexaploid wheat were identified.
- b. Conduct a preliminary analysis of response to *F. graminearum* in these lines at the University of North Texas. In a parallel approach at University of Maryland, TILLING mutants were identified at the *Lpx3* homeologs on chromosomes 4A and 4B in the tetraploid Kronos and their response to *F. graminearum* characterized.

2) Specific objectives

- a. Identify TILLING lines containing mutations in each of the Lpx3 homeologs on chromosomes 4A, and 4B, and 4D in tetraploid wheat variety Kronos.
- b. Characterize the reaction of these mutant lines compared to the parental variety Kronos

TILLING mutants in hexaploid variety Cadenza were targeted at the University of North Texas.

3) Significant results

- a. Compared to the wheat variety Bobwhite, the variety Kronos exhibits higher level of resistance to *F. graminearum* infection.
- b. Preliminary results with point inoculations of homozygous 4A and 4B mutants confirmed the former to have higher level of resistance as compared to the wild type parent Kronos.
- c. Further confirmation of the previous results is being done in the fall of 2018.

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 FHB.

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

Training: A graduate student associated with this project received training in molecular plant pathology. During the course of this project, these personnel received training on the application of molecular methods for studying *Fusarium* infection, in planning of experiments, data collection and recording, and data analysis and interpretation. The graduate student was provided training in developing scientific writing and presentation skills. The graduate student was enrolled in dissertation hours under the PI.

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

Professional Development: This project has further contributed to the professional development of the graduate student. The project provided opportunities to the grad student for presentation skill development. PI has worked individually with the graduate student towards achieving the student's long-term professional goal in academia.

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

Results were disseminated to the local community through a poster presentation at University of Maryland's College of Agriculture symposium on Healthy Food systems PI Rawat also used the resources developed in this project to teach her graduate-senior undergraduate level course: Principles of Plant Pathology.

Results associated with this project were also disseminated to the wheat and barley scab community via a poster at the 2017 Annual USWBSI Forum in Milwaukee, WI.

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Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17 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 FY17 award period?**

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 FY17 award period?**

If yes, how many?

- 3. Have any post docs who worked for you during the FY17 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**

If yes, how many?

- 4. Have any post docs who worked for you during the FY17 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?

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 FY17 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

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Publications, Conference Papers, and Presentations

Instructions: Refer to the FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17 grant. Only include citations for publications submitted or presentations given during your award period (9/6/17 - 9/5/18). If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each reference/citation, you must indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in publication/presentation.

Journal publications.

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

Other publications, conference papers and presentations.

Chhabra B., Singh L., Shah J., Tiwari V.K., Rawat N. 2018. Using the mediators for winning the fight against Fusarium Head Blight

Status: Poster Presented by graduate student Chhabra.

Acknowledgement of Federal Support: YES (poster)

Shah J., Alam, S., and Rawat, N. 2017. Targeting Wheat Genes Associated with Susceptibility to *Fusarium graminearum* for Enhancing FHB Resistance. In: Proceedings of the 2017 National Fusarium Head Blight Forum. Milwaukee, WI.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (poster), YES (abstract)