USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY19 Performance Report
Due date: July 24, 2020

Cover Page

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Fiscal Year:	2019
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Production of Widely-adapted FHB-resistant Barley Germplasm and Novel Information and Tools via Conventional and Transgenic Breeding Approaches
FY19 USDA-ARS Award Amount:	$ 51,831

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
BAR-CP	Identification, Characterization and Development of Widely-Adapted FHB-Resistant Germplasm	$ 21,831
GDER	Down with DON: Stable Expression of Proven Genes in a Marker-Free Background	$ 30,000
	FY19 Total ARS Award Amount	**$ 51,831**

KATHY ESVELT KLOS

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Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
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

Project 1: *Identification, Characterization and Development of Widely-Adapted FHB-Resistant Germplasm*

1. **What are the major goals and objectives of the research project?**
   a. Identify resistant lines in elite winter germplasm
   b. utilize existing spring resistance sources for new crosses to a) create mapping populations and b) broaden the adaptability of Aberdeen FHB-resistant malting germplasm by introducing broad-spectrum disease resistance.
   Added objective: Investigate qPCR fungal biomass measurements as a proxy for DON

   This project supplements the FHB resistance evaluation and breeding (spring malting) by Gongshe Hu

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

   Winter germplasm screening

   a) Major Activities:

   -Procedures established in 2019 for testing vernalized, transplanted-seedling hills of winter barley were used in a second year of screening at Aberdeen, ID on 200 winter barleys. This procedure allows late spring planting that results in heading being delayed until warmer, FHB-conducive weather occurs.

   --200 winter barley lines were also screened in 2019/2020 at Cornell, Virginia Tech (VT) and, in collaboration with Juliet Marshall at the University of Idaho, in the Southern Idaho mist nursery at Kimberly.

   -- A spring barley training population was evaluated in the 2019 Aberdeen, ID nursery and GWAS analysis performed, suggesting the presence of genetic variation contributing to FHB severity.

   --This work enables meeting objectives necessary to accomplish our goal of screening for resistance, namely putting in place new testing infrastructure, securing cooperation for access to existing infrastructure, and using these resources for phenotyping.

   b) Significant Results:

   --Useful phenotyping data were returned from 2018/2019 trials at VT, Aberdeen and Kimberly, suggesting useful levels of FHB resistance in some Aberdeen winter barley lines.

   --2019/2020 trials in progress at Cornell, VT, Aberdeen, and Kimberly. Disease severity was low in VT trials, so grain was not harvested for DON testing.

   c) Key Outcomes

   --The available data indicate that there is variation for FHB resistance within the Aberdeen spring and winter lines and opportunity to select for superior lines.

Mapping and development of new populations

a) Major Activities:

-- 95SR316A/Gadsby cross completed and advanced by single-seed descent to F5-derived RILs.

--95SR316A/ND Genesis population advanced using the doubled haploid (DH) technique by OSU cooperators. In 2018/2019 seed was produced from >200 fully-fertile plants.

--Seed of both crosses planted in headrows in Aberdeen, ID to produce seed for phenotypic evaluation.

b/c) Significant Results/Key Outcomes:

--This work has not advanced to the point where meaningful data can be collected. However, these populations represent useful, new parental combinations. 95SR316A is stripe rust-resistant line with good agronomic and malting characteristics, and potentially useful partial FHB resistance. These populations represent resources that may contain novel FHB resistance alleles, and will be segregating for alleles for foliar pathogen resistance.

PCR estimates of fungal biomass and relationship to DON

a) Major Activities:

--Unprocessed grain samples were obtained from cooperators at Idaho, Minnesota, North Dakota, Virginia Tech, and Cornell (7 locations total).

--A dip-inoculation method of inducing a uniformly high-severity FHB infection under controlled conditions was evaluated on a set of 15 elite barley lines with varying levels of FHB susceptibility. Variation in DON accumulation where FHB severity was >90% suggests the potential for evaluating type V resistance (resistance to DON accumulation) separately from type I resistance (Resistance to initial infection).

--Our specific objective is an improved method of FHB infection severity (vs. visual scoring) that gives us a better idea of the level of mycotoxin contamination

b/c) Significant Results/Key Outcomes

--Assessment of 2019 grain samples for DON and fungal biomass was delayed by COVID-19-related personnel shortages, but proceeds.

--More work is needed to determine the potential for using fungal biomass, with or without dip-inoculation, as a selection method. There is potential for using it to identify and eliminate lines that are most susceptible to mycotoxin contamination prior to screening them for DON via GC-MS.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

Yes. Processing of grain samples from 2018/2019 FHB nurseries for DON and fungal biomass evaluation was delayed by reduced support personnel in the facility as maximal teleworking status was implemented.

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

At Aberdeen, the PIs and the technicians continue to gain proficiency in the nuances of FHB infection characteristics and methods for phenotyping, including the mechanics of running successful misting systems suited to promoting disease in the Idaho climate. The work is providing professional development opportunities for two post-doctoral researchers.

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

Reports of the preliminary field phenotyping data and of the qPCR biomass estimates were presented as posters at the 2019 FHB Forum. Results have also been related to producers via the UI Small Grains Extension Report.

