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

FY22 Performance Progress Report

Due date: July 26, 2023

Cover Page

USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Genomic and germplasm infrastructure for FHB breeding
Principle Investigator (PI):	Jason Fiedler
Institution:	USDA-Agricultural Research Service
Institution UEI:	N/A
Fiscal Year:	2022
FY22 USDA-ARS Award Amount:	$414,094
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Period of Performance:	May 1, 2022 - April 30, 2023
Reporting Period End Date:	April 30, 2023

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	A Low-Cost Genotyping Platform to Develop FHB-Resistant Barley	$120,000
DUR-CP	Transcriptome Analysis of Durum with Superior Scab Resistance and Performance	$23,934
VDHR-SPR	Centralized Genomic Selection Resources for FHB-Resistant Spring Wheat Breeding	$150,160
VDHR-SPR	Leveraging the Pangenome to Investigate Genetic Background Effects on the Fhb1 Locus	$120,000
	FY22 Total ARS Award Amount	$414,094

I am submitting this report as an: ☒ Annual 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.

JASON FIEDLER

Digitally signed by JASON FIEDLER
Date: 2023.07.25 14:06:35 -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: A Low-Cost Genotyping Platform to Develop FHB-Resistant Barley

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

The objectives of the research project are to:

1) Characterize population sub-structure and diversity of US breeding germplasm.
2) Identify a set of evenly spaced markers for a low-cost assay
3) Evaluate dual-hybridization mode of Infinium multi-species array

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

Objective 1:

a) What were the major activities?
Analysis of 50K and 9K genotyping of ~12,000 breeding lines from the US.

b) What were the significant results?
We identified nine distinct, but related sub-groups overlapping with geographic growing area.

c) List key outcomes or other achievements.
NA

Objective 2:

a) What were the major activities?
The identification of 3,089 SNPs for barley that represent the variation is as many geographic areas as possible. Pilot studies of all the Barley CP PIs with several hundred of their breeding lines were run (totaling 2,304 samples). The fine-tuning of cluster-files for genotype calling and development of pipelines to make processing at the Fargo Genotyping Lab semi-automatic. Users have been evaluating their own pilot data and providing feedback on usefulness. Imputation evaluation has also been conducted.

b) What were the significant results?
We now provide ready-to-use genotyping files for the Barley CP PIs without the need for additional analysis (unless desired). Additionally, the 3K set of SNPs provide enough information to accurately impute the higher-density 50K array (99.9% median taxa accuracy on 17,000 markers with maf >= 0.05)

c) List key outcomes or other achievements.
Barley CP members that have used the 3K array are happy with the results and have shown that genomic selection models built with it are equivalent to models built with higher density marker data.

Objective 3:

a) What were the major activities?

A dual -hybridization pilot of 384 Barley/Wheat, 768 Barley/Oat, 96 Barley/Durum and 384 Barley/Oat/Wheat mixed samples were run on the 3K array and evaluated. Custom calling scripts were developed to aid in high-throughput clustering.

b) What were the significant results?

Clusters migrate compared to single-mode and require an orthologous technique to determine the proper cluster positions and genotype call. In some case, the clusters merge into one another. Approximately 5-10% of markers cannot be used in dual/multi mode.

c) List key outcomes or other achievements.

A pipeline was developed to assess single-vs dual mode for any set of germplasm and provide cluster call thresholds for accurate genotyping. Every multi-project in the future can "trained" with this pipeline to determine the most accurate call at the lowest price.

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

Nothing to Report

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

This array is commercially available directly from Illumina. Most customers know about this array through direct communication. We have established a collaboration with the genotyping company SGS (formally TraitGenetics) where they have been exploring running this array for their large commercial partners.

Project 2: Transcriptome Analysis of Durum with Superior Scab Resistance and Performance

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

1) Identify genes that are associated with FHB resistance.
2) Integrate transcriptome with genome haplotypes and develop high-throughput assays.

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

Objective 1:

a) What were the major activities?
In Fall 2022, a FHB greenhouse was established to collect RNA before and after inoculation. Four durum (2 resistance and 2 susceptible) lines were included. RNA was extracted from rachis and spikelets at 0 dpi, 5 dpi and 5 dpi of mock inoculated spikes. RNA reads were mapped to the Sumai3 genome and differentially expressed genes were identified.

b) What were the significant results?
In total, 68 RNA samples (4 lines x 3 treatments x 2 tissues x 3 replicates – a few failures) were extracted. The pipelines worked well to produce RNA with amounts and quality sufficient for sequencing library generation and sequencing. Paired with the new annotations, initial investigation identified several genes in key regions that were associated with disease resistance. Additionally, analysis is underway to finalize the set of genes for this year.

c) List key outcomes or other achievements.
NA

Objective 2:

a) What were the major activities?
Once an initial set of differentially-expressed genes is finalized, haplotype information will be conducted.

b) What were the significant results?
NA

c) List key outcomes or other achievements.
NA

3. What opportunities for training and professional development has the project provided?
The graduate student assisting with this project is learning bioinformatic skills to process and analyze this information.

