USDA-ARS | U.S. Wheat and Barley Scab Initiative

FY21 Performance Progress Report

Due date: July 26, 2022

Cover Page

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Fiscal Year:	2021
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Pedigree Based Association Analysis of Novel Sources of FHB Resistance in Durum Wheat
FY20 USDA-ARS Award Amount:	$62,512
Recipient Organization:	USDA-ARS Cereal Disease Laboratory 1551 Lindig Street, University of Minnesota St. Paul, MN 55108
DUNS Number:	N/A
EIN:	N/A
Project/Grant Period:	5/1/21 - 4/30/22
Reporting Period End Date:	4/30/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
DUR-CP	Enhancing FHB Resistance by Epigenetic Modification of Durum Cultivars	$62,512
	FY21 Total ARS Award Amount	$62,512

I am submitting this report as an: ☑ Annual Report ☐ Final Report

I certify to the best of my knowledge and belief that this report is correct and complete for performance of activities for the purposes set forth in the award documents.

SHAHRYAR KIANIAN Digitally signed by SHAHRYAR KIANIAN Date: 2022.07.18 14:35:18 -05'00'

Principal Investigator Signature

Date Report Submitted

‡ BAR-CP – Barley Coordinated Project
DUR-CP – Durum Coordinated Project
EC-HQ – Executive Committee-Headquarters
FST-R – Food Safety & Toxicology (Research)
FST-S – Food Safety & Toxicology (Service)
GDER – Gene Discovery & Engineering Resistance
HWW-CP – Hard Winter Wheat Coordinated Project
MGMT – FHB Management
MGMT-IM – FHB Management – Integrated Management Coordinated Project
PBG – Pathogen Biology & Genetics
TSCI – Transformational Science
VDHR – Variety Development & Uniform Nurseries
NWW –Northern Soft Winter Wheat Region
SPR – Spring Wheat Region
SWW – Southern Soft Red Winter Wheat Region

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

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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 lines produced by altering the DNA methylation patterns,
2. profile the transcriptome changes that have occurred as a result of epigenetic modification in resistant durum lines, and
3. validate altered gene expression patterns and characterize candidate genes for use as perfect molecular markers in breeding.

The ultimate objective of this project is to enhance FHB 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 these three items below.)*

a) What were the major activities?

Further testing of backcross derived lines (crossing M4 resistant lines to the susceptible parent, advancing up to BC1:F3 without selection) for FHB resistance and agronomic performance (Objective 1)

Transcriptome analysis of additional tissue samples from the resistant M4 lines and susceptible parents/checks (Objective 2)

Quantitative real time PCR analysis of candidate genes with additional tissue/samples (objective 3)

Identification of durum mutant lines for candidate genes (objective 3)

b) What were the significant results?

As reported previously 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. This experiment was replicated with additional testing and results were further confirmed.

The top 50 resistant backcross (BC) derived lines along with the parental lines were further tested at two field locations, Saint Paul & Rosemount, MN for agronomic traits such as heading date, plant height, total number of spikes per plant, 30 spike seed count and seed weight. The measurements were performed in 8 replicates, 4 replicates at Saint Paul and 4 at Rosemount, MN. Values for all traits measured (heading date, height, total number of spikes, seed count per 30 spikes and seed weight per 30 spikes) for the backcross lines fell within the range of durum checks and in line with the susceptible

parent. In table below average values for each line across location/rep is presented (more detailed analysis/data is available). These lines were released to the NDSU durum breeding program for their evaluation and use. The parental lines, which were sent to the NDSU durum breeding program several years ago, were found to be resistant and were evaluated in field nurseries and Preliminary Yield Trial (PYT). At that stage it was determined that the lines need further improvement related to quality traits. We suspect the backcross derived lines also need improvement related to quality traits as the susceptible parents are outdated relative to current durum cultivars.

