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:	59-0206-1-201
USDA-ARS Agreement Title:	Management and Innovative Research of FHB in Barley
FY20 USDA-ARS Award Amount:	$24,718
Recipient Organization:	North Dakota State University Department of Plant Pathology PO Box 6050, Dept. 7660 Fargo, ND 58108-6050
DUNS Number:	80-388-2299
EIN:	45-6002439
Recipient Identifying Number or Account Number, if any:	
Project/Grant Period:	6/01/21 -5/31/23
Reporting Period End Date:	5/31/2022

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Coordinated Fungal Biomass Measurements of FHB in Barley and Microbial Fingerprinting	$24,718
	FY21 Total ARS Award Amount	$24,718

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.

7/26/2022

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: Coordinated Fungal Biomass Measurements of FHB in Barley and Microbial Fingerprinting

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

Major goals of this project are to establish the biomass measurement by qPCR pipeline for samples harvested from the FHB nursery. This includes development and validation of qPCR primers and probes to improve the cost and efficiency of the qPCR. Additionally, the Barley CP committee recommended exploring biomass measurements by qPCR as an indicator of potential DON accumulation through the malting process.

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?

Major activities include the following:

1. Redesigning and validating primer probe combinations for better qPCR sensitivity and efficiency targeting *TRI5* from FHB causing *Fusaria* and *Actin* from barley
2. Grind, extract, and store DNA samples from four programs: NABSEN 2021, Training Population from Kimberly (2020), Hulless Barley Diversity Panel (Dr. Smith), and the Microbiome project (Dr. Geddes). Provide samples to James Gillespie for DON analysis
3. Test contaminated malt barley batches prior to malting and compare to DON accumulation after malting.
4. Extra activities include test individual kernels from 4 barley varieties in two locations (Fargo and Kimberly) to compare visual rating to biomass in the field.

b) What were the significant results?

1. Primer/probe design and validation

The following primer/probes were designed and validated, and compared to Sybergreen primers for efficiency against purified *Fusarium graminearum* DNA, a mixture of *F. graminearum* DNA and *Hordeum vulgare* DNA, and infected seed samples. The primer/probes sets are listed in table 1. They were selected based on their efficiencies determined with the software program Primer3.

Table1 : qPCR primers and probes for Taqman assay vs. primers used for sybergreen assay

Primers/Probes		
Fg	TRI5QPF2	CTCACCCAGGAAACCCTACA

Fg	TRI5QPP2	GATGGTTGCTGTCTTCTCGG
Fg	TRI5QPr2	CATCACCTGAGGGTCCTTGT
Hv	ActinQPF2	CCAGGTATCGCTGACCGTAT
Hv	ActinQPP2	GAAGATCAAGGTCGTCGCTC
Hv	ActinQPR2	GCTGAGTGAGGCTAGGATGG

Primers for SYBR		
Fg	TMT_fw	5'-GATTGAGCAGTACAACTTTGG-3'
Fg	TMT_rev	5'-ACCATCCAGTTCTCCATCTG-3'

*SYBR green primers were used in Dr. Zhao Jin's publication https://www.mdpi.com/2072-6651/10/9/369

Comparation of SYBR green and Taqman probe assay was completed by testing 13 samples and standard curve based on 150 ng/ul stock DNA from purified, see table 2. The reaction was run at standard recommended qPCR protocols for iTaq ™ from Bio-rad on a 96 CFX system (Bio-rad). Data output is from Maestro CFX software (Bio-rad). Figure 1 shows the standard curves for both SYBR and Taqman to be within acceptable ranges for qPCR analysis for *Tri5*. Test were also run for *Actin* Taqman probes, no barley DNA was added and no detection was found (Data not shown). Overall, this shows no cross reactivity for *F. graminearum* DNA and Actin for these primer sets.

Table 2: Concentrations of purified *F. graminearum* DNA run for initial standard curve
[1] 75 ng/ul
[2] 25 ng/ul
[3] 15 ng/ul
[4] 7.5 ng/ul
[5] 2.5 ng/ul
[6] 0.75 ng/ul
[7] 0.25 ng/ul
[8] 0 ng/ul (negative control)

