

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:	Response of Transgenic Wheat Altered in Defense Metabolites to Head Scab
FY20 USDA-ARS Award Amount:	\$45,000
Recipient Organization:	USDA-ARS Forage and Bioenergy Research Unit 251 Filley Hall/Food Industry Complex, University of Nebraska-East Campus Lincoln, NE 68583-0937
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
GDER	Discovering Gene Expression Changes Linked to Phenylpropanoid-based FHB Resistance	\$45,000
FY21 Total ARS Award Amount		\$45,000

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.

DEANNA FUNNELL-HARRIS Digitally signed by DEANNA FUNNELL-HARRIS
Date: 2022.07.21 09:40:00 -05'00'

7-21-2022

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: Discovering Gene Expression Changes Linked to Phenylpropanoid-based FHB Resistance

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

- Obj. 1) Investigate response of wheat lines with both (stacked) constitutive-expression (CE) constructs [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 and Type 2 (to spread) resistance following artificial inoculations under greenhouse conditions in Lincoln, NE, and Minneapolis, MN.
- Obj. 2) Conduct global gene expression using RNA-Seq on the CE 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.)

Obj. 1.

a) What were the major activities?

Stacked lines with both constructs were generated and lines with SbC3H in moderately resistant backgrounds were developed. Because of restricted access to workspaces during 2020, reduced personpower in workspaces in 2020 and part of 2021, and no access for foreign visitors to federal occupied workplaces until May 31, 2022, we focused on generating the materials for RNA-sequencing (Obj. 2). The experiment was highly controlled to reduce environmental effects on gene expression, and only one head on a single plant per pot was inoculated for Type I and Type II resistance. Plants of the two moderately resistant lines, Alsen and Sumai 3, the susceptible line, Wheaton, and the moderately-susceptible recipient line CB037 and two lead homozygous events with each construct were inoculated with *Fusarium graminearum* or a mock control.

b) What were the significant results?

As measured by the Area Under the Disease Progress Curve (AUDPC) and Fusarium damaged kernels (FDK), only Alsen and Sumai 3 exhibited Type I resistance. For Type II resistance, the one SbCCoAOMT CE line had an AUDPC not significantly different from those of Alsen and Sumai 3 and both CE lines had FDK not significantly different from those of Alsen and Sumai 3.

c) List key outcomes or other achievements.

This work presented solid evidence that increased expression of CCoAOMT in wheat could provide a source of quantitative resistance to spread of *F. graminearum* infection in heads.

Obj. 2.

a) What were the major activities?

Wheat heads of CB037 and the two SbCCoAOMT lines (one head per plant and one plant per pot) were point-inoculated with a suspension of *F. graminearum* or the mock control and were collected at 12 and 72 hr after inoculation. The flash-frozen tissues were ground under liquid nitrogen and total RNA was extracted. The RNA was

sent to University of Nebraska Medical Center Genomics Core Facility for sequencing. The data were filtered for quality, differentially expressed genes (DEGs) were identified then analyzed using heatmaps, volcano plots, Venn diagrams, and weighted correlation network analysis (WGCNA).

b) What were the significant results?

Surprisingly, no common genes were differentially-expressed in both of the CE lines, as compared with CB037, but over 470 differentially-expressed genes were shared by the CE lines. These genes were shown to encode proteins in families that include genes involved in defense against fungal pathogens and cellular exportation or detoxification (i. e. of deoxynivalenol). These proteins include ABC transporters, glutathione S-transferases, chitin-recognition and chitinases, leucine rich repeats, cytochrome P450s, UDP-glucuronosyl and UDP-glucosyl transferases, cell wall-associated receptor kinases and those with WRKY domains.

c) List key outcomes or other achievements.

-This research provides evidence that CE expression of SbCCoAOMT affects multiple defense, transporter and detoxification pathways three days following infection by *F. graminearum*.

-This research may provide several molecular targets for screening germplasm for increased quantitative resistance to spread of *F. graminearum* infection.

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

The monies provided helped support training of a M. Sci. student (Mr. Shiv Singla) who performed all the inoculation studies, collection and preparation of materials for RNA-sequencing and analyses of sequencing data. Presentation at the virtual professional meetings North Central Division American Phytopathological Society (NCD APS) Annual Meeting (6/2021) and Fusarium Head Blight Forum (12/2021) was also supported. Additionally, travel to Plant Health 2022 is being supported, where Mr. Singla will be presenting these results. Mr. Singla's training gave him the opportunity to present a virtual seminar to the Department of Plant Pathology, University of Nebraska (UNL) (3-2021). Finally, Mr. Singla's training provided him the opportunity to be selected for an internship at Bayer Corporation (St. Louis) for Summer, 2022.

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

During 2021 and the first part of 2022, travel was still being restricted, resulting in delayed or cancelled in-person meetings and seminars. Some meetings and seminar series were conducted virtually and this research was presented in this manner at the NCD APS Annual Meeting (6-21) and the Fusarium Head Blight Forum (12-21) and for UNL's Department of Plant Pathology (3-21). This research also was included in an in-person seminar for the same department (10-21) and will be presented in person for Plant Health 2022. The two papers resulting from the research supported by USWBSI are currently in preparation.

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.

Singla, S., Duray, Z., Dill-Macky, R., O'Neill, P., Bernhardson, L., Tatineni, S., Sattler, S., Wegulo, S. and Funnell-Harris, D. (2021) Phenylpropanoid based Fusarium head blight resistance in wheat. Proceedings of the 2021 National Fusarium Head Blight Forum; Virtual. December 6-7, 2021. Retrieved from: <https://scabusa.org/forum/2021/2021NFHBFForumProceedings.pdf>.

Funnell-Harris, D. "Modifying cell wall phenylpropanoids of small grains for increased usability, and disease and drought resistance. (2021) Invited talk. October 2021. University of Nebraska, Department of Plant Pathology.

Singla, S., Duray, Z., Dill-Macky, R., O'Neill, P., Bernhardson, L., Tatineni, S., Sattler, S., Wegulo, S. and Funnell-Harris, D. "Investigating Phenylpropanoid-based Fusarium Head Blight (FHB) Resistance in Wheat". (2021) American Phytopathological Society North Central Division Annual Meeting, 2021, Virtual, June 15, 2021. (Poster)

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." (2020) 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.

Funnell-Harris, D., Sattler, S., Khasin, M. and Wegulo, S. "Modifying Cell Wall Phenylpropanoids of Small Grains for Increased Usability and Disease and Drought Resistance." (2020) Invited talk. Sept. 2020. University of Nebraska, Complex Biosystems Seminar Series.