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:	Breeding Potential for Barley Microbiomes
FY20 USDA-ARS Award Amount:	$7,624
Recipient Organization:	USDA-ARS National Center for Agricultural Utilization Research 1815 N University St., Peoria, IL 61604
DUNS Number:	N/A
EIN:	N/A
Recipient Identifying Number or Account Number, if any:	
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
TSCI	Breeding Potential for Microbiome Protection against Fusarium Head Blight	$7,624
	FY21 Total ARS Award Amount	$7,624

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.

BRIANA WHITAKER Digitally signed by BRIANA WHITAKER Date: 2022.07.25 16:09:02 -05'00' 07/25/2022

Principal Investigator Signature | Date Report Submitted

ǂ 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: Breeding Potential for Microbiome Protection against Fusarium Head Blight

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

The major objective (Objective 1) of this research project was to: ***identify FHB recruited and genotype-responsive microbes in barley***.

This will be accomplished by: 1) Identification of groups of microbes (taxa) that are recruited in response to FHB disease using diverse germplasm from a training population. and 2) Identification of groups of microbes with differential abundance across barley genotypes.

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?

Objective 1: In the summer of 2021, we sampled head tissue from four separate mist-inoculated, breeding nurseries. We collected head samples from 10 genotypes per site and scored visual symptoms of disease. Whole plot toxin samples are also being obtained from across all locations (ongoing).

Head samples for microbiome processing were brought back to NDSU, ground to fine powder, and sampled for both toxin and DNA extractions. DNA was extracted from all 400 samples (4 sites x 10 genotypes x 2 disease status [yes/no] x 5 replicates). At NDSU, all steps for the bacterial microbiome prep were optimized and >90% of the samples successfully sequenced. A small number of samples will need to be re-sequenced due to low quality read contamination or low sequencing depth overall. Preliminary data analysis for the bacterial microbiome results has begun and is ongoing.

In addition, Co-PI Baldwin's lab has been working to optimize qPCR primers for amplifying barley host DNA as a paired comparison with known *Fusarium graminearum* qPCR primer sets. The results of the qPCR will be used to obtain Fusarium biomass in all samples and a quantitative metric of disease.

After extraction of DNA from all samples and quality testing, an aliquot of the normalized DNA extraction was shipped to Co-PI Whitaker at the USDA-ARS in Peoria for fungal microbiome library optimization and sequencing. At the USDA-Peoria, preliminary sequencing efforts have been performed to identify a set of fungal primers that will reduce non-target host genomic amplification and capture the greatest degree of inherent fungal diversity in samples.

b) What were the significant results?

During the 2021 field season, much of the upper Midwest experienced severe drought conditions and as a result disease severity was exceptionally low at three of the four

sites tested (Figure 1). The St. Paul Minnesota site was the only site with visible disease scores above 50% on average.

