USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Annual Performance Progress Report
Due date: July 29, 2021

Cover Page

Principle Investigator (PI):	Jason Fiedler
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Phone:	701-412-3246
Fiscal Year:	2020
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Improving the Genomic Resources of Fusarium Resistance in Spring and Durum Wheat
FY20 USDA-ARS Award Amount:	$ 250,515
Project/Grant Reporting Period:	5/1/20 - 4/30/21
Reporting Period End Date:	4/30/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
DUR-CP	Identity Novel Haplotypes in Durum with Superior Scab Resistance and Performance	$ 37,492
VDHR-SPR	A Pan-Genome of Scab Resistance Sources in Wheat: Uncovering Hidden Information in the Sequences	$ 213,023
	FY20 Total ARS Award Amount	**$ 250,515**

JASON FIEDLER Digitally signed by JASON FIEDLER
Date: 2021.07.23 12:29:35 -05'00'

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R- Research
S – Service (DON Testing Labs)
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

FY20 Annual Performance Progress Report
PI: Fiedler, Jason
USDA-ARS Agreement #: N/A
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Project 1: *Identity Novel Haplotypes in Durum with Superior Scab Resistance and Performance*

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

   The two main objectives of this projects are to 1) Generate high-density genotypes from unique FHB-resistant durum lines and 2) Identify haplotype blocks and genotype these blocks in related populations and map FHB resistance 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?

   1) We have collected lines from collaborators, conducted homogeneity tests and increased the seed. We have installed equipment and evaluated laboratory methods necessary to extract DNA from lines and acquire genotypes.
   2) We have evaluated analysis methods to identify haplotype blocks and collected existing genotype information from a few of the lines.

   b) What were the significant results?

   1) The homogeneity tests showed that most of the lines provided by collaborators were pure, and DNA from a single plant was chosen to be genotyped at high density. Methods to extract epigenotypes on populations were shown to be very low efficiency, so single-line bisulfite sequencing will be used to identify epi-alleles. The BluePippin instrument is useful to size-select DNA for long-read nanopore sequencing. With this combination, we routinely obtained N50 values of around 21 kb fragment sizes. The commercial X-drop technique for targeted long read sequencing was evaluated and looks to be useful to identify a region of approximately 50 kb around the FHB1 region in check cultivars, however, the method was fairly low-throughput.
   2) The Practical Haplotype Graph implemented in TASSEL was validated and will be a useful method to organize and visualize haplotype blocks of the sequenced lines.

   c) List key outcomes or other achievements.

   1) We have validated the methods we will use to achieve this objective. We have also identified and increased single plants that will be used to genotype at high-density. Population-wide epigenetic genotyping methods will need to be assessed later as the single line method will be sufficient to achieve our goals, and can be validated with single-marker methods. The original timeline has been delayed by the COVID-19 pandemic, but we are on track to achieve the objective this year as soon as the contract with the sequencing vendor is finalized.

2) We have validated the methods we will use to achieve this objective and anticipate moving quickly after the achievement of objective 1.

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.

This research was negatively impacted by the COVID-19 pandemic due to the max telework posture that the USDA-ARS and university shutdowns that occurred in March of 2020. This limited the number of people who could be in the lab at a given time and slowed down requests for germplasm from collaborators. These two things kept us from adhering to our expected timeline.

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

This project has provided the postdoctoral researcher with training opportunities to become more proficient in wet lab skills such as DNA extraction, liquid handling robot usage and next-generation sequencing library construction. He has also been trained in bioinformatic analysis skills such as sequence processing, Practical Haplotype Graph construction, and clustering.

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

Nothing to Report

Project 2: *A Pan-Genome of Scab Resistance Sources in Wheat: Uncovering Hidden Information in the Sequences*

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

The two main objectives of this project are to 1) Generate a pangenome of FHB resistance sources with full genome sequences of three lines and re-sequencing data from several other lines that have been central to breeding efforts. Objective 2) is to utilize this pangenome and associated expression analysis to identify genomic differences that can predict resistance and develop assays to specifically target these to accelerate breeding efforts.

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) We have collected FHB-resistant source lines from collaborators, performed homogeneity tests and increased the seed of single lines. We have installed equipment and evaluated laboratory methods necessary to extract DNA/RNA from lines for resequencing and expression analysis. The three lines to be sequenced with PacBio were sent to our collaborator. A postdoctoral researcher was hired to conduct this work.
2) We have evaluated analysis methods to identify haplotype blocks and collected existing genotype information from a few of the lines. We have also evaluated laboratory and analysis methods to identify the differentially expressed genes from the RNA samples.

3) What were the significant results?

1) The homogeneity tests showed that most of the lines provided by collaborators were pure, and DNA from a single plant was chosen to be sequenced. The BluePippin instrument is useful to size-select DNA for long-read nanopore sequencing. With this combination, we routinely obtained N50 values of around 21 kb fragment sizes. The commercial X-drop technique for targeted long read sequencing was useful to identify a region of approximately 50 kb around the FHB1 region in check cultivars. Spring wheat cultivar Rollag was the first to be sequenced with PacBio and we obtained a 26x coverage contig-level assembly with a N50=32 Mb from 30 flowcells.
2) The Practical Haplotype Graph implemented in TASSEL was validated and will be a useful method to organize and visualize haplotype blocks of the sequenced lines. Additional software packages were evaluated to identify structural variations once the genomes are finished.

3) List key outcomes or other achievements.

1) We have validated the methods we will use to achieve this objective. We have also identified and increased the single plants that will be used to investigate further. The original timeline has been delayed by the COVID-19 pandemic, but we are on track to achieve the objective this year.
2) We have validated the methods we will use to achieve this objective and anticipate moving quickly after the achievement of objective 1.

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.

This research was negatively impacted by the COVID-19 pandemic due to the max telework posture that the USDA-ARS and university shutdowns that occurred in March of 2020. This limited the number of people who could be in the lab at a given time and slowed down requests for germplasm from collaborators. These two things kept us from adhering to our expected timeline.

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Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (5/1/20 - 4/30/21). 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 FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

3. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

4. **Have any post docs who worked for you during the FY20 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
   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 FY20 award period (5/1/20 - 4/30/21). 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	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
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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
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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 PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (5/1/20 - 4/30/21**) 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:

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.

Nothing to Report

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

None.

Other publications, conference papers and presentations.

None.