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:	59-0206-6-005
USDA-ARS Agreement Title:	Novel Genes for FHB Resistance
FY19 USDA-ARS Award Amount:	$ 25,000
Recipient Organization:	Rutgers, The State University of New Jersey Division of Grant and Contract Accounting ASB 111, 3 Rutgers Plaza New Brunswick, NJ 08901·8559
DUNS Number:	00-191-2864
EIN:	22-6001086
Recipient Identifying Number or Account Number:	439564 / 804524
Project/Grant Reporting Period:	4/24/19 - 4/23/20
Reporting Period End Date:	4/23/2020

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Novel Genes for FHB Resistance	$ 25,000
	FY19 Total ARS Award Amount	**$ 25,000**

Nilgun Tumer

July 22, 2020

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

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Project 1: *Novel Genes for FHB Resistance*

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

Our overall goal is to identify and characterize novel genes for Fusarium head blight (FHB) resistance in small grain cereals. We are also investigating trichothecene mechanisms of action to understand how these virulence factors function in plants. In addition to the inhibition of translation, trichothecene mycotoxins induce toxic levels of ROS. A better understanding of how the plant counteracts the induction of ROS by trichothecenes like DON would help improve resistance to the fungus. In particular, we are investigating the impact that overexpressing non-specific lipid transfer proteins (nsLTPs) in wheat has for FHB resistance and DON content.

This project addresses the following FY19-20 priorities of GDER: 1) Identify wheat or barley gene variants that improve FHB resistance; 2) Develop assays that can be used to rapidly validate candidate wheat and barley genes for resistance against FHB and/or reduced DON accumulation; 3) Develop effective FHB resistance and/or reduced DON accumulation through transgenic strategies. Stakeholders will benefit from this research through identification of elite wheat cultivars and barley lines that are resistant to FHB, identification of novel markers for breeding programs and important insights into the mode of action of trichothecene mycotoxins.

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

a) What were the major activities?

The major activities involved field testing (Rosemount, MN) of transgenic wheat overexpressing nsLTP (AtLTP4.4-GFP and TaLTP3-GFP) for FHB severity and DON content, greenhouse testing of transgenic wheat after point inoculation of *F. graminearum*, testing both wheat seedling leaf tissue and protoplasts for ROS response to trichothecene exposure. We also purified AtLTP4.4 from *Pichia pastoris* for *in vitro* testing.

b) What were the significant results?

Greenhouse and field testing identified transgenic wheat lines that significantly improved resistance to FHB and significantly reduced DON content. Greenhouse testing using point inoculated wheat (transgenic lines vs non-transgenic controls) identified transgenic lines overexpressing nsLTPs which had reduced fungal biomass compared to non-transgenic controls. AtLTP4.4-GFP protein isolated from *Pichia* was shown to bind lipids and inhibit fungal growth using both zone of inhibition assays and fungal bioassays. In addition, we showed that transgenic wheat overexpressing AtLTP4.4-GFP shows a significantly less accumulation of reactive oxygen species (ROS) when leaf tissue or isolated protoplasts are exposed to DON.

c) List key outcomes or other achievements.

In May we submitted a research paper to Phytopathology that presents the impact of over-expressing nsLTPs in wheat. This was an extensive effort requiring growth chamber, greenhouse, and field studies. The key finding of the work was that nsLTPs have a dual role in protecting plants from *Fusarium graminearum*. Overexpression of nsLTPs reduce both the fungal growth and the accumulation of ROS upon exposure to trichothecene mycotoxins. We also showed that the purified nsLTP protein was able to inhibit fungal growth.

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.

The project was negatively impacted by COVID-19 because we were not able to work in the labs between March 13 and July 1, 2020.

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

The project has provided for the training for four undergraduate students (Maha Kahn, Noura AlDarwish (RISE Scholar), Waner Zheng (Douglas Scholar), and Jeffrey Garcia-Sanchez (McNair Scholar). The students have learned how to grow *Fusarium graminearum*, grow wheat/barley in the greenhouse, isolate *Fusarium* conidia and count the spores with a hemocytometer and flow cytometry, how to inoculate plants, how to perform protein isolation and SDS-PAGE/Western blot analysis. They learned how to isolate high quality plant DNA and how to perform qRT-PCR. They participated in the isolation of plant protoplasts and performed ROS assays using both wheat leaf tissue sections and protoplasts. The students gave presentations during lab meetings and learned how to prepare and present posters. Jeffrey Garcia-Sanchez successful defended his Honors Thesis project working with us on this project. The title of his undergraduate thesis was "Combating *Fusarium graminearum* in wheat: Testing the overexpression of nsLTPs and measuring the impact of exposure to green leaf volatiles." Jeffrey will attend the Biological Sciences Molecular, Cellular, & Developmental Biology program at the University of South Carolina this fall.

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

This research was presented at the 2019 USWBSI meeting and a manuscript was submitted to *Phytopathology*.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY19 award period (4/24/19 - 4/23/20). 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?**
   No.

   If yes, how many?

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 (4/24/19 - 4/23/20**) 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:

De Wolf, E., D. Shah, P. Paul, L. Madden, S. Crawford, D. Hane, S. Canty, R. Dill-Macky, D. Van Sanford, K. Imhoff and D. Miller. 2019. "Impact of Prediction Tools for Fusarium Head Blight in the US, 2009-2019." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 12), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Journal publications.

McLaughlin, J.E. Al Darwish, N., Tyagi, N., Trick, H.N, McCormick, S., Dill-Macky, R., & Tumer, N.E. Overexpression of non-specific lipid transfer proteins enhances wheat resistance to *Fusarium graminearum* and trichothecene mycotoxins. *Submitted to Phytopathology*.
Status: Submitted
Acknowledgement of Federal Support: YES

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

Other publications, conference papers and presentations.

John E. McLaughlin, Neerja Tyagi, Harold N. Trick, Susan McCormick, Ruth Dill-Macky and Nilgun E. Tumer. 2019. "Non-specific Lipid Transfer Proteins (nsLTPs) have Antifungal and Anti-ROS Properties that Enhance Resistance of Wheat to *Fusarium graminearum* Infection and Deoxynivalenol Exposure." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 46), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (Abstract and Poster)

Ruth Dill-Macky, Rebecca D. Curland, Beheshteh Zargaran, Gary J. Muehlbauer, Gerit Bethke, Deanna Funnell-Harris, Jyoti Shah, John McLaughlin and Nilgun Tumer. 2019. "Testing Transgenic Spring Wheat and Barley Lines for Reaction to Fusarium Head Blight: 2019 Field Nursery Report." In: S. Canty, A. Hoffstetter, H. Campbell and R. Dill-Macky (Eds.), *Proceedings of the 2019 National Fusarium Head Blight Forum* (p. 54), Milwaukee, WI; December 8-10. University of Kentucky, Lexington, KY.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (Abstract and Poster)