USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY14 Final Performance Report
July 15, 2015

Cover Page

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Fiscal Year:	FY14
USDA-ARS Agreement ID:	59-0206-1-121
USDA-ARS Agreement Title:	A Genome-Wide Screen to Identify Novel Genes for FHB Resistance.
FY14 USDA-ARS Award Amount:	$ 65,175

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	Novel Plant Genes for FHB Resistance.	$ 65,175
	FY14 Total ARS Award Amount	**$ 65,175**

Nilgun Tumer

7/10/15

Principal Investigator Date

* MGMT – FHB Management
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
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
WES-CP – Western 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 major problem or issue is being resolved relevant to Fusarium head blight (scab) and how are you resolving it?

Our goal is to develop wheat plants resistant to trichothecenes and *Fusarium graminearum*. We worked on the trichothecene mechanism of action to identify novel genes for resistance to DON. We showed that overexpression of an *Arabidopsis* non-specific lipid transfer protein (nsLTP) gene, *AtLTP4.4,* in both yeast and *Arabidopsis* provided enhanced resistance to trichothecin (Tcin) and other trichothecenes.

In collaboration with Dr. Harold Trick, we generated transgenic wheat (Bobwhite) plants containing *AtLTP4.4.* These plants were evaluated in the field at Rosemount, MN for *Fusarium graminearum* resistance by Dr. Ruth Dill-Macky during the 2014 field season. However, in that test any potential resistance conferred by the transgene might have been masked by the extremely high level of disease which impacted Bobwhite.

Preliminary results suggested that the expression level of *AtLTP4.4* had to be improved in Bobwhite. To increase expression, we generated a monocot codon optimized version of *AtLTP4.4* tagged with His and HA epitopes for protein detection. The codon optimized version of *AtLTP4.4* was cloned into pACH17 and transformed into Forefront, RB07 and Bobwhite by Dr. Harold Trick and colleagues at Kansas State University. In May 2015 we received 14 transgenic lines in Forefront, 23 transgenic lines in RB07, and 14 transgenic lines in Bobwhite. We are now analyzing these plants for expression the LTP gene by immunoblot analysis. Seed increases will be performed on all lines showing high levels of expression of the LTP protein. This material will be tested in the greenhouse against DON and *Fusarium graminearum* prior to field testing by Dr. Ruth Dill-Macky at the University of Minnesota.

2. List the most important accomplishments and their impact (i.e. how are they being used) to minimize the threat of Fusarium Head Blight or to reduce mycotoxins. Complete both sections; repeat sections for each major accomplishment:

Accomplishment:

1) Our results in *Arabidopsis* confirmed the mechanism of cytotoxicity we identified in yeast and showed that ROS induction due to trichothecene exposure contributes to trichothecene toxicity in plants.

2) We identified the chloroplast as a prominent source of ROS in leaf tissue exposed to Tcin or DON.

3) We showed that toxicity of trichothecenes in plants can be substantially alleviated by treatment with antioxidants.

4) Overexpression of *AtLTP4.4* in both yeast and *Arabidopsis* significantly attenuated ROS levels due to trichothecene exposure.

5) Using a sensitive glutathione (GSH) bioassay, we identified that overexpression of the cysteine-rich *AtLTP4.4* in both yeast and *Arabidopsis* increased the cellular glutathione content.

6) We confirmed that exogenous addition of GSH significantly improved resistance of *Arabidopsis* to Tcin while the addition of buthionine sulfoximine, an inhibitor of GSH synthesis, increased sensitivity to the mycotoxin.

7) We generated transgenic wheat, Forefront, RB07, and Bobwhite lines containing the monocot codon optimized version of *AtLTP4.4*.

Impact:

1) We identified a potential mechanism of resistance in plants overexpressing *AtLTP4.4*. We showed that increasing the glutathione content of wheat should enhance resistance to trichothecenes by reducing oxidative stress.

2) Our results indicate that expression of *AtLTP4.4* needs to be optimized in wheat to increase resistance to FHB in the field. We have generated transgenic plants with the codon optimized version of *AtLTP4.4* and are in the process of testing them.

3) Dr. Tumer gave an invited talk at the recent 2015 Mycotoxins and Phycotoxins Gordon conference (Easton, MA) on June 14-19, 2015.

4) Our manuscript entitled "A Lipid transfer protein increases the glutathione content and enhances *Arabidopsis* resistance to a trichothecene mycotoxin" is published in PLoS One (see references below).

Training of Next Generation Scientists

Instructions: Please answer the following questions as it pertains to the FY14 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 FY14 award period?**

   No, but currently one MS student, Daniel Finn, is working on the FHB project in our lab.

   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 FY14 award period?**

   Yes.

   If yes, how many? Dr. Anwar Bin Umer.

3. **Have any post docs who worked for you during the FY14 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 FY14 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?

Include below a list of all germplasm or cultivars released with full or partial support of the USWBSI during the FY14 award period. List the release notice or publication. Briefly describe the level of FHB resistance. *If not applicable because your grant did NOT include any VDHR-related projects, enter N/A below.*

N/A

Include below a list of the publications, presentations, peer-reviewed articles, and non-peer reviewed articles written about your work that resulted from all of the projects included in the FY14 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

McLaughlin, JE, Bin Umer, A, Widiez, T., Finn, D., McCormick, S. and Tumer, NE. 2015. A Lipid transfer protein increases the glutathione content and enhances *Arabidopsis* resistance to a trichothecene mycotoxin. PLoS One. Jun 9;10(6):e0130204.

Nilgun Tumer. 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.

McLaughlin, JE, Bin Umer, A, Widiez, T., Finn, D., McCormick, S. and Tumer, NE. A lipid transfer protein increases the glutathione content and improves Arabidopsis resistance to a trichothecene mycotoxin. 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
Poster 48.

Dill-Macky, R., Elakkad, A.M., Muehlbauer, G.J., Li, X., Dahleen, L.S., Skadsen, R.W., Bregitzer, P.P., McLaughlin, J.E., and Tumer, N.E. Testing transgenic spring wheat and barley lines for reaction to Fusarium Head Blight: 2014 Field nursery report. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri, USA. December 7-9, 2014. Poster 20.

McLaughlin, JE, Bin Umer, A, McCormick, S. and Tumer, NE. Non-specific lipid transfer proteins provide enhanced resistance to trichothecenes by reducing oxidative stress in yeast and Arabidopsis. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri, USA. December 7-9, 2014. Poster 24.