

**USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY13 Final Performance Report
July 15, 2014**

Cover Page

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Fiscal Year:	FY13
USDA-ARS Agreement ID:	59-0206-1-121
USDA-ARS Agreement Title:	A Genome-Wide Screen to Identify Novel Genes for FHB Resistance.
FY13 USDA-ARS Award Amount:	\$ 63,291

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	A Genome-wide Screen to Identify Novel Genes for FHB Resistance.	\$ 63,291
	FY13 Total ARS Award Amount	\$ 63,291

Nilgun Tumer 7/14/14
 Principal Investigator Date

* MGMT – FHB Management
 FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain
 GDER – Gene Discovery & Engineering Resistance
 PBG – Pathogen Biology & Genetics
 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: *A Genome-wide Screen to Identify Novel 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 FHB. We identified two novel Arabidopsis genes encoding non-specific lipid transfer proteins (nsLTPs), which were overexpressed in an activation tagged Arabidopsis line that showed resistance to trichothecin (Tcin). Transgenic Arabidopsis lines individually overexpressing *AtLTP4.4* or *AtLTP4.5*, showed enhanced resistance to Tcin relative to the non-transgenic or vector control lines, with *AtLTP4.4* providing the greatest protection. Overexpression of either gene in *Saccharomyces cerevisiae* also enhanced resistance to Tcin, DON, T2-toxin and DAS.

We have constructed an expression vector with *AtLTP4.4* downstream of the maize ubiquitin promoter in pAHC17 and provided this vector to Harold Trick for transformation into wheat. We identified seven independently derived transgenic Bobwhite wheat lines containing *AtLTP4.4*. Seed from the seven transgenic wheat lines was sent to Ruth Dill-Macky at the University of Minnesota and is currently being evaluated for *Fusarium* resistance at the Rosemount Agricultural Experiment Station in Minnesota. Harold Trick has regenerated 18 additional Rollag and RB07 lines with *AtLTP4.4*:pAHC17, which are being increased at Rosemount, MN. We have constructed a codon-optimized version of *AtLTP4.4* for wheat expression and cloned it into pAHC17. The codon optimized *AtLTP4.4*:pAHC17 is being transformed into Bobwhite, Rollag, RB07, as well as spring adapted lines by Harold Trick. We plan to increase these lines in the greenhouse this fall for preparation for the 2015 field season.

To identify novel cellular genes involved in trichothecene resistance, we screened the *Saccharomyces cerevisiae* deletion library for increased sensitivity to nonlethal concentrations of Tcin and identified 121 strains exhibiting higher sensitivity than the parental strain. The largest group of sensitive strains had significantly higher reactive oxygen species (ROS) levels relative to the parental strain, suggesting that oxidative stress contributes to trichothecene sensitivity. A dose-dependent increase in ROS levels was observed in the parental strain treated with different trichothecenes, but not in a petite version of the parental strain or in the presence of a mitochondrial membrane uncoupler, indicating that mitochondria are the main site of ROS production due to toxin exposure.

We showed that cytotoxicity of trichothecenes is alleviated after treatment of Arabidopsis or yeast with antioxidants, suggesting that oxidative stress contributes to trichothecene toxicity. In both Arabidopsis and yeast overexpression of *AtLTP4.4* significantly attenuated ROS levels upon exposure to Tcin relative to the vector controls. These results revealed a role for mitochondrial oxidative stress in trichothecene toxicity. We found that overexpression of specific nsLTPs improves resistance to trichothecenes by protecting plants against trichothecene-induced oxidative stress.

We showed that elimination of trichothecene-damaged mitochondria by mitophagy improves resistance to trichothecenes. These results demonstrated for the first time that increased mitophagy is a cellular protection mechanism against trichothecene-induced mitochondrial oxidative stress and a potential target for trichothecene resistance. A manuscript describing these results is in press in PNAS (see attached).

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:

- We showed that trichothecenes induce reactive oxygen species (ROS) and mitochondria are the main site of ROS production due to toxin exposure.
- Overexpression of *AtLTP4.4* improves resistance to trichothecenes by significantly attenuating ROS levels, indicating that it protects plants against trichothecene-induced oxidative stress.
- We have generated transgenic wheat plants expressing *AtLTP4.4*, which are being evaluated against FHB in the field.
- We carried out a genome-wide screen to identify cellular protection mechanisms against trichothecenes and showed that elimination of trichothecene-damaged mitochondria by mitophagy improves trichothecene resistance by protecting against mitochondrial oxidative stress.

Impact:

- We showed that mitochondrial oxidative stress is a mechanism for trichothecene toxicity *in vivo* and is a target for resistance to FHB.
- Overexpression of a lipid transfer protein gene increases trichothecene resistance by reducing mitochondrial oxidative stress. Transgenic wheat lines overexpressing this gene are currently being evaluated in the field in Rosemount, MN.
- We identified a number of new cellular protection mechanisms against trichothecene toxicity and showed that mitophagy increases resistance to trichothecenes by reducing mitochondrial oxidative stress.
- A manuscript describing these results is in press in PNAS.

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 FY13 grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

- Bin-Umer, A. McLaughlin, J.E., Butterly, M. McCormick, S. and N.E. Tumer. Elimination of damaged mitochondria through mitophagy reduces mitochondrial oxidative stress and increases tolerance to trichothecenes. *Proc. Natl. Acad. Sci. USA*, in press. Accepted June 27, 2014.

Three manuscripts related to this work are also in progress:

- McLaughlin JE, Bin-Umer MA, Widiez, T., Finn D., McCormick, SE and Tumer, NE. (2014). Two Novel Non-Specific Lipid Transfer Proteins Provide Enhanced Resistance to a Trichothecene Mycotoxin by Reducing Oxidative Stress. In preparation.
- Bin-Umer MA, McLaughlin JE, Butterly M, McCormick S and Tumer, NE. (2014) Novel nonspecific lipid transfer proteins protect yeast from trichothecene toxicity by preventing mitochondrial damage. In preparation.

The following presentations have been made related to this work:

- Bin Umer, A. Trichothecene Exposure Leads to Mitochondrial ROS-Mediated Cell Death in Yeast. Annual meeting of the National Fusarium Head Blight Forum, Milwaukee, WI USA. 3-5 December, 2013. Flash and Dash Talk.
- McLaughlin, J. An Arabidopsis Non-specific Lipid Transfer Protein Provides Enhanced Resistance to a Trichothecene Mycotoxin by Reducing Oxidative Stress. Annual meeting of the National Fusarium Head Blight Forum, Milwaukee, WI USA. 3-5 December, 2013. Flash and Dash Talk.

Poster Abstracts:

- Bin-Umer, A. McLaughlin, J.E., Basu, D. McCormick, S. and N.E. Tumer. “Trichothecene Exposure Leads to Mitochondrial ROS-Mediated Cell Death in Yeast.” In: S. Canty, A. Clark, Y. Salat, and D. Van Sanford (Eds.), *Proceedings of the 2013 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 63.
- McLaughlin, J., Bin-Umer, A., Widiez, T., McCormick, S., and N.E. Tumer. “An Arabidopsis Non-Specific Lipid Transfer Protein Provides Enhanced Resistance to a Trichothecene Mycotoxin by Reducing Oxidative Stress.” In: S. Canty, A. Clark, Y. Salat, and D. Van Sanford (Eds.), *Proceedings of the 2013 National Fusarium Head Blight Forum*. East Lansing, MI/Lexington, KY: U.S. Wheat & Barley Scab Initiative. p. 74.