USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY20 Annual Performance Progress Report
Due date: July 29, 2021

Cover Page

Principle Investigator (PI):	Deanna Funnell-Harris
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Fiscal Year:	2020
USDA-ARS Agreement ID:	N/A
USDA-ARS Agreement Title:	Response of Transgenic Wheat Altered in Defense Metabolites to Head Scab
FY20 USDA-ARS Award Amount:	$ 45,000
Project/Grant Reporting Period:	5/1/20 - 4/30/21
Reporting Period End Date:	4/30/2021

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Discovering Gene Expression Changes Linked to Phenylpropanoid-based FHB Resistance	$ 45,000
	FY20 Total ARS Award Amount	**$ 45,000**

DEANNA FUNNELL-HARRIS Digitally signed by DEANNA FUNNELL-HARRIS Date: 2021.07.19 15:19:31 -05'00'

Principal Investigator Date

* MGMT – FHB Management
FST – Food Safety & Toxicology
R- Research
S – Service (DON Testing Labs)
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

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PI: Funnell-Harris, Deanna
USDA-ARS Agreement #: N/A
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Project 1: *Discovering Gene Expression Changes Linked to Phenylpropanoid-based FHB Resistance*

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

1) Investigate response of lines with both (stacked) constitutive-expression constructs [sorghum caffeoyl CoA 3-O-methyltransferase (SbCCoAOMT) and p-coumarate 3-hydroxylase (SbC3H)] in the CB037 background and SbC3H in resistant backgrounds, SuMai No. 3 and Alsen, for Type I (to infection) resistance under greenhouse conditions in Lincoln, NE, and Minneapolis, MN.
2) Conduct global gene expression using RNA-Seq on the constitutive expression lines (SbCCoAOMT and/or SbC3H) and wild-type.

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?

Obj. 1: Due to limitations imposed by COVID-19 (see below) collaboration was established with Dr. Robert Mitchell (USDA-ARS, Lincoln, NE) to analyze biomass from transgenic lines constitutively-expressing SbMyb60 (transcription factor), Sb4CL (4-coumarate:CoA ligase), as well as SbCCoAOMT and SbC3H, using Near Infrared Spectroscopy (NIRS). Biomass from uninoculated plants (transgenic lines and the recipient line, CB037) and F. graminearum- and mock-inoculated plants (transgenic lines, CB037, and FHB resistant and susceptible checks, Sumai 3 and Wheaton, respectively) was analyzed using equipment in Dr. Mitchell's laboratory. This required only one individual working at any given time, thus adhering to COVID-19 restrictions.

Obj. 2: Experiments were conducted to confirm genotypes of transgenic lines, and to establish a protocol for extracting high-quality RNA usable for RNA-Seq.

b) What were the significant results?

Obj. 1: The data obtained from NIRS will be used to compare uninoculated (baseline) and inoculated (F. graminearum or mock) transgenic plants, with CB037 and susceptible and resistant checks, for ash contents, nitrogen levels, different forms of lignin, and digestibility. The data are currently being analyzed.

Obj. 2: Genotyping of two events each of SbCCoAOMT and SbC3H constitutive expression lines showed that grain stocks were homozygous (true breeding). An extraction protocol for high-quality RNA from individual wheat heads was established.

c) List key outcomes or other achievements.

Obj. 1. Following analyses of data from NIRS, a manuscript detailing biochemical traits and responses to FHB of constitutive expression lines will be prepared and submitted for publication, crediting the Initiative.

Obj. 2. Preliminary analyses have been completed. A greenhouse experiment that will be analyzed using RNA-Seq is being performed.

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

Yes. USDA-ARS at the University of Nebraska Lincoln (UNL) was in lock-down for 10 weeks from late March into early June, 2020, with essentially no admittance into laboratories and greenhouses other than checking on freezers and maintaining plants and cultures. From June, 2020, until the present, USDA-ARS has been mandated for limited (<25%) building capacity. Procedures that require a concerted team effort in an enclosed space (laboratory, greenhouse or growth chamber) are severely limited and require approval by the USDA-ARS Plains Area Biosafety/Biocontrol Officer and Area Director before proceeding. Additionally, during 2020, Executive Orders put in place to restrict foreign travel and admittance of foreign visitors to federal facilities, in order to limit spread of COVID-19, significantly delayed identifying a qualified graduate student.