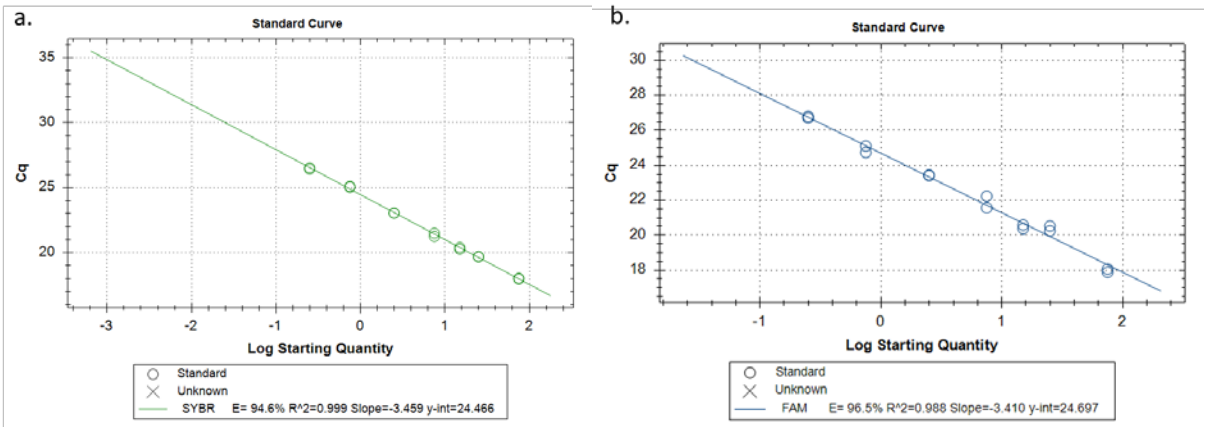

Figure 1: (a) Standard curve for Tri5 SYBR green. Efficiency (E) at 94.6% and R^2 at 0.999% are reported. (b) Standard curve FAM and Hex for Tri5 Taqman assays reported E = 96.5 and R^2 = 0.988.

Next, we attempted several levels of optimization for the qPCR assay. The multiplex of *Tri5* and *Actin* was run to ensure that amplification does not affect each other (not inhibitory to the other reaction). Figure 2 shows the results of the dual probe assay prior to optimization. Primer/probe pairs testing for *Tri5* performed as expected, however the *Actin* primer/probes were out of range for efficiencies (E) at 106.6%. Additionally, the lower RFU in the Actin-duplex reaction from the Actin-singleplex reaction (data not shown) indicates some competition for the reagents in the reaction. The Actin-singleplex reaction had an acceptable efficiency of 101.6% (data not shown). Further optimization of *Tri5* and *Actin* primer/probes concentration and annealing temperatures were accomplished for the dual probe assay. Effects on annealing temp and supermix volume did not greatly affect the performance of the *Actin* primer/probe assay in the dual assay. Although changing the annealing temperature did have an effect on the *Tri5* assay, priority was given to the *Actin* assay with optimal annealing temperature of 62 °C at 5 ul supermix per reaction.

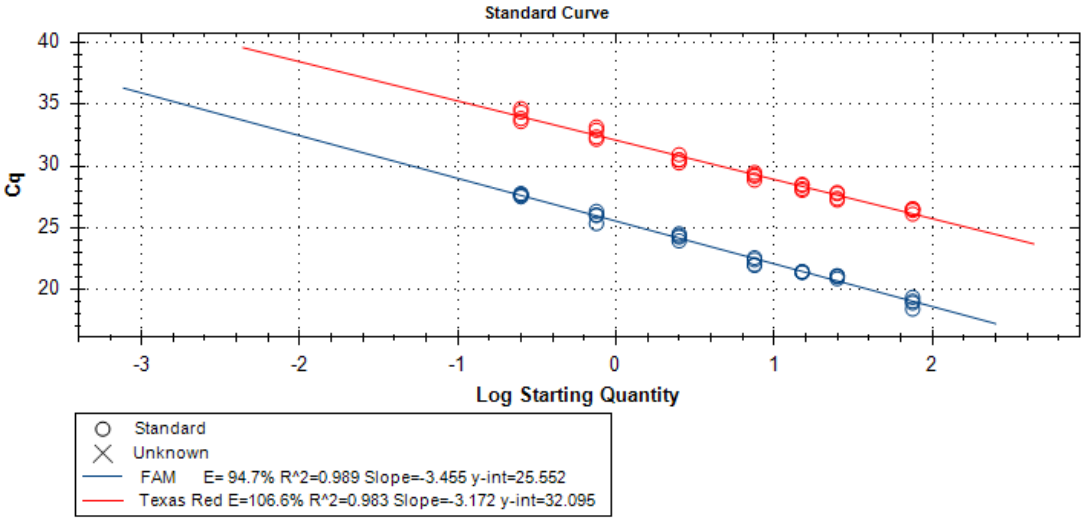

Figure 2: Dual probe assay with Tri5 (FAM) and Actin (Texas Red). Standard curve generated with a 50/50 mixture of *F. graminearum* DNA and barley DNA.

Testing Fusarium biomass before and after malting. Using the new primer sets 7 samples of contaminated barley were measure before and after malting for biomass concentrations. This was an effort lead by Dr. Zhao Jin in my laboratory. The results from this limited study (Figure 3) demonstrates that the initial amount of fusarium biomass in the barley was indicative of end biomass in the malt. Malting increased the Fusarium biomass roughly 10 -fold.

