USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY15 Final Performance Report
Due date: July 15, 2016

Cover Page

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Fiscal Year:	2015
USDA-ARS Agreement ID:	59-0206-1-121
USDA-ARS Agreement Title:	A Genome-Wide Screen to Identify Novel Genes for FHB Resistance.
FY15 USDA-ARS Award Amount:	$ 65,112
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
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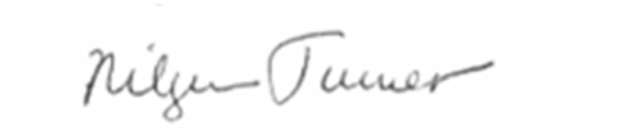
Recipient Identifying Number or Account Number:	433617
Project/Grant Reporting Period:	04/24/15-04/23/16
Reporting Period End Date:	04/23/16

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Novel Plant Genes for FHB Resistance.	$ 65,112
	FY15 Total ARS Award Amount	**$ 65,112**



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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 Plant 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 plants resistant to trichothecenes and FHB. We are also investigating trichothecene mechanism of action to understand how these virulence factors function in plants.

In collaboration with Harold Trick we are generating 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 to determine if expression of these genes in transgenic wheat will confer FHB resistance.

2. What was accomplished under these goals?

1) major activities

We identified and *Arabidopsis* and a wheat non-specific lipid transfer protein (nsLTP) gene, *AtLTP4.4* and *TaLTP3* (A wheat nsLTP gene: AY226580), respectively.

We showed that expression of a fusion protein containing EGFP fused to the C-terminus of *AtLTP4.4* (AtLTP4.4:EGFP) in yeast and *Arabidopsis* provided enhanced resistance to trichothecin (Tcin) and other trichothecenes.

We showed that expression of the wheat nsLTP gene, *TaLTP3* in yeast conferred resistance to trichothecenes.

In collaboration with Harold Trick we generated transgenic wheat plants overexpressing codon optimized *AtLTP4.4* in the pAHC17 vector in four different wheat backgrounds, Bobwhite, RB07, Rollag, and Forefront. We were not able to detect protein expression in transgenic wheat lines, possibly because *AtLTP4.4* is not stable in wheat when it is expressed by itself. To overcome this problem, we have made fusions with enhanced green fluorescent protein (EGFP) and showed that this fusion protein is expressed in transgenic *Arabidopsis* plants.

To overexpress *AtLTP4.4* and *TaLTP3* as an EGFP fusion in transgenic wheat and barley, we constructed new transformation vectors. For wheat expression, 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-GFP fusion.

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.

2) specific objectives: To determine if overexpression of *AtLTP4.4* and *TaLTP3* in wheat confers resistance to trichothecenes.

3) significant results: Four overexpression vectors (two for wheat and two for barley) containing the *AtLTP4.4* and *TaLTP3* genes were generated. Expression of the *AtLTP4.4* and *TaLTP3* genes was tested using transient expression assays in tobacco leaves. All four vectors containing the nsLTP genes were found to express the fusion proteins in tobacco leaves.

To generate transgenic wheat plants *TALTP3* and *AtLTP4.4* constructs were provided to Dr. Harold Trick. *TALTP3* bombarded into wheat (Bobwhite) four times and *ATLTP4* three times. *TALTP3* was bombarded into Forefront and RBO7 callus. The plants are on selection media.

4) key outcomes or other achievements: We have identified the cause of the low *AtLTP4.4* expression in transgenic wheat plants and constructed new vectors to overcome this problem.

We have generated a yeast (*Pichia*) strain that overexpresses *AtLTP4.4,* which will be used to isolate protein for the production of antibodies.

Analysis of *Arabidopsis* protoplasts overexpressing *AtLTP4.4* showed reduced reactive oxygen species (ROS) generation at the basal level and after Tcin exposure compared to the non-transgenic control. This result provides further evidence that *AtLTP4.4* protects cells against oxidative stress due to trichothecenes.

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

The project has provided for the training of one MS student (Daniel Finn) and one undergraduate student (Mario Pinzas). Mr. Finn is using *Arabidopsis* protoplasts to investigate the impact of trichothecenes on *Arabidopsis.* He is also screening an *Arabidopsis* activation tagging library to identify additional trichothecene resistant mutants.

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 2015.

Dr. Nilgun Tumer presented an invited talk at the Mycotoxins & Phycotoxins Gordon Research Conference: Stonehill College, Easton, MA. June 14-19, 2015.

Dr. Tumer was an invited speaker at the Corn Utilization and Technology Conference June 6-8, 2016 in St. Louis, MO.

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY15 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 FY15 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 FY15 award period?** No.

   If yes, how many?

3. **Have any post docs who worked for you during the FY15 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 FY15 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 FY15 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

Refer to the FY15-FPR_Instructions for listing publications/presentations about your work that resulted from all of the projects included in the FY15 grant. If you did not have any publications or presentations, state 'Nothing to Report' directly above the Journal publications section.

Journal publications.

J. E. McLaughlin, M. A. Bin-Umer, T. Widiez, D. Finn, S. McCormick, N. E. Tumer (2015). A Lipid Transfer Protein Increases the Glutathione Content and Enhances Arabidopsis Resistance to a Trichothecene Mycotoxin. *PLoS One*. 10(6):e0130204.
Status: Published
Acknowledgement of Federal Support: YES

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

Nothing to report.

Other publications, conference papers and presentations.

McLaughlin, J., Finn, D., Trick, H., McCormick, S. and Tumer, NE. Expression of a lipid transfer protein in wheat to alleviate oxidative stress induced by trichothecenes - A possible mechanism to increase resistance to FHB. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri, USA. December 6-8, 2015. Poster 21.
Status: Abstract published and poster presented
Acknowledgement of Federal Support: YES

Tumer. N. E. Trichothecene mechanism of action and plant resistance to trichothecenes. Mycotoxins & Phycotoxins Gordon Research Conference: The Biology, Chemistry and Ecology of Naturally Occurring Fungal and Algal Toxins with Public Health and Economic Impacts. Stonehill College, Easton, MA. June 14-19, 2015. Invited Talk.
Status: Talk presented.
Acknowledgement of Federal Support: YES

Tumer, N. E. Expression of a lipid transfer protein alleviates oxidative stress induced by trichothecenes and confers resistance to Fusarium Head Blight. Corn utilization and Technology Conference June 6-8, 2016, St. Louis, MO. Invited talk.
Status: Abstract published and talk presented.
Acknowledgement of Federal Support: YES