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

-One undergraduate student was trained in basic agronomy and plant pathology skills.
-One biological science technologist was trained in all steps of wheat culturing, FHB inoculations and disease scoring, and preparation of materials for further analyses.
-Mr. Shiv Singla, M. Sci. graduate student funded by this grant, began in Dr. Funnell-Harris' laboratory Jan. 1, 2021. Mr. Singla completed two courses in plant pathology and one in bioinformatics at UNL. Additionally, he was trained by Dr. Funnell-Harris and her staff, and Dr. Wegulo and his staff at UNL, in wheat culturing, all aspects of FHB inoculation and disease scoring, experimental design, plant genotyping and RNA extraction. Additionally, with Dr. Funnell-Harris' instruction, Mr. Singla made a public oral presentation on this research.

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

Due to travel restrictions (which are still in place) and delayed or cancelled in-person meetings, results from this research were presented via Zoom or other on-line platforms. These included two public seminars to the University of Nebraska community, and presentation at the Fusarium Head Blight Forum.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY20 award period (5/1/20 - 4/30/21). 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 FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY20 award period?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

3. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant taken faculty positions with universities?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

4. **Have any post docs who worked for you during the FY20 award period and were supported by funding from your USWBSI grant gone on to take positions with private ag-related companies or federal agencies?**
   ☐Yes ☒No
   If yes, how many? Click to enter number here.

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 FY20 award period (5/1/20 - 4/30/21). 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	FHB Rating (0-9)	Year Released
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
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Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year
Click here to enter text.	Select Grain Class	Select what represents your most resistant check	Enter as text 0-9 rating	Select Year

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

Publications, Conference Papers, and Presentations

Instructions: Refer to the PR_Instructions for detailed more instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY20 grant award. Only citations for publications published (submitted or accepted) or presentations presented during the **award period (5/1/20 - 4/30/21**) 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. See example below for a poster presentation with an abstract:

Z.J. Winn, R. Acharya, J. Lyerly, G. Brown-Guedira, C. Cowger, C. Griffey, J. Fitzgerald, R.E. Mason and J.P. Murphy. 2020. "Mapping of Fusarium Head Blight Resistance in NC13-20076 Soft Red Winter Wheat." In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum (*p. 12.), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

Nothing to report.

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

Nothing to report.

Other publications, conference papers and presentations.

Funnell-Harris, D., Duray, Z., Dill-Macky, R., O'Neill, P., **Sattler, S., Wegulo, S. and Tatineni, S.**; **"**Discovering Gene Expression Changes Linked to Phenylpropanoid-based FHB Resistance." In: S. Canty, A. Hoffstetter, and R. Dill-Macky (Eds.), *Proceedings of the 2020 National Fusarium Head Blight Forum (*p. 12.), Virtual; December 7-11. Online: https://scabusa.org/pdfs/NFHBF20_Proceedings.pdf.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Funnell-Harris, D., **Sattler, S.,** Khasin, M. and **Wegulo, S.** 2020. "Modifying Cell Wall Phenylpropanoids of Small Grains for Increased Usability and Disease and Drought

Resistance." Invited talk. Sept. 2020. University of Nebraska, Complex Biosystems Seminar Series.

Status: Abstract Published and Seminar Given.
Acknowledgement of federal support: YES (Seminar)

Singla, S. "Investigating Phenylpropanoid-based Fusarium Head Blight (FHB) Resistance in Wheat." Accepted talk. Mar. 2021. University of Nebraska, Department of Plant Pathology Spring Seminar Series.

Status: Seminar Given
Acknowledgement of federal support: YES (Seminar)