USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Performance Report
Due date: July 24, 2020

Cover Page

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Fiscal Year:	2019
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Pedigree Based Association Analysis of Novel Sources of FHB Resistance in Durum Wheat
FY19 USDA-ARS Award Amount:	$ 53,140

USWBSI Individual Project(s)

USWBSI Research Category[*]	Project Title	ARS Award Amount
DUR-CP	Enhancing FHB Resistance by Epigenetic Modification of Durum Cultivars	$ 53,140
	FY19 Total ARS Award Amount	**$ 53,140**

Shahryar Kianian

7/24/20

Principal Investigator Date

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[*] 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

Project 1: *Enhancing FHB Resistance by Epigenetic Modification of Durum Cultivars*

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

The specific objectives of this project are to:

1. characterize the stability and inheritance of epigenetic changes in FHB resistant durum cultivars produced by altering the DNA methylation patterns, and
2. profile the transcriptome changes that have occurred as a result of epigenetic modification in resistant durum lines.

The ultimate objective of this project is to enhance the resistance in durum cultivars by removal of persistent suppression mechanism. Through this project we aim to develop lines with enhanced FHB resistance and associated molecular markers that can be incorporated into durum breeding programs.

2. What was accomplished under these goals or objectives? *(For each major goal/objective, address items a-b) below.)*

a) What were the major activities?

- Crossing and advancement of most resistant epigenetic mutants for characterization of the stability of resistance (Obje. 1)

- Transcriptome analysis of tissue from most resistance and susceptible parents (Obje. 2)

b) What were the significant results?

Eight advanced durum-breeding lines were treated with 5-methyl-azacytidine to test the feasibility of generating sources of Fusarium head blight (FHB) resistance. A total of 415, of the 800 treated seeds, germinated and were advanced up to four (M4) generations by selfing. Thirty-two of the 415 M4 lines were selected following preliminary screening and were further tested for FHB resistance for multiple years (3 years), field locations (2 locations), and in the greenhouse. Five of the 32 M4 lines showed less than 30% disease severity, as compared to the parental lines and susceptible checks. Fusarium-damaged kernels and deoxynivalenol analyses supported the findings of the field and greenhouse disease assessments. The overall methylation levels (%) were compared using FASTmC method, which did not show a significant difference between M4 and parental lines.

Two of the most resistant M4 lines were crossed to a susceptible parent, advanced up to third generation (BC_1:F_3) and were tested for stability and inheritance of the resistance. About, one third of the BC_1:F_3 lines showed FHB resistance similar to their M4 parents in replicated field testing. Further evaluation of selected lines in the greenhouse confirmed the stability of resistance.

One of the resistant M4 lines, E.25.10, along with a susceptible check (Ben) was used in transcriptome analysis. The analysis revealed differential expression (log2 value) of many novel candidate genes and the known genes in the M4 as compared to Ben. Gene

Ontology analysis of differentially expressed common genes revealed elevated expression of genes related to ion binding, cation binding, metal ion binding, defense response, cell wall organization and modification, hydrolase activity, enzyme regulator activity and other activities in the M4 line, as compared to Ben (Figure 1), that could play essential roles in conferring FHB resistance.

