USDA-ARS
U.S. Wheat and Barley Scab Initiative
FY17 Final Performance Report
Due date: July 31, 2018

Cover Page

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Fiscal Year:	2017
USDA-ARS Agreement ID:	59-0206-6-005
USDA-ARS Agreement Title:	Novel Genes for FHB Resistance.
FY17 USDA-ARS Award Amount:	$ 64,922
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:	4/24/17 - 4/23/18
Reporting Period End Date:	04/23/18

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Novel Genes for FHB Resistance.	$ 64,922
	FY17 Total ARS Award Amount	$ 64,922

Nilgun Tumer 7-31-18

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: *Novel Genes for FHB Resistance.*

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

Our goal is to identify novel genes for Fusarium head blight (FHB) resistance and develop wheat and barley resistant to trichothecenes and FHB. We are also investigating trichothecene mechanisms of action to understand how these virulence factors function in plants.

In collaboration with Harold Trick we generated transgenic wheat plants overexpressing *Arabidopsis* and wheat non-specific lipid transfer protein (nsLTP) genes, *AtLTP4.4* and *TaLTP3* (A wheat nsLTP gene: AY226580) in different wheat genetic backgrounds including Bobwhite, RB07, Rollag, and Forefront. We are quantifying FHB resistance in these lines in the greenhouse using a qPCR method that allows us to measure the abundance of fungal DNA relative to wheat DNA in inoculated wheat floral tissue. Field testing of these lines is also being performed this summer at the University of Minnesota (Rosemount) under the direction of Dr. Ruth Dill-Macky.

In collaboration with Dr. Jochen Kumlehn at the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany we have generated transgenic barley plants containing *AtLTP4.4*, *TaLTP3*, and the vector control, pB835. We have advanced these lines in our greenhouse and have analyzed them for expression of the transgenes.

2. What was accomplished under these goals? *Address items 1-4) below for each goal or objective.*

1) major activities

We have identified thirteen homozygous lines expressing *AtLTP4.4* and *TaLTP3*. We have tested these lines in the greenhouse for *Fusarium graminearum* resistance. We have shifted away from testing detached leaves and have focused our resistance testing using inoculated floral tissues. The qPCR analysis of fungal DNA in the floral tissue is used to quantify resistance. This technique quantifies fungal DNA relative to wheat DNA. The field testing at Minnesota will include FHB resistance scores and DON concentrations of the transgenic lines relative to the non-transgenic controls.

We are analyzing transgenic barley lines for expression of *AtLTP4.4* and *TaLTP3* genes. We are in the process of testing transgenic barley plants in the greenhouse.

We have expressed Arabidopsis LTP4.4 protein in *Pichia pastoris* for purification and developed a protein purification method using the GE Healthcare AKTA purification system.

2) specific objectives

   1) Determine if transgenic wheat lines overexpressing *AtLTP4.4* show improved resistance to FHB and reduced DON accumulation.

   2) Determine if overexpression of *AtLTP4.4* will reduce DON accumulation in transgenic barley.

3) significant results

   We have found that overexpression of either *AtLTP4.4* or *TaLTP3* in transgenic wheat enhances resistance to *Fusarium graminearum* when floral tissue is point inoculated with 20,000 spores. The development of the fungus is significantly reduced compared to the controls at 7, 14, and 21 days after inoculation (DAI).

   We have identified transgenic barley lines expressing high levels of *AtLTP4.4* and *TaLTP3* genes.

   We have purified the mature version of the AtLTP4.4 protein and showed that it is able inhibit growth of *Fusarium graminearum* in vitro. We have also tested two wheat nsLTPs and found that only one of them is able to strongly inhibit *Fusarium* growth, while the other has no impact on fungal growth at the concentrations tested.

