USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY18 Performance Report
Due date: July 12, 2019

Cover Page

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Fiscal Year:	2018
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Response of Transgenic Wheat Altered in Defense Metabolites to Head Scab.
FY18 USDA-ARS Award Amount:	$ 35,000

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Response of Wheat Constitutively Expressing Lignin Genes to Fusarium Head Blight.	$ 35,000
	FY18 Total ARS Award Amount	**$ 35,000**

DEANNA FUNNELL-HARRIS Digitally signed by DEANNA FUNNELL-HARRIS Date: 2019.06.25 10:58:48 -05'00'

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: *Response of Wheat Constitutively Expressing Lignin Genes to Fusarium Head Blight.*

1. **What are the major goals and objectives of the project?**
   a. Screen transgenic lines constitutively expressing genes (*SbMyb60, SbCCoAOMT, Sb4CL* and *SbC3H*) in the monolignol biosynthesis pathway for resistance following artificial inoculations under field conditions.
   b. Determine FHB resistance in lines with two stacked transgenes in the CB037 background, or with a single transgene in a highly susceptible or moderately-resistant background, and constitutively expressing monolignol biosynthesis genes.

2. **What was accomplished under these goals?**
   a. Screen transgenic lines for resistance following artificial inoculations under field conditions.
      1) major activities-In collaboration with Dr. Ruth Dill-Macky, lines were to be screened using spray inoculations with *Fusarium graminearum* under conducive conditions in the field and compared with the recipient parent line, CB037, the moderately resistant cultivars Alsen and Sumai No. 3 and the susceptible cultivars Norm, Wheaton and Roblin. The 2018 screening has been completed while the 2019 screen is currently ongoing.
      2) specific objectives-
         i. FHB disease incidence and severity, visual scabby kernels (VSK) and DON levels were to be determined.
         ii. Results were to be compared with responses to pathogen under greenhouse conditions with individual lines.
      3) significant results-Transgenic lines overexpressing the enzyme coumaroyl shikimate 3-hydroxylase or the transcription factor Myb60 from *Sorghum bicolor* (SbC3H and SbMyb60) showed improved resistance over CB037 when spray-inoculated with *F. graminearium* under field conditions, considering disease index (DI) (one line each of two), VSK (one of two SbC3H and both SbMyb60 lines) and DON levels (one of two SbC3H and both SbMyb60 lines).
      4) key outcomes or other achievements- All overexpression lines tested with spray inoculations in the greenhouse appeared to be highly susceptible to the pathogen as measured by Area Under the Disease Progress Curve; greenhouse point inoculations of SbC3H overexpression lines exhibited increased resistance while SbMyb60 lines were susceptible. One of two lines overexpressing *S. bicolor* enzyme caffeoyl-Coenzyme A 3-O-methyltransferase (SbCCoAOMT) exhibited reduced DI in the field (but not VSK and DON) and increased resistance to point inoculation in the greenhouse. Both lines overexpressing *S. bicolor* 4-coumarate-Coenzyme A ligase (Sb4CL) were susceptible under all conditions.

   b. Determine FHB resistance in lines with two stacked transgenes in the CB037 background, or with a single transgene in a highly susceptible or moderately-resistant background.
      1) major activities
         i. Crossing transgenic lines carrying different constructs to stack transgenes.
         ii. Crossing transgenic lines carrying single overexpression constructs with elite susceptible and resistant cultivars.

2) specific objectives
   i. Combining lignin pathway overexpression constructs may increase levels of antimicrobial phenolics within plant cell walls with the goal of further increasing resistance.
   ii. Combining individual overexpression constructs with desirable quality traits in susceptible lines, may lead to increased resistance in elite backgrounds.
   iii. Combining individual overexpression constructs with elite, moderately resistant lines to recombine resistant genes and the overexpression constructs, with the goal to further increase resistance.

3) significant results
   1. F1 from 15 sets of crosses between transgenic plants carrying different overexpression constructs were screened for presence of two constructs from parental lines. Mature grain was collected from those plants carrying the two expected constructs. The F2 have not yet been screened for responses to FHB.
   2. A total of 54 crosses between transgenic lines carrying each of the overexpression constructs, and susceptible lines Wheaton, Roblin and Samson and moderately resistant lines SuMai No.3, CITR11215 (selection) and Alsen, have been performed. Progeny have not yet been screened for responses to FHB.