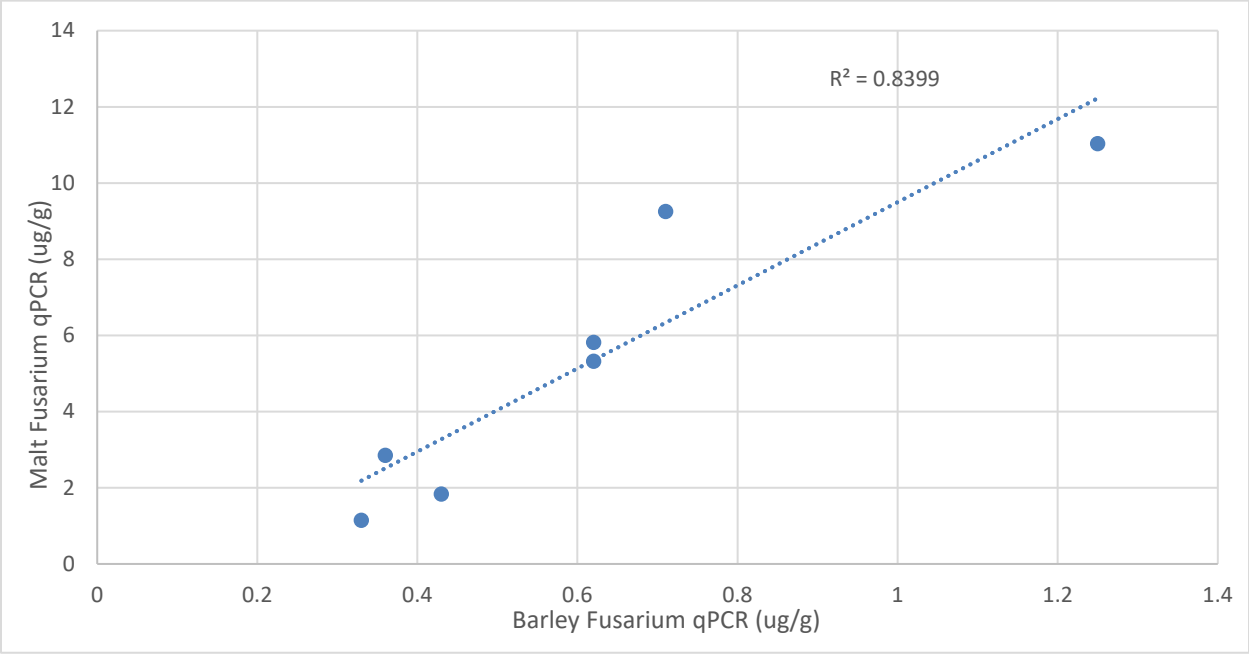

Figure 3: Fusarium biomass before malting (barley Fusarium qPCR) and after malting (Malt Fusarium qPCR).

3. List key outcomes or other achievements.

Tri5 detection (primers and probes) were found to be very flexible, can work with any concentration and annealing temperature. Issues with the negative and NTC amplification were found but solved by shorting the total Ct from 40 to 35 cycles. *Actin* detection (primers and probes) were not so flexible. They work well with same conditions of *Tri5*, however, the efficiency is beyond the ideal range and hence needs improvement. As a result, low sensitivity of this assay might be the limiting factor. A comparison of various supermixs including iTaq and SSO supermix have been accomplished, but the dual hybrid assay is still out of range for the full standard curve of 5 points, 10 -fold in *Actin*. A limited standard curve of 5 concentrations, 4-fold, gave an efficiency of 95.1% and an R^2 of 0.902 (data not shown). This placed the *Actin* assay within acceptable range for the dual assay. Given the accepted higher levels of barley

DNA compared to *Fusarium* DNA in the infected seed a shorter and higher DNA concentration range of efficiencies should be acceptable for most tests. We have sent our detection protocol to three laboratories for confirmation and further optimization.

NABSEN 2021, the Training population for Kimberly 2020, The Hulless barley diversity panel and the microbiome project have been ground, extracted for DNA, and quantified. The ground samples were measured to 1 gram and delivered to James Gillespie for DON analysis. Within the next month they will be measured for Fusarium biomass and the analysis will be delivered to the individual PIs for their analysis. Another useful purpose of this general DNA extraction is keeping a repository for the potential use of microbial fingerprinting. This is a direct output from Dr. Geddes's project on the microbiome of barley in relation to FHB.

The initial results testing Fusarium biomass in barley prior to malting are encouraging to further develop the qPCR test as a quality measure for malt barley. Further studies are necessary to determine the relationship between malting, biomass, and DON production.

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

This project supported training and opportunities for one graduate student to work on qPCR as a screening, breeding tool and as an end-use product quality check. The work supported the graduate student to attend the North Central Division of APS and will support them to travel to the USWBSI to present results on biomass assays.

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

Mostly the results have been disseminated through personal communications, however, multiple publications are in development to share our results.

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.