4. How have the results been disseminated to communities of interest?
Nothing to report.

Project 3: Centralized Genomic Selection Resources for FHB-Resistant Spring Wheat Breeding

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

   1) Develop a low-cost assay that is useful in US breeding programs.
   2) Generate a standard pipeline for phenotyping and prediction.

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

   Objective 1:

   a) **What were the major activities?**
   Sub-setting of exome capture markers, population sub-structure analysis of US breeding germplasm, pilot studies of 3K wheat array (> 6,000 samples), testing of multi-species hybridization, and evaluation of imputation to "upgrade" the 3K set to a higher-density set of genotypes.

   b) **What were the significant results?**
   At least 2,000 markers are non-fixed in every hexaploid wheat breeding germplasm pool tested. This is sufficient to build genomic selection models that are equivalent to those built with higher-density genotypes. Imputation accuracy varied from 93-98% median taxa accuracy. In dual and multi-mode, the clusters migrate, but with the aid of new helper scripts, 90-95% percent of the markers can still be reliably called and used with decreasing the cost to $4.67/sample in tri-mode.

   c) **List key outcomes or other achievements.**
   The 3K array has shown itself to be a useful genotyping platform for its intended purposes and has already drawn attention from other crops to build similar resources. Additionally a marker-assisted report is now provided for every project that provides predicted alleles (traits) for

   Objective 2:

   a) **What were the major activities?**
   Genomic coordinator position advertised, and candidate selected. Person started 06/01/2023. Phenotype and genotype data (90K and 3K) assembled

   b) **What were the significant results?**
   Genotyping data from lines grown in 2010-2023 were collected phenotype data from the reports were assembled into a format more easily processed together.

   c) **List key outcomes or other achievements.**
   NA

3. **What opportunities for training and professional development has the project provided?**
   Nothing to Report

4. **How have the results been disseminated to communities of interest?**
   The Wheat 3K array has been discussed with wheat breeding customers.

Project 4: Leveraging the Pangenome to Investigate Genetic Background Effects on the Fhb1 Locus

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

1) *Introgress a minimal Sumai 3 Fhb1 segment in elite germplasm.*
2) *Develop mapping populations to evaluate specific background effects.*
3) *Evaluate background gene expression effects on Fhb1.*

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

Objective 1:

a) What were the major activities?
ND2710(Sumai3/Wheaton/Grandin) was used as the source of Sumai3 FHB1. In Spring of 2022, initial crosses between this line and non-FHB1 adapted germplasm from the 3 major HRSW breeding programs (MDHRS13-0273-0036, MT1621 and MN15005-4) was conducted. A backcross to the individual recurrent parent was performed in Fall of 2022 (~30 crosses total for 16-20 plants). In the Spring of 2023, backcross # 2 was conducted to the progeny that possessed FHB1 in heterozygous state (~15 crosses total for 8-11 plants.)

b) What were the significant results?
FHB1 genotyping works well to rapidly identify the lines that maintain heterozygous state after crossing.

c) List key outcomes or other achievements.
Backcrossing is progressing as expected.

Objective 2:

a) What were the major activities?
In Spring 2023, Vida was crossed to ND744, SD4343, and Lang to establish mapping populations that segregate for specific linkage blocks previously derived with Sumai3.

b) What were the significant results?
Only 2-5 plants were crossed, but enough F1 seed was obtained to establish the mapping population we desire.

c) List key outcomes or other achievements.
NA

Objective 3:

a) What were the major activities?
In Fall 2022, a FHB greenhouse was established with the sole purpose of collecting RNA before and after inoculation. Four HRSW lines were included in this to test out the

protocol for acquiring high-quality RNA from rachis and spikelets at 0 dpi, 5 dpi and 5 dpi of mock inoculated spikes. RNA reads were mapped to the Sumai3 genome and differentially expressed genes were identified.

b) What were the significant results?

In total, 68 RNA samples (4 lines x 3 treatments x 2 tissues x 3 replicates – a few failures) were extracted. The pipelines worked well to produce RNA with amounts and quality sufficient for sequencing library generation and sequencing. Paired with the new annotations, initial investigation identified several genes in key regions that were associated with disease resistance.

c) List key outcomes or other achievements.

NA

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

The postdoc that led this project for the year learned several important skills in data science and bioinformatics in one-on-one training by the mentor. The postdoc also presented this work at the FHB Forum scientific conference In December of 2022

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

Nothing to Report

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 FY22 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 May 1, 2022 – April 30, 2023?

X Yes, I've included the citation reference in listing(s) below.

☐ No, I have nothing to report.

Journal publications as a result of FY22 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 FY22 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 FY22 award

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

Jason Fiedler, Nicholas Tinker, Mandy Waters, Nandety, Raj, Deven See, Guihua Bai, Gina Brown-Guedira, Eduard Akhunov and Qijian Song. A new rapid low-cost genome- wide genotyping platform to support genomics in small grains. The Plant and Animal Genome Conference. San Diego, CA. Poster presentation 01/13/2023 – federal support acknowledged: yes.

And an invited Illumina-sponsored webinar at the IWGSC seminar series:
Jason Fiedler, A multi-species, low-cost, genome-wide genotyping platform to support molecular breeding in small grains, IWGSC Webinar series, 01/26/2023 – federal support acknowledged: yes.