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

Cover Page

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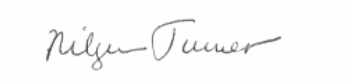
Fiscal Year:	2018
USDA-ARS Agreement ID:	59-0206-6-005
USDA-ARS Agreement Title:	Novel Genes for FHB Resistance.
FY18 USDA-ARS Award Amount:	$ 25,000
Recipient Organization:	Rutgers, The State University of New Jersey Division of Grant and Contract Accounting ASB 111, 3 Rutgers Plaza New Brunswick, NJ 08901·8559
DUNS Number:	00-191-2864
EIN:	22-6001086
Recipient Identifying Number or Account Number:	439564 / 804524
Project/Grant Reporting Period:	04/24/18 - 04/23/19
Reporting Period End Date:	04/23/19

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Novel Genes for FHB Resistance.	$ 25,000
	FY18 Total ARS Award Amount	$ 25,000



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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 lines resistant to trichothecenes and FHB. We have identified a novel non-specific lipid transfer protein (nsLTPs) gene in a screen of *Arabidopsis* for resistance to trichothecenes and are investigating the impact of overexpressing this gene in wheat and barley for FHB resistance and reduction in DON content.

This project addresses the following FY18-19 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? *Address items 1-4) below for each goal or objective.*

1) Major activities
In collaboration with Dr. 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 genetic backgrounds including Bobwhite, RB07, Rollag and Forefront. We increased seed from thirteen different homozygous lines expressing *AtLTP4.4* and *TaLTP3*. We quantified FHB resistance in the greenhouse at Rutgers and in the field at the University of Minnesota (Rosemount) and determined the DON levels in wheat under the direction of Dr. Ruth Dill-Macky.

Resistance testing in the greenhouse was based on a quantitative PCR method which measures the fungal biomass of inoculated floral tissues. This technique quantifies fungal DNA relative to wheat DNA and serves as an excellent measure of resistance. The field testing at Minnesota included determination of FHB severity scores and DON levels of the transgenic wheat lines relative to the non-transgenic controls.

We expressed *Arabidopsis* LTP4.4 protein in *Pichia pastoris* and purified it. We determined the 50% inhibitory concentration (IC_{50}) against *F. graminearum* in bioassays.

We identified transgenic barley plants expressing high levels of *AtLTP4.4* and *TaLTP3* in collaboration with Dr. Jochen Kumlehn at the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany and increased seed for a second season in our

greenhouse. We identified strong expressing lines using Western blot analysis and are testing the impact of the nsLTPs on fungal growth and ROS levels.

2) Specific objectives

1) Develop elite wheat cultivars expressing *AtLTP4.4* and *TaLTP3* that show FHB resistance
2) Identify *AtLTP4.4* and *TaLTP3* expressing FHB resistant transgenic barley lines

3) Significant results

We showed that overexpression of either *AtLTP4.4* or *TaLTP3* significantly enhances *F. graminearum* resistance in wheat when floral tissue is point inoculated with spores and fungal growth is determined at 7, 14, and 21 days after inoculation (DAI).

We evaluated a total of eight different transgenic wheat lines overexpressing *TaLTP3* and *AtLTP4.4* in the field in 2018 at Rosemount Minnesota. The plants were arranged in a randomized complete block design with four replications. Four of the wheat transgenic lines were in the Bobwhite genetic background and four were in the RB07 genetic background along with untransformed parental controls. Two transgenic Bobwhite lines showed significant reduction in FHB severity relative to the controls. All four transgenic lines in the Bobwhite background showed significant reduction in DON content (ppm) relative to the controls. DON levels in the nontransgenic RB07 controls and the transgenic lines were very low and therefore we could not see a difference between the transgenic lines and controls.

We expressed the mature form of AtLTP4.4 in *Pichia pastoris* and have purified the recombinant protein. We showed that AtLTP4.4 protein is able to inhibit *Fusarium* growth *in vitro*. We also tested two wheat nsLTPs in the bioassay and found that TaLTP9 is able to strongly inhibit *Fusarium* growth, while TaLTP7 has no impact on fungal growth at the concentrations tested.

We found that *AtLTP4.4* overexpression in transgenic barley plants is able to reduce fungal growth of leaf cuttings relative to vector control suggesting that *AtLTP4.4* may improve resistance of barley to FHB.

4) Key outcomes or other achievements

We identified transgenic wheat lines expressing nsLTP genes from wheat and *Arabidopsis*, which showed resistance to FHB and reduced DON accumulation in the field.

Purified AtLTP4.4 was shown to directly inhibit the growth of *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 for four undergraduate students (Maha Kahn, Noura Al Darwish (RISE Scholar), Waner Zheng (Douglas Scholar), and Jeffrey Garcia-Sanchez (McNair Scholar). Noura Al Darwish has been accepted into the Ph.D. program at the University of Arizona. The students have learned growing *Fusarium graminearum*, isolating *Fusarium* conidia and counting the spores, growing wheat and barley plants in the greenhouse, inoculating plants with *Fusarium*, performing protein isolation and analysis using SDS-PAGE and Western blot analysis, isolating high quality plant DNA, and quantitative PCR. The students gave presentations during lab meetings and at Rutgers Symposia and learned how to prepare and present posters.

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

Dr. John McLaughlin presented our current work at the Annual Meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri, USA December 2-4, 2018 and at the Gordon conference on Mycotoxins and Phycotoxins at Stonehill College, Massachusetts, June 16-21, 2019. He was also member of the forum organization committee and arranged for the three speakers in the Gene Discovery and Engineering Resistance section of the conference.

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 (04/24/18 - 04/23/19). 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 presentation with an abstract:

> 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)

Journal publications.

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

Other publications, conference papers and presentations.

McLaughlin, J. E., Tyagi, N., Trick, H. N., McCormick, S., Dill-Macky, R. and Tumer, N. E. Arabidopsis and Wheat Non-specific Lipid Transfer (nsLTP) Proteins Inhibit *Fusarium graminearum* and Confer Enhanced Resistance to FHB: Greenhouse, Field and *in vitro* evidence. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri, USA December 2-4, 2018. Poster 34.
Status: Abstract Published and Poster Presented.
Acknowledgement of Federal Support: Yes (Poster), Yes (Abstract)

Dill-Macky, R., Elakkad, A. M., Zargaran, B., Muehlbauer, G. J., Bethke, G., McLaughlin, J., Tumer, N. E. and Funnell-Harris, D. Testing Transgenic Spring Wheat and Barley Lines for Reaction to Fusarium Head Blight: 2018 Field Nursery Report. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri, USA December 2-4, 2018. Poster 31.
Status: Abstract Published and Poster Presented.
Acknowledgement of Federal Support: Yes (Poster), Yes (Abstract)

McLaughlin, J. E. Tyagi, N., Trick, H. N., McCormick, S., Dill-Macky, R. and Tumer, N. E. *Arabidopsis* and wheat non-specific lipid transfer (nsLTP) proteins provide enhanced resistance to FHB and reduce DON accumulation. Gordon Research Conference (GRC) on Mycotoxins and Phycotoxins. Stonehill College, Massachusetts, June 16-21, 2019. Poster 81.
Status: Poster Presented.
Acknowledgement of Federal Support: Yes (Poster)