USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY18 Performance Report
Due date: September 23, 2019

Cover Page

Principle Investigator (PI):	Nidhi Rawat
Institution:	University of Maryland
E-mail:	nrawat@umd.edu
Phone:	301-405-9744
Fiscal Year:	2018
USDA-ARS Agreement ID:	59-0200-6-018
USDA-ARS Agreement Title:	Investigating Sources of Fusarium Head Blight Resistance from Wheat and its Wild Relatives.
FY18 USDA-ARS Award Amount:	$ 37,925
Recipient Organization:	University of Maryland Office of the Comptroller Contract and Grant Accounting RM 4101, Chesapeake Bldg College Pard, MD 20742-3141
DUNS Number:	790934285
EIN:	52-6002033
Recipient Identifying Number or Account Number:	KFS 5258230
Project/Grant Reporting Period:	9/5/18 - 9/4/19
Reporting Period End Date:	09/05/19

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Wheat Variants Deficient in a FHB Susceptibility Factor.	$ 25,620
MGMT	Analyzing Commercial Wheat and Barley Cultivars for FHB Reaction in MD/DE.	$ 12,305
	FY18 Total ARS Award Amount	$ 37,925

Rawat

9/27/2019

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

1. **What are the major goals and objectives of the project?**
The long-term goal of this project is to identify native wheat gene variants that improve FHB resistance and/or reduce DON accumulation, with the aim that these could provide novel genetic material for integration into FHB resistance breeding programs. The major goals of the proposed work are to establish whether (i) the FHB resistance promoting effect of *Lpx3* knockdown is also effective in wheat backgrounds other than Bobwhite, (ii) one or more *Lpx3* homeolog(s) in wheat contribute towards susceptibility to *Fusarium graminearum*, and (iii) nonsense and/or missense *Lpx3* variants can provide a non-GMO (genetically-modified organism) strategy that in the future can be utilized by breeding programs to enhance FHB resistance in wheat. The part of project

The specific objectives for FY18 were to:

1. Backcross *Lpx3* variant lines to clear background mutations.
2. Characterize the response of homozygous *Lpx3* variants to *Fusarium graminearum*.
3. Develop wheat lines with mutant combinations at more than one *Lpx3* homeologous loci.

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

1) Major activities

The activities in the project at UMD have been carried out in tetraploid wheat cultivar Kronos. Specific activities conducted under the above goals were:

a) Procurement of knock-out mutants from UC Davis TILLING resource: Two knock-out mutants of Lpx3 gene each for the A and B genomes of Kronos were identified from the TILLING database. These mutants were procured from UC Davis and confirmed by Sanger Sequencing for the mutations.

b) Testing of Homozygous mutants and Back-crossing: Cel-1 assays and/or dCAPS markers were used for identifying homozygous mutants for all the four mutations. The homozygous mutants were tested for the phenotype using point inoculations with Fusarium graminearum GZ3639 macroconidia. B genome knock-out mutants were found to have significantly lower disease severity 21 days after inoculation.

c) Back-crossing Lpx3 variant lines to clear background mutations: As there are numerous mutations in the background of each variant line, backcrosses were initiated with wild type Kronos to clear up the background mutations. We are currently growing the back-cross progeny with mutations in heterozygous conditions in the greenhouse, for one more back-cross this season. Next season, these individuals will be selfed following which, homozygous mutants with >75% parental background recovered will be grown to test the effect of the target mutations.

d) Combining A and B genome mutants: Crosses were made to combine the A and B genome mutations. F1 plants are currently growing in the greenhouse, and some are being allowed to self. F2 individuals with both the mutations present in homozygous condition will be selected next season from these plants. Also some of the F1 plants

are being crossed with wild type Kronos to reduce the number of background mutations. The back-crossed individuals will be allowed to self, for the combined homozygous knock-out mutations with reduced background mutations.