Project 2: *Down with DON: Stable Expression of Proven Genes in a Marker-Free Background*

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

Overall project goals:

1) Reduce FHB and DON in *F. graminearum* (*Fg*)-infected barley via expression of double-stranded (ds) RNA homologous to *Fg* genes for mycotoxin synthesis and/or pathogenicity.
2) Precisely deliver single-copy transgenes via novel methods: direct *Ds* transposition mediated delivery and recombinase mediated cassette exchange (RMCE).

The specific objectives for FY 18 and FY19 were to:

--Demonstrate RMCE functionality

--Produce transgenic barley with antifungal sequences (inverted repeats [IRs]) targeting *TRI6* and *NOXA*

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

a) Major Activities:

--While transgenic plants with site=specific recombination platforms were sucesfully produced, no exchange was observed. This limits the effectiveness of the Ds transposition mediated delivery platform for guiding insertions to the appropriate site. Focus was shifted, therefore, to collaborative development of a meristem-based transformation system.

--Collaboration with the University of Wisconsin, Wisconsin Crop Improvement Center produced a meristem-based transformation system which was demonstrated using stable expression of GUS in the spring two-row malting barley 'Gemcraft'. Current work on plants from these efforts are underway to estimate the level of somaclonal variation.

--Introduction of vectors containing inverted repeats of *TRI6* and *NOXA* are in progress.

b/c) Significant Results and Key Outcomes:

--Meristem-based transformation is expected to be less genotype-dependent than methods requiring regeneration from callus tissue. This should provide researchers the ability to perform functional genomic studies in a cultivar relevant to industry. Transcript effects can then be observed in the context of specific agronomic and quality parameters. This method should also reduce the problem of somaclonal variation caused by going through tissue culture. Somaclonal variation can complicate identifying target gene function by introducing variation unconnected to the gene system under study.

--We will use this method to investigate the effects of antifungal inverted repeat sequences targeting *TRI6* and *NOXA* on FHB infection and DON accumulation.

3. Was this research impacted by the COVID-19 pandemic (i.e. university shutdowns, reduced or lack of support personnel, etc.)? If yes, please explain how this research was impacted or is continuing to be impacted.

Yes. University of Wisconsin shutdowns have delayed production of transformants expressing inverted repeats targeting *NOXA*. This activity has been extended into 2020/2021.

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

Over the life of this project, there have been several undergraduate summer students from Idaho State University that have contributed to this project and to whom we have provided basic training on molecular procedures such as PCR and selection of plants based on molecular phenotyping. Ann Caspersen, the senior technician working on this project, has gained expertise in various approaches to transformation, and has become proficient in a number of advanced molecular recombinant DNA and PCR techniques, including digital droplet PCR. Post-Doctoral researcher Tom Baldwin has provided novel data to the fungal genetics and FHB research communities as a result of his involvement with this project. This project has been a significant contributor to his professional development as a *Fusarium* expert.

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

Presentations at USWBSI Forums

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (N/A). 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 FY19 award period?**
   No

   If yes, how many?

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY19 award period?**
   No

   If yes, how many?

3. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   Yes

   If yes, how many? 1

4. **Have any post docs who worked for you during the FY19 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   No

   If yes, how many?

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 FY19 award period. 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** (S, MS, MR, R, where R represents your most resistant check)	**FHB Rating** (0-9)	**Year Released**

Add rows if needed.

NOTE: List the associated release notice or publication under the appropriate sub-section in the 'Publications' section of the FPR.

Abbreviations for Grain Classes

Barley - BAR
Durum - DUR
Hard Red Winter - HRW
Hard White Winter - HWW
Hard Red Spring - HRS
Soft Red Winter - SRW
Soft White Winter - SWW

Publications, Conference Papers, and Presentations

Instructions: Refer to the FY19-FPR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY19 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (N/A**) 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.

Journal publications.

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

Other publications, conference papers and presentations.

Mndolwa, E., C.A. Griffey, E. Kress, J. Fitzgerald, J. Marshall, M.E. Sorrells, S.A. Baldwin, T. Baldwin, P. Bregitzer, K. Esvelt Klos. 2019. "Evaluation of Aberdeen Barley (*Hordeum Vulgare*) Germplasm for Fusarium Head Blight Resistance." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum*, Milwaukee, WI; December 8-10 University of Kentucky, Lexington, KY. p. 103.
Status: Abstract and Poster Presented
Acknowledgement of Federal Support: Yes (Abstract and Poster)

Baldwin, T., E. Mndolwa, E. Kress, K. Esvelt Klos. P. Bregitzer. 2019. "High vs. low DON accumulating lines of barley evaluated via dip inoculation of Fusarium head blight." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum*, Milwaukee, WI; December 8-10 University of Kentucky, Lexington, KY. p. 85
Status: Abstract and Poster Presented
Acknowledgement of Federal Support: Yes (Abstract and Poster)

Bregitzer, P. 2019.Ds transposition in barley: a dual-purpose tool for transposon tagging and transgene delivery. https://ambainc.org/wp-content/uploads/2019/10/Bregitzer.pdf
Status: Online presentation
Acknowledgement of Federal Support: Yes