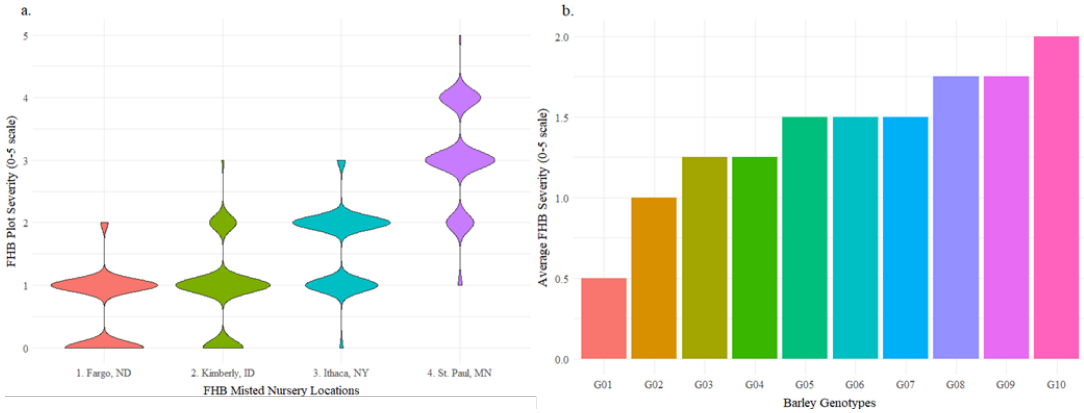

Figure 1: *Average FHB visual severity measurements for the Aberdeen training population a) grown in 4 misted FHB evaluation nurseries and b) across 10 barley genotypes. The four misted nurseries spanned four States in the U.S. including North Dakota (ND), Idaho (ID), New York (NY), and Minnesota (MN). FHB Severity (0-5 scale) represents the visual severity of 248 barley genotypes planted in each location. Statistical significance was tested by ANOVA ($p < 0.05$).*

For a full detailing of significant results from the bacterial microbiome sequencing effort see 'FY21-PPR_Geddes_B' Report

At the USDA-Peoria, barley microbiome samples were processed in conjunction with samples from two other grain crops (wheat and corn). The proportion of Illumina sequences that came back as non-fungal (i.e., plant genomic ITS sequences) in barley ranged from 0-90% of the total reads (Fig 2a). Comparisons with the two other grain crops (wheat and corn) showed the least plant genomic ITS amplification in corn, but similar plant genomic ITS amplification in wheat. There was marginally higher plant genomic ITS amplification in primer set #3 for barley samples relative to the three other primer sets tested (Fig 2b).

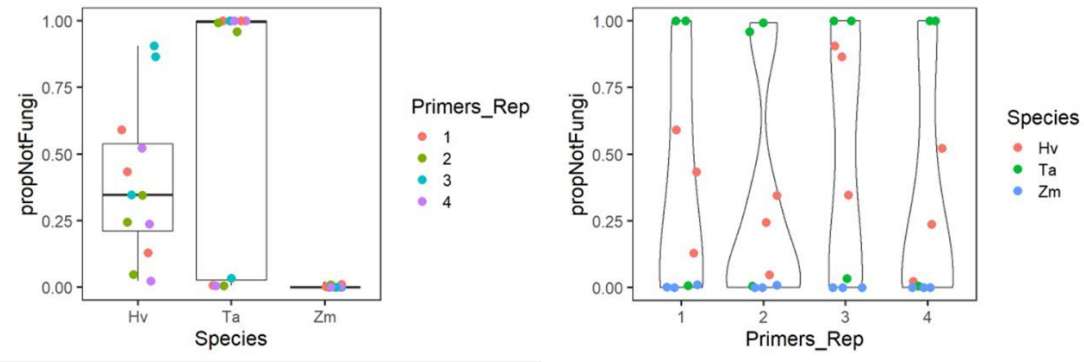

Figure 2: *Proportion of sequencing reads that were not fungal in origin ('propNotFungi') sorted by host species (A) and by primer set tested (B). The host species were Hordeum vulgare (Hv, Barley), Triticum aestivum (Ta, wheat), and Zea mays (Zm, corn). All four primer sets targeted the ITS2 region of the fungal genome, but non-target amplification of plant genomic ITS sequences was frequently detected across samples.*

Additionally, there was a strong correlation between the proportion of plant genomic ITS reads per sample with the total sequencing read depth per sample (Fig 3). In other words, this result is a strong indication that there was very low fungal biomass relative to plant biomass across the barley samples and is likewise indicative of very low fungal diversity in these drought-stricken barley samples and sites (as was seen in the bacterial results from the 'FY21-PPR_Geddes_B' Report).

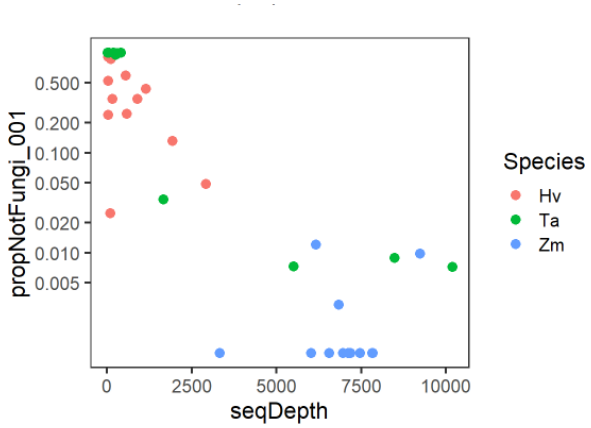

Figure 3: *Proportion of sequencing reads that were not fungal in origin ('propNotFungi_001') as a function of the sequencing depth per sample (a metric of library quality). Each point represents a single sample and is colored by host species, as in Fig 2.*

