USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY16 Final Performance Report
Due date: July 28, 2017

Cover Page

Principle Investigator (PI):	Nilgun Tumer
Institution:	Rutgers University
E-mail:	tumer@aesop.rutgers.edu
Phone:	732-932-8165 x215
Fiscal Year:	2016
USDA-ARS Agreement ID:	59-0206-6-005
USDA-ARS Agreement Title:	Novel Genes for FHB Resistance.
FY16 USDA-ARS Award Amount:	$ 65,049
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:	4/24/16 - 4/23/17
Reporting Period End Date:	04/23/17

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Novel Genes for FHB Resistance.	$ 65,049
	FY16 Total ARS Award Amount	**$ 65,049**

Nilgun Tumer

7-17-17

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?

The major goals of this project are:
1) To determine if transgenic wheat lines expressing AtLTP4.4 show reduced DON accumulation and improved resistance to FHB.
2) Determine if overexpression of AtLTP4.4 will reduce DON accumulation in transgenic barley.

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

1) major activities

For wheat expression, we have generated new constructs containing the Arabidopsis (*AtLTP4.4*) and wheat (*TaLTP3*) nonspecific lipid transfer protein genes as EGFP fusions. Both genes were cloned into B712p7o2x35s-UbiZmF-LGFP (DNA Cloning Services, Germany), which has the Bar gene driven by the CaMV35S promoter and the maize ubiquitin promoter driving expression of the transgene-EGFP fusion. 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, Rollag, Rb07 and Forefront to determine if expression of these genes in transgenic wheat will confer FHB resistance. We are currently using greenhouse experiments to test resistance to both trichothecenes and *Fusarium graminearum*.

For barley transformation both genes were cloned into the B835p6o2x35s-UbiZm-LGFP overexpression vector, which has hygromycin (HPTII) selectable marker, and the maize ubiquitin promoter driving expression of the transgene:GFP fusion. This material will be screened for gene and protein expression in September.

2) specific objectives

To determine if overexpression of *AtLTP4.4* and *TaLTP3* in wheat and barley will confer resistance to trichothecenes and *Fusarium graminearum*.

3) significant results

Dr. Harold Trick's team provided us with multiple transgenic lines containing *AtLTP4.4* and *TaLTP3* in the Bobwhite, Rb07, and Forefront backgrounds. A total of 25 transgenic lines were identified. These included 9 Bobwhite lines containing *AtLTP4.4*, 4 Bobwhite lines containing *TaLTP3*, 2 Rb07 lines containing *AtLTP4.4*, 3 Rb07 lines containing *TaLTP3*, 2 Forefont lines containing *AtLTP4.4*, and 5 Forefront lines containing *TaLTP3*. These lines have been grown out in the greenhouse and screened by leaf painting for herbicide resistance by confocal microscopy and by Western blot analysis for identification of the GFP fusion protein. The Bobwhite transgenics are in the T2 generation and the Rb07 and Forefront transgenics are in the T1 generation. We have identified Bobwhite lines expressing high

levels of *AtLTP4.4* and *TaLTP* using both confocal microscopy and Western blot analysis and are in the process of screening the Rb07 and Forefront transgenics for expression.

We have initiated a collaboration with Dr. Jochen Kumlehn at the Leibniz Institute of Plant Genetics and Crop Plant Research in Gatersleben, Germany for barley transformation using *AtLTP4.4* and *TaLTP*. His team has recently developed transgenic barley (Golden Promise) containing *AtLTP4.4*, *TaLTP3*, and the vector control pB835. This material is currently in the T0 generation and will be ready for and testing the T1 generation in the Rutgers greenhouse in September.

4) key outcomes or other achievements
We have used detached leaf assays and floret inoculation assays to determine if the transgenic wheat lines are resistant to *F. graminearum*. In addition, we have tested the impact of trichothecenes on wheat head development by point inoculation of wheat florets by DON. Preliminary analysis indicated that overexpression of the nsLTP proteins reduces wheat head bleaching due to DON relative to the nontransgenic controls. The detached leaf assays using the *AtLTP4.4* overexpressing Bobwhite leaves showed reduced *F. graminearum* growth.

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

One MS student (Daniel Finn), three undergraduate students (Mario Pinzas, Divakar Rajeswaran, Maha Kahn) and one high school student (Adelina Branescu) were trained on this project. Mr. Finn successfully completed his M.S.

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

Dr. John McLaughlin presented results at the annual National fusarium Head Blight Forum. December 4-6, 2016.

Dr. John McLaughlin presented results at the Mycotoxins & Phycotoxins Gordon Research Conference. Stonehill College, Easton, MA. June 18-23, 2017.

Dr. Nilgun Tumer presented results at the Mycotoxins & Phycotoxins Gordon Research Conference. Stonehill College, Easton, MA. June 18-23, 2017.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY16 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 FY16 award period?** Yes.

   If yes, how many? One.

2. **Did any graduate students in your research program supported by funding from your USWBSI grant earn their Ph.D. degree during the FY16 award period?** No.

   If yes, how many?

3. **Have any post docs who worked for you during the FY16 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 FY16 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 FY16 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 FY16-FPR_Instructions for detailed instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY16 grant. Only include citations for publications submitted or presentations given during your award period (4/24/16 - 4/23/17). 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 presented at the FHB Forum:

Journal publications.

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

Other publications, conference papers and presentations.

McLaughlin, J.E., Finn, D., Tyagi, N., Trick, H. McCormick, S. and Tumer. N.E. 2016. "Expression of an *Arabidopsis* nonspecific lipid transfer protein in *Pichia Pastoris* and wheat." In: S. Canty, A. Clark, K. Wolfe and D. Van Sanford (Eds.), Proceedings of the 2016 National Fusarium Head Blight Forum (p. 110). East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. Poster 53.
Status: Abstract Published and Poster Presented
Acknowledgement of Federal Support: YES (poster), NO (abstract)