4) key outcomes or other achievements

   In the greenhouse experiments *Fusarium graminearum* conidia was used for inoculation of wheat floral tissue to determine resistance at 7, 14, and 21 days after inoculation (DAI). We have identified transgenic lines in both the Bobwhite and RB07 backgrounds that overexpress the *AtLTP4.4* and *TaLTP3* genes and confer enhanced resistance. The resistance was quantified using a qPCR fungal biomass vs plant biomass assay. Purified AtLTP4.4 protein was shown to directly inhibit *Fusarium graminearum* when applied to a liquid suspension of spores.

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

The project has provided for the training of four undergraduate students (Mario Pinzas, Divakar Rajeswaran, Maha Kahn, and Noura AlDarwish) and two high school students (Adelina Branescu and Priyanka Bhatnagar). 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, inoculate plants, isolate high-quality plant DNA, perform qPCR analysis and perform protein isolation and analysis using SDS-PAGE and Western blot. The students presented their work during lab meetings and learned how to prepare and present posters.

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

Dr. John McLaughlin presented our results at the annual National Fusarium Head Blight Forum in December 2017 entitled: Enhanced resistance to Fusarium graminearum by expression of non-specific lipid transfer proteins in wheat. Annual meeting of the National Fusarium Head Blight Forum, Milwaukee, Wisconsin, USA. December 3-5, 2017. A poster of this work was also presented at the national meeting in addition to at the Mycotoxins & Phycotoxins, Gordon Research Conference (June 2017). Our work was also presented at a Big 10 lipid conference hosted here at Rutgers University (November 2017).

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY17 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 FY17 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 FY17 award period?** No.

   If yes, how many?

3. **Have any post docs who worked for you during the FY17 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 FY17 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 FY17 award period. All columns must be completed for each listed germplasm/cultivar. Use the key below the table for Grain Class abbreviations. *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 FY17-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY17 grant. Only include citations for publications submitted or presentations given during your award period (4/24/17 - 4/23/18). 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.

Journal publications. Nothing to report.

Books or other non-periodical, one-time publications. Nothing to report.

Other publications, conference papers and presentations.

Invited talk:

Enhanced resistance to *Fusarium graminearum* by expression of non-specific lipid transfer proteins in wheat. Annual meeting of the National Fusarium Head Blight Forum, Milwaukee, Wisconsin, USA. December 3-5, 2017. Presentation slides in PDF format can be found at:
https://scabusa.org/pdfs/nfhbf17_GS3_McLaughlin.pdf
Status: Abstract Published and Talk Presented
Acknowledgement of Federal Support: YES (slides)

McLaughlin, JE, Finn, D., Tyagi, N., Trick, H., McCormick, S. and Tumer, NE.
Resistance to Fusarium mycotoxins by expression of an Arabidopsis nonspecific lipid transfer protein in wheat. Mycotoxins & Phycotoxins, Gordon Research Conference. Understanding the Exposure and Global Health Risks Associated with Naturally Occurring Environmental Biotoxins in a Rapidly Changing World. Stonehill College, Easton, MA. June 18-23, 2017. Poster 62.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), No (abstract)

McLaughlin, JE, Finn, D., Tyagi, N., Trick, H., McCormick, S., Welti, R., and Tumer, NE. Expression of a non-specific lipid transfer protein in wheat for resistance to Fusarium mycotoxins. Rutgers Center for Lipid Research Annual Symposium: Lipid Biology and Disease. New Brunswick, NJ. November 3rd, 2017.
Status: Poster Presented
Acknowledgement of Federal Support: YES (poster)

McLaughlin, J., Tyagi, N., Trick, H., McCormick, S. and Tumer, NE. Resistance to *Fusarium graminearum* and Fusarium mycotoxins by expression of Arabidopsis and wheat non-specific lipid transfer proteins in wheat. Annual meeting of the National Fusarium Head Blight Forum, Milwaukee, Wisconsin, USA. December 3-5, 2017. Poster 25.

Status: Abstract Published and Poster Presented

Acknowledgement of Federal Support: YES (poster), YES (abstract)