2) Specific objectives

a) Backcross *Lpx3* variant lines to clear background mutations: Back-crosses were made for all four mutations with the wild type parent Kronos. The backcrossed individuals with mutations in heterozygous condition will be crossed again with the parent this season. Next season these BC2 individuals will be selfed to retrieve mutations in homozygous condition, which will be tested for the phenotype as described above.

b) Characterize the response of homozygous *Lpx3* variants to *Fusarium graminearum*: Homozygous mutations were tested for disease reaction by point inoculations and the B genome mutations had significantly lower FHB severity. However, it is important to note here that there are thousands of mutation sin the background of these homozygous mutants, which should be first cleared up to reach to an ultilate conclusion.

c) Develop wheat lines with mutant combinations at more than one *Lpx3* homeologous loci: Crosses were made to combine mutations in the A and B genomes. F1 individuals with both mutations combined are being grown in the greenhouse, and backcrosses will be made to reduce the background mutations. At the same time, some F1 individuals will be allowed to self to develop F2 population, from which progeny with both mutations in homozygous condition will be retrieved for testing the phenotype.

3) Significant results

a) F1 individuals having the four combinations of A and B genome knock-out mutations have been developed. Back-crossing of these individuals will reduce the number of back-ground mutations.

b) Homozygous mutants were phenotyped and the B genome knock-out mutation provided significant reduction in FHB severity

c) Back-cross individuals for clearing up background mutations were developed. They will be further backcrossed once more and then homozygous individuals will be retrieved for final testing of the knock-out mutations for individual genomes, which will also be compared with the combined effect of A and B genome knock-out mutant.

4) Key outcomes or other achievements

Preliminary phenotyping indicates that the B genome knock-out mutations of the Lpx3 gene may reduce the FHB severity. However, we are clearing up background mutations to make robust conclusions. Also the additive effect of combining A and B genome mutations needs to be investigated.

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

This work is being conducted by an MS student Bhavit Chhabra, and is a part of his MS thesis research. He has been trained with Fusarium inoculations and phenotyping in greenhouse, as well as scoring FHB severity and incidence in field conditions. He has received extensive training in marker design, PCR, sequencing, Cel-1 assays, and statistical analysis of results. Professional development opportunities provided to him include attending regional and national meetings. He made an oral presentation at the Mid-Atlantic ASPB symposium-2019, and a poster presentation in American Phytopathological Society meeting in Cleveland-2019. He also received training on SAS in Cleveland in a workshop conducted for graduate students.

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

The results have been disseminated through oral presentations and poster presentations at various platforms, including: National FHB forum 2018, Mid-Atlantic ASPB meeting 2019, and American Phytopathological Society meeting in Cleveland-2019.

Project 2: *Analyzing Commercial Wheat and Barley Cultivars for FHB Reaction in MD/DE.*

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

The major goal of this project is to conduct misted nursery to assess variety response to FHB in Maryland (MD) and Delaware (DE) cultivars.

Project Objective are:

a) Conduct misted nursery for local wheat and barley varieties from Maryland and Delaware.
b) Make the results available to the growers in a timely manner so that they can use them in making planting decisions in the growing season.
c) Organize field day to make growers aware of the importance of planting resistant varieties in management of FHB.

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

1) Major activities:

a) Maryland and Delaware wheat and barley varieties were procured for FHB evaluations and DON content measurement from seed companies. Last season (2017-2018) we tested only wheat varieties, and the reviewers asked us to include barley as well in our screening, therefore, this year, 12 barley varieties were included in the test.
b) FHB misted nursery was conducted at Beltsville Research Station of the University of Maryland. Data on FHB severity, incidence, FDKs, test weight, and DON content was measured and statistically analyzed.
c) Results were published as factsheet and shared with growers via various platforms including Maryland Commodity Classic, Extension network, UMD Extension website, USWBSI website, UMD Agriculture Newletter.
d) Field visits were organized for growers and stakeholders during FHB evaluation.

2) Specific objectives

a) Maryland and Delaware wheat and barley varieties were evaluated for their FHB response under high disease pressure environment. The latter was created by inoculating the field with infected corn kernels and misting the field for four weeks during flowering time of wheat. Grain samples were sent to Dr. Yanhong Dong at University of Minnesota, which is the lab assigned to the PI for DON estimation.
b) The results were shared with growers and stakeholders using multiple platforms of outreach.
c) Field visits for farmers and stakeholders were organized by the team during evaluation of disease index

3) Significant results

a) Significant differences were found in levels of resistance in different cultivars. The varieties with moderate resistance mostly had Fhb1 or native resistance in their pedigree.
b) A two-row barley variety Calypso was found to be late and having significantly lower FHB index and DON content. It is being screened again this year for confirmation of the last year's results.

