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

Cover Page

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Fiscal Year:	2019
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
FY19 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:	9/6/19 - 9/5/21
Reporting Period End Date:	9/5/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
MGMT	Analyzing Commercial Wheat and Barley Cultivars for FHB Reaction in MD/DE	$ 12,305
GDER	Wheat Variants Deficient in a FHB Susceptibility Factor	$ 25,620
	FY19 Total ARS Award Amount	**$ 37,925**

8/27/2021

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

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

The goal of this project is to evaluate popular wheat and barley cultivars in Maryland and Delaware for their reaction to FHB and DON content in misted irrigation trials. FHB is the most important disease of these crops in the region, and growers need information on the level of resistance to FHB and DON accumulation in deciding on planting. Public sources of such information are invaluable on account of being unbiased towards the FHB and DON ratings. With the private commercial companies releasing their wheat and barley varieties every year, it becomes all the way more critical for growers to have an impartial source of information on the level of resistance in these varieties, which might otherwise be desirable. We will also be organizing a field-visit/ field-day to the FHB misted nursery by the local growers and stakeholders to increase awareness about the importance of planting resistant varieties. Field visit will also enable them to appreciate the efforts of USWBSI in supporting the scab research for the benefit of local growers. We have been conducting misted nurseries for FHB and DON content assessments for past two years in the region, and the growers have used the information generated in their planting decisions.
The specific objectives are:

1) Conduct misted nursery for local wheat and barley varieties from Maryland and Delaware.
2) 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.
3) 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 or objectives?** *(For each major goal/objective, address these three items below.)*

a) What were the major activities?

1) Procure Maryland and Delaware wheat and barley varieties for FHB evaluations and DON content measurement
2) Conduct FHB misted nurseries, Obtain data on FHB severity, incidence, FDKs, test weight, and DON content
3) Statistical analyses and Publication of results at various platforms for use by growers

b) What were the significant results?

A misted nursery was conducted to maintain a high FHB pressure artificially and a thorough analysis of ~80 commercial wheat and barley varieties was conducted over two years. Results were shared with the growers and stakeholders to make their planting decisions for Fall season as Factsheets published in July end.

c) List key outcomes or other achievements.

Factsheets providing ratings of various commercial cultivars of wheat and barley published.

Results of the work shared with farmers and stakeholders

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.

Field visits were planned for farmers and stakeholders interested in seeing the varietal performance in high FHB pressure nurseries. However, due to Covid, these could not be arranged.

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

Two graduate students were trained in the project with isolation of fungal pathogen, corn inoculum preparation, and disease recording. Seven undergraduate students helped with planting and disease data recording over the two-year period.

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

As Factsheets emailed to all the commodity boards, extension agents, and farmers. Oral presentation was made by the PI at the Maryland Commodity Classic meeting, the largest meeting of farmers, stakeholders and extension agents in the state. Factsheets were also presented at Maryland Commodity Classic meeting booth by the lab students.

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

1. What are the major goals and objectives of the research 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. With past funding from the USWBSI, we showed that 9-lipoxygenases (9-LOXs) in Arabidopsis thaliana are susceptibility factors that are targeted by Fusarium graminearum to facilitate infection [22, 23]. Wheat and barley contain genes that are homologous to the Arabidopsis 9-LOXs and are expressed in floral tissues [22]. We demonstrated that RNA-interference (RNAi)-mediated knockdown of 9-LOX-encoding genes enhanced FHB resistance in the hexaploid wheat cv Bobwhite [22, 23]. In particular, the silencing of the Lpx3 gene conferred resistance that was characterized by lack of spread of infection from the inoculated spikelet. The 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.

Several TILLING (Targeting Induced Local Lesions in Genomes) mutants covering all three Lpx3 homeologs on chromosomes 4A, 4B and 4D have been identified in the hexaploid and tetraploid wheat varieties Cadenza and Kronos, respectively. Mutations in these TILLING lines are predicted to yield prematurely truncated Lpx3 protein, or strong missense alleles. With the availability of these genetic resources, the specific objectives for FY18 and FY19 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 or objectives? *(For each major goal/objective, address these three items below.)*

a) What were the major activities?

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.

b) What were the 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.

c) List 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. 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.

Yes, Covid-19 impacted this project significantly. We lost the plants planted to make back-crosses in March in the greenhouse, as we could not access the greenhouses at that time. This process was completed in the following Fall and Spring season. However, it delayed our plant material development plans by at least a year.

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

A graduate student has been involved at UMD in this collaborative project. He has been trained with Fusarium culture preparation, 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.

One undergraduate students has been trained in the lab, with the lab and greenhouse techniques involved in the project.

5. 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: Potomac Division APS meeting (March 2020), summer GDER project update meeting in May 2021, and at various academic talks delivered by the PI.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the **FY19 award period (9/6/19 - 9/5/21)**. 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? 1 MS student

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? Click to enter number here.

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 (9/6/19 - 9/5/21)**. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations.

Not applicable

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
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
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
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
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
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 (9/6/19 - 9/5/21**) should be included. If you did not publish/submit or present anything, state 'Nothing to Report'.

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.