4) key outcomes or other achievements-
   Based on responses of transgenic lines carrying single overexpression constructs, it is likely we would focus our future FHB screens on progeny from crosses involving SbMyb60 and SbC3H lines. It is unlikely we would continue pursuing FHB responses with lines containing Sb4CL.

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

-We have trained three undergraduate students in basic microbiology, agronomy and plant pathology skills. These include wheat harvesting and threshing techniques, procedures for grinding biomass samples, and scoring wheat seed for Fusarium Head Blight. One student has also been trained in inoculum preparation while another has been trained in wheat emasculations.

-Our post-MS technologist (Mr. Z. Duray) had opportunities to give oral presentations at the National FHB meeting and locally, on research performed on this grant. As of April 1, 2019, he obtained a permanent technologist position. We are currently in the process of rehiring this position, in order to complete the research.

-We have trained a second biological science technologist in all steps of wheat culturing, and FHB inoculations, disease scoring and preparation of materials for further analyses.

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

-Two poster presentations were made at the National FHB forum during FY18 and FY19. The FY19 poster was selected for a Flash-and-Dash talk given by the project's technologist, Mr. Duray (early career).

-Results from this research were given in an oral presentation to the Nebraska Wheat Board, Nov. 16, 2018.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY18 award period. 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 FY18 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 FY18 award period?** No.

   If yes, how many?

3. **Have any post docs who worked for you during the FY18 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?** No.

   If yes, how many?

4. **Have any post docs who worked for you during the FY18 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 FY18 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 FY18-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY18 grant. Only include citations for publications submitted or presentations given during your award period. If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

NOTE: Directly below each reference/citation, you must indicate the Status (i.e. published, submitted, etc.) and whether acknowledgement of Federal support was indicated in publication/ presentation. See example below for a poster presented at the FHB Forum:

> Conley, E.J., and J.A. Anderson. 2018. Accuracy of Genome-Wide Prediction for Fusarium Head Blight Associated Traits in a Spring Wheat Breeding Program. In: Proceedings of the XXIV International Plant & Animal Genome Conference, San Diego, CA.
> Status: Abstract Published and Poster Presented
> Acknowledgement of Federal Support: YES (poster), NO (abstract)

Other publications, conference papers and presentations.

Funnell-Harris, D. L., Duray, Z., Graybosch, R. A., Sattler, S. E., Wegulo, S. N. and Clemente, T. E. 2017. "Response of Wheat Constitutively Expressing Monolignol Biosynthesis Genes to Fusarium Head Blight." In: S. Canty, B. Wiermer, and D. Van Sanford (Eds.), *Proceedings of the 2017 National Fusarium Head Blight Forum* (p. 45). East Lansing, MI/Lexington, KY: U.S. Wheat and Barley Scab Initiative.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), YES (abstract)

Funnell-Harris, D. L. 2018. "Fungal and Bacterial Diseases of Wheat." Presentation made to the Nebraska Wheat Board (https://nebraskawheat.com/wp-content/uploads/2019/06/November-2018-Board-meeting-minutes.pdf), Nov. 16, 2018, Lincoln, NE.
Status: Presented
Acknowledgement of Federal Support: YES (verbal)

Duray, Z., Funnell-Harris, D. L., Graybosch, R. A., Sattler, S. E., Wegulo, S. N. and Clemente, T. E. 2018. "Response of Wheat Constitutively Expressing Lignin Genes to Fusarium Head Blight." In: Canty, S., A. Hoffstetter, B. Wiermer and R. Dill-Macky (Eds.), *Proceedings of the 2018 National Fusarium Head Blight Forum* (p. 70). East Lansing, MI/Lexington, KY: U.S. Wheat and Barley Scab Initiative.
Status: Abstract Published, Poster and Talk Presented
Acknowledgement of Federal Support: YES (poster), YES (talk), YES (abstract)