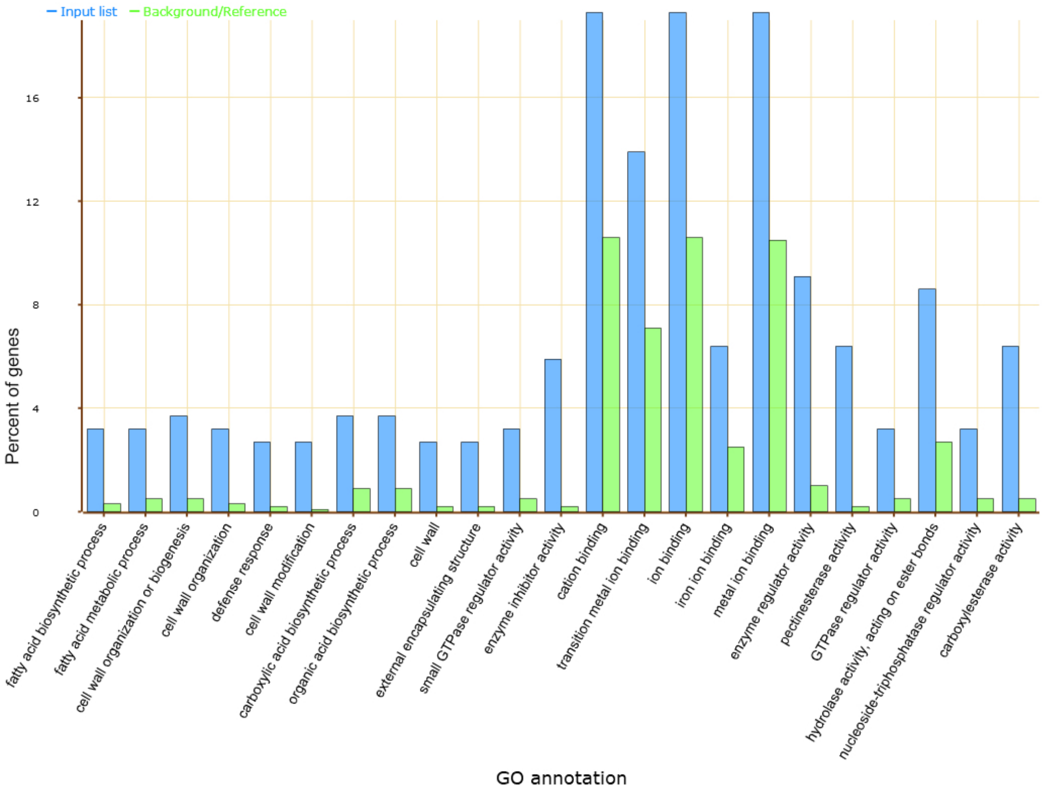

Figure 1. Bar graph representation of enrichment of gene ontology (GO) term for genes commonly up-regulated in M4 line as compared to Ben. X-axis represents GO annotation for predicted functions of the genes and the Y-axis shows percent of genes for each functions. Blue bars represent percent of genes in input list and the green bars represent genes in reference list.

Thus, transcriptome analysis of a M4 line revealed significant number of differentially expressed genes related to biosynthesis of secondary metabolites, MAPK signaling, photosynthesis, starch and sucrose metabolism, plant hormone signal transduction and plant-pathogen interaction pathways, which may have helped in improved FHB resistance.

c) List key outcomes or other achievements.

- Identification of FHB resistant durum lines by epigenetic modification
- Identification of a number of genes possibly underlying the FHB resistance in these modified lines by initial transcriptome analysis
- Initial analysis of backcross lines indicating stability of FHB resistance
- Further evaluation of backcross lines for agronomic traits in preparation for germplasm release

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

USDA Maximized telework posture and University shutdowns hindered our ability to conduct a number of projects related to FHB laboratory and field research. As much of the laboratory work could be put on hold, they were. The local field experiments were handled according to various guidelines; however, distant field work was inhibited due to travel restrictions. Additionally, due to shift in focus of various genome centers on Covid-19 testing/analysis many our molecular analysis work was put on hold. Thus, we expect the impact of this maximized telework posture to be significant.

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

Dr. Jitendra Kumar is the postdoctoral scientist on this project. Drs. Bradeen and Kianian have been actively advising/mentoring Dr. Kumar as he advances through his career. He has actively participated at various on-campus meeting (e.g., Department of Plant Pathology Seminar series). He has made several oral presentations to various groups (e.g., departmental, Cereal Disease Laboratory, and lab groups) and has been active in preparing publications from his research.

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

Through presentations and publication of outcomes.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (N/A). 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?**
   NO.
   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 FY19 award period?**
   NO.

   If yes, how many?

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?**
   No

   If yes, how many?

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?**
   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 FY19 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 FY19-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 (N/A)** 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:

> De Wolf, E., D. Shah, P. Paul, L. Madden, S. Crawford, D. Hane, S. Canty, R. Dill-Macky, D. Van Sanford, K. Imhoff and D. Miller. 2019. "Impact of Prediction Tools for Fusarium Head Blight in the US, 2009-2019." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p.12), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Kumar, J., R.M. Krishan, S. Pirseyedi, E.M. Elias, S. Xu, R. Dill-Macky and S.F. Kianian. 2020. Epigenetic regulation of gene expression improves Fusarium head blight resistance in durum wheat. Scientific Reports
Status: Submitted
Acknowledgement of Federal Support: YES

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

Other publications, conference papers and presentations.

Kumar, J., R.M. Krishan, S. Pirseyedi, S. Xu, E.M. Elias, R. Dill-Macky and S.F. Kianian. 2019. "Epigenetic modifications: A novel source of FHB resistance in durum wheat." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p.98), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract published and Oral Presentation Given
Acknowledgement of Federal Support: YES