Line	HD	HT (inches)	#spikes	#seed per 30 spikes	Seed weight (30 spikes)
BC-D	39	33.7	115.5	965.1	31.3
BC-E	40.5	38.1	119.8	818.9	32
M4-E	42	38.9	96.9	792.9	28.5
M4-D	37.5	35.6	107.5	855.9	25.4
Ben	41	37	108.6	916.4	32.9
Carpio	43	34.6	119.9	902.6	32.2
Divide	42.8	34.5	105.8	928	29.7
Joppa	37.5	34.6	115.6	938.4	34.6
ND-Grano	40.8	33	124.8	908.5	30.9
ND-Riverland	42.5	36.4	118.6	880.4	32.4

We also performed additional RNA sequencing data from various tissues with and without Fusarium infections of mutant and parental lines to determine what changes are responsible for the enhanced resistance. We also performed transcriptome analysis by comparing between the M4 line (41708-72, E.25.10 and E.25.23) and the parental lines (D0-41708 and E.25) at two time points, 12h and 48h. Comparison of M4 with the parental lines provided significant details on the acquired resistance (we are finalizing a manuscript for submission). In addition to regulatory genes such as BZIP, MYB, and NAC transcription factor, several other genes including alpha-amylase inhibitor protein, disease resistance protein and detoxification superfamily proteins were found differentially expressed in M4 lines. The three M4 lines varied in their gene expression, as expected for lines derived from different durum varieties, but have some common set of genes that may be responsible for FHB resistance. A total of 25 genes with significantly altered gene expression patterns have been identified that could play a critical role in the resistance mechanism. Among these are some novel genes that are significantly altered in expression pattern compared with the parental check (see table below for a few examples).

Novel Genes	**E.25.48H vs E25.10.48H**	**E25.48H vs E25.23.48H**
TRITD4Av1G046970.1	7.12	7.02
TRITD4Bv1G122210.1	6.49	3.72
TRITD7Bv1G058860.1	6.35	5.33
TRITD7Av1G084400.1	5.43	5.18

To further confirm our transcriptome results we have been systematically performing quantitative real-time PCR analysis of the genes identified above in RNA obtained from tissues representing more detailed time course of infection. Tagged durum mutants for the 25 genes have been identified and will be planted in the fall greenhouse for seed purification and initial testing of mutation identity prior to characterization for disease reaction.

c) List key outcomes or other achievements.

1) Identification of FHB resistant durum lines through epigenetic modification
2) Demonstration that the resistance is heritable
3) Demonstration that the resistance is not associated with poor agronomic (e.g., late heading, or taller plants) performance
4) Identification of possible candidate genes for FHB resistance through transcriptome analysis

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

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

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

Through presentations and publication of outcomes

Publications, Conference Papers, and Presentations

Please include a listing of all your publications/presentations about your FHB work that were a result of funding from your FY21 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period** should be included.

Did you publish/submit or present anything during this award period?

☐ Yes, I've included the citation reference in listing(s) below.
☑ No, I have nothing to report.

Journal publications as a result of FY21 grant award

List peer-reviewed articles or papers appearing in scientific, technical, or professional journals. Include any peer-reviewed publication in the periodically published proceedings of a scientific society, a conference, or the like.

Identify for each publication: Author(s); title; journal; volume: year; page numbers; status of publication (published [include DOI#]; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Books or other non-periodical, one-time publications as a result of FY21 grant award

Report any book, monograph, dissertation, abstract, or the like published as or in a separate publication, rather than a periodical or series. Include any significant publication in the proceedings of a one-time conference or in the report of a one-time study, commission, or the like.

Identify for each one-time publication: Author(s); title; editor; title of collection, if applicable; bibliographic information; year; type of publication (book, thesis or dissertation, other); status of publication (published; accepted, awaiting publication; submitted, under review; other); acknowledgement of federal support (yes/no).

Other publications, conference papers and presentations as a result of FY21 grant award

Identify any other publications, conference papers and/or presentations not reported above. Specify the status of the publication.