4) Key outcomes or other achievements

a) Farmers were provided with ratings of FHB indices and DON content of the local popular varieties in a timely manner, which was used extensively by them in making their planting decisions for 2019-2020 season.

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

A PhD student has been involved with inoculum preparation and scoring of data in field, providing him training on field evaluation of genetic resistance against FHB. Professional development opportunities provided to him include attending regional and national meetings. He made a poster presentations at the Mid-Atlantic ASPB symposium-2019, and American Phytopathological Society meeting in Cleveland-2019. He also received training on SAS in Cleveland in a workshop conducted for graduate students.

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

The results have been disseminated through oral presentations, field visits and publication as factsheets. Results were shared with growers and stakeholders via various platforms including Maryland Commodity Classic, Extension network, UMD Extension website, USWBSI website, and UMD Agriculture Newletter.

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?** Yes

   If yes, how many? 1 MS student enrolled and should be graduating in 2020.

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?** Yes

   If yes, how many? 1 PhD student enrolled and should be graduating in 2021.

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?** No

   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?** 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 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 (9/5/18 - 9/4/19). 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. See example below for a poster presentation with an abstract:

> Conley, E.J., and J.A. Anderson. 2018. Accuracy of Genome-Wide Prediction for Fusarium Head Blight Associated Traits in a Spring Wheat Breeding Program. In: Proceedings of the XXIV International Plant & Animal Genome Conference, San Diego, CA.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (poster), NO (abstract)

Journal publications.

Singh, L., Anderson, J., Chen, J., Gill, B.S., Tiwari, V.K., **Rawat, N.** (2019). Development and Validation of a perfect KASP Marker for Fusarium Head Blight resistance gene *Fhb1* in Wheat. The Plant Pathology Journal. 35(3): 200–207
Status: Published
Acknowledgement of Federal Support: YES

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

Rawat, N. 2019. FHB Scab update. Anne Arundel County Agriculture Newsletter, May 2019: Pp 6.
Status: Published
Acknowledgement of Federal Support: YES

Rawat, N., Wight, J. 2019. Fusarium Head Blight Screening Nursery Factsheet. https://psla.umd.edu/sites/psla.umd.edu/files/_images/Wheat%20and%20Barley%20FHB%20ratings%202019.pdf
Status: Published
Acknowledgement of Federal Support: YES

Rawat, N., Wight, J. 2018. Fusarium Head Blight Screening Nursery Factsheet. https://psla.umd.edu/sites/psla.umd.edu/files/_docs/MD_CROPS/FHB%20Misted%20Nursery%20Factsheet-2018.pdf
Status: Published
Acknowledgement of Federal Support: YES

Other publications, conference papers and presentations.

Oral presentations:

Rawat, N. 2019. Developing genetic solutions to small grain diseases of the Mid-Atlantic region. APS Potomac Division meeting, Rehoboth Beach, Delaware, USA.
Status: Presented
Acknowledgement of Federal Support: YES

Rawat, N. 2019. Designing resistance against hemi-biotrophic pathogens of small grain crops. Institute of Bioscience and Biotechnology Research. Shady Grove, MD, USA.
Status: Presented
Acknowledgement of Federal Support: YES

Rawat, N. 2019. North Atlantic Invitation to Innovation meeting, organized by Syngenta. Annapolis, Maryland, USA.
Status: Presented
Acknowledgement of Federal Support: YES

Rawat, N. 2018. *Fusarium graminearum:* Can we really outsmart the sly pathogen? UC Berkeley. Buchanan Endowed lecture, Berkeley, CA, USA.
Status: Presented
Acknowledgement of Federal Support: YES

Rawat, N. 2018. Till-D: An *Aegilops tauschii* Specific Tilling Resource for Gene Discovery and Wheat Improvement. Crop Science Society of America, Baltimore, MD, USA.
Status: Presented
Acknowledgement of Federal Support: YES

Singh, LPS, Rawat, N. 2018. Evaluating the application window for wheat using Syngenta's new Fusarium Head Blight fungicide 'Miravis'. APS Potomac Division meeting, Ocean City, Maryland, USA.
Status: Presented
Acknowledgement of Federal Support: YES

Rawat, N. 2018. Overcoming the fungal foes of wheat. AGNR cornerstone event: Global Challenges: Building Healthy Food Systems. University of Maryland College Park, MD, USA.
Status: Presented
Acknowledgement of Federal Support: YES