Chhabra, B., Singh, L., Wallace, S., Schoen, A., Dong, Y., Tiwari, V.K., Rawat, N. 2021. Screening of an EMS mutagenized population of a wheat cultivar susceptible to Fusarium head blight identifies resistant variants. Plant Disease DOI: 10.1094/PDIS-03-21-0670-RE.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES

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

None.

Other publications, conference papers and presentations.

Mittal, I., Alam, S., Chhabra, B., Shulaev, E., Mohan, V., Alam, Bhavit Chhabra, Elena Shulaev, Vijee Mohan, Rawat, N., Shah, J. (2020). Targeting wheat genes associated with susceptibility to Fusarium graminearum for enhancing FHB resistance. In: S. Canty, A. Hoffstetter, Dill-Macky, R. (Eds.), Proceedings of the 2020 National Fusarium head blight Forum (p.69), 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)

Luis, J.M., Ng S.J., Bergsrom, G., Bissonnette, K., Bowen, K., Bradley, C., Byamukama, E., Chilvers, M., Collins, A., Cowger, C., Darby, H., DeWolf, E., Dill-Macky, R., Esker, P., Friskop, A., Kleczewski, N., Koehler., A., Langston, D.B., Madden, L., Marshall, J., Mehl, H., Moraes, W., Nagelkirk, M., Rawat, N., Smith, D., Telenko, D., Wegulo, S., Young-Kelly H., Paul, P.A. (2020). Fusarium head blight Management Coordinated Project: Integrated Management Trials- Wheat 2018-2020. In: S. Canty, A. Hoffstetter, Dill-Macky, R. (Eds.), Proceedings of the 2020 National Fusarium head blight Forum (38-42), 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)

Luis, J.M., Ng S.J., Bergstrom, G., Bissonnette, K., Bowen, K., Bradley, C., Byamukama, E., Chilvers, M., Collins, A., Cowger, C., Darby, H., DeWolf, E., Dill-Macky, R., Esker, P., Friskop, A., Kleczewski, N., Koehler., A., Langston, D.B., Madden, L., Marshall, J., Mehl, H., Moraes, W., Nagelkirk, M., Rawat, N., Smith, D., Telenko, D., Wegulo, S., Young-Kelly H., Paul, P.A. (2020). Fusarium head blight Management Coordinated Project: Integrated Management Trials- Barley 2018-2020. In: S. Canty, A. Hoffstetter, Dill-Macky, R. (Eds.), Proceedings of the 2020 National Fusarium head blight Forum (43-48), 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., Alam, S., Chabra, B., Mohan, V., Shulaev, E., Nagarajan, A., Gill, J., Rawat, N., Tyagi, N., Lee, H., and H. N. Trick (2019). 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 (p. 56), Milwaukee, WI, Dec. 8-10, 2019. U.S. Wheat & Barley Scab Initiative, Online: https://scabusa.org/pdfs/NFHBF19_Proceedings_Web.pdf.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Abstract and Poster)

Paul, P., Ng, S.J., Bergstrom, G., Bissonnette, K., Bradley, C., Byamukama, E., Chilvers, M.I., Collins, A., Cowger, C., Darby, H.M., DeWolfe, E., Dill-Macky, R., Esker, P., Friskop, A., Kleczewski, N., Koehler, A., Madden, L.V., Marshall, J., Mehl, H., Moraes, W., Nagelkirk, M., Rawat, N, Smith, D., Talenko, D., Wegulo, S., Young-Kelly, H.M. (2019). Fusarium head blight management coordinated project: Uniform fungicide trials 2018-2019. Proceedings of the 2019 National Fusarium head blight Forum (p. 20-24). Milwaukee, WI, Dec. 8-10, 2019. U.S. Wheat & Barley Scab Initiative, Online: https://scabusa.org/pdfs/NFHBF19_Proceedings_Web.pdf.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Abstract and Poster)

Paul, P., Ng, S.J., Bergstrom, G., Bissonnette, K., Bradley, C., Byamukama, E., Chilvers, M.I., Collins, A., Cowger, C., Darby, H.M., DeWolfe, E., Dill-Macky, R., Esker, P., Friskop, A., Kleczewski, N., Koehler, A., Madden, L.V., Marshall, J., Mehl, H., Moraes, W., Nagelkirk, M., Rawat, N., Smith, D., Talenko, D., Wegulo, S., Young-Kelly, H.M. (2019). Fusarium head blight management coordinated project: Uniform fungicide trials 2018-2019. Proceedings of the 2019 National Fusarium head blight Forum (p. 25-29). Milwaukee, WI, Dec. 8-10, 2019. U.S. Wheat & Barley Scab Initiative, Online: https://scabusa.org/pdfs/NFHBF19_Proceedings_Web.pdf.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Abstract and Poster)

Rawat, N. Factsheet: 2020 Maryland wheat variety disease ratings https://psla.umd.edu/sites/psla.umd.edu/files/files/documents/Extension/Small%20Grains%20in%20MD/2020%20Disease%20Ratings%20Wheat%20Varieties%20(1).pdf

Status: Published and disseminated

Acknowledgement of Federal Support: YES

Rawat, N., Wight, J. 2019. Fusarium head blight screening nursery Factsheet. https://cpb-us-e1.wpmucdn.com/blog.umd.edu/dist/a/434/files/2019/08/Wheat-and-Barley-FHB-ratings-2019.pdf

Status: Published and disseminated

Acknowledgement of Federal Support: YES