By comparison, the corn samples clearly had higher fungal biomass and captured the greatest amount of fungal diversity and least non-target plant genomic ITS amplification. Primer set #2 showed the highest fungal richness (i.e., number of unique taxa) across all samples, including in the barley samples (Fig 4).

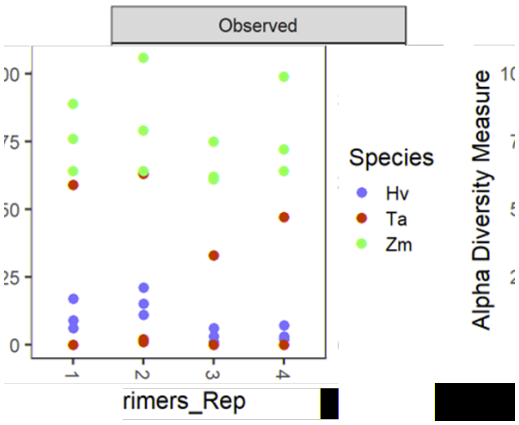

Figure 4: *Observed fungal richness (i.e., number of unique fungal sequences) as a function of primer set tested. Each point represents a single sample and is colored by host species, as in Fig 2.*

c) List key outcomes or other achievements.

A major effort at the beginning of this work involved optimizing the DNA extraction from barley heads, the library preparation protocols for 16S and ITS amplicon sequencing and bioinformatic analysis pipelines.

Preliminary results from the fungal amplicon library prep have identified a primer set with reduced plant amplification and highest capture rate of fungal diversity (Primer set #2). Ongoing tests will work to optimize sequencing parameters to achieve highest sequencing read depth, capture the highest fungal diversity, and least plant genomic ITS amplification per sample.

Using optimized protocols, we have now successfully sequenced the bacterial microbiome of >90% of samples using our optimized methodology. Preliminary data analysis of these samples shows encouraging support for the presence of genotype-responsive microbes. However, we note that lack of disease severity associated with drought conditions may limit our power to identify disease-recruited microbes from this data-set in three of the four locations.

Although we identified strong support for genotype-responsive taxa, a key outcome from this work indicates that higher disease severity across more sites would improve our statistical power to identify disease-recruited taxa. Early disease reports indicate much higher disease severity across the four breeding nurseries in 2022. Therefore, we plan to seek permission to refocus our funded Year 2 proposal towards this goal. We believe this can be accomplished with the currently approved budget.

A second year of funding, in a higher disease year, would provide us with the necessary statistical power to identify microbial taxa that are both disease-recruited and genotype-responsive, a current limitation to the 2021 data. These data would establish the potential for utilizing breeding to manipulate the microbiome for improving barley resilience to FHB disease.

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

During the first year of this project, a graduate student Brooke Benz, was partially funded by this grant. Brooke's research activities included gaining experience performing field research and FHB scoring, molecular techniques such as DNA extractions and library preparations, and statistical analyses. In addition, Brooke has presented this research at one local and one national research forum (see below for details).

Two undergrads, Amber Kalvoda and Ashley Potter, gained research training as part of this project in: planting, sorting seed, preparing for harvest, processing samples, and performing DNA extractions.

Four early career scientists (Geddes, Baldwin, Whitaker, Banerjee) performed research during the first year of this project.

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

The graduate student, Brooke Benz, presented this research at the Asilomar Fungal Genetics Conference (FY21), as well as at the graduate student research seminar series for the Department of Plant Pathology at NDSU.

Co-PI Whitaker presented preliminary research and directions for this grant to the Gene Discovery and Engineering Resistance (GDER) CP in May 2021.

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.