USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY12 Final Performance Report
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Cover Page

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Fiscal Year:	FY12
USDA-ARS Agreement ID:	59-0206-1-121
USDA-ARS Agreement Title:	A Genome-Wide Screen to Identify Novel Genes for FHB Resistance.
FY12 USDA-ARS Award Amount:	$ 63,353

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	A Genome-wide Screen to Identify Novel Genes for FHB Resistance.	$ 63,353
	Total ARS Award Amount	**$ 63,353**

Nilgun Tumer 7/15/13

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?

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. Both nsLTPs belong to type IV nsLTP family. The function nsLTPs in plants is not well understood. Currently we are investigating if expression of type IV nsLTPs improves resistance to trichothecene mycotoxins in transgenic Arabidopsis and in transgenic wheat plants. We are working on identifying the host cellular processes that are targeted by trichothecene mycotoxins and determining if nsLTP expression will improve resistance of wheat to trichothecenes. We are investigating the mechanism of nsLTP-mediated resistance to determine how cellular functions that are affected by trichothecenes are selectively protected by AtLTP4.4 and AtLTP4.5 expression. In addition, we are using yeast to identify additional genes that may provide resistance to trichothecenes when expressed in plants.

2. List the most important accomplishment and its impact (i.e. how is it 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 expression of Arabidopsis nsLTP genes, AtLTP4.4 and AtLTP4.5 in transgenic Arabidopsis plants improves resistance to trichothecenes. We have constructed an expression vector with the AtLTP4.4 gene downstream of the maize ubiquitin promoter in pACH17 and provided this vector to Dr. Harold Trick for transformation into wheat.

We showed that expression of AtLTP4.4 and AtLTP4.5 in yeast conferred resistance to lethal doses of DON and trichothecin (Tcin), as well as type A trichothecenes, T-2 and DAS. Both genes protected yeast against growth inhibition by trichothecenes, but not against other translation inhibitors. AtLTP4.4 and AtLTP4.5 genes were associated with mitochondria and reduced inhibition of mitochondrial translation by Tcin, DON, T-2 and DAS, but not cytosolic translation. Mitochondrial membrane fragmentation by trichothecenes was also significantly reduced in yeast expressing AtLTP4.4 and AtLTP4.5 genes. These results indicated that expression of AtLTP4.4 and AtLTP4.5 genes protect mitochondrial translation and mitochondrial membrane integrity against the inhibitory effects of trichothecenes.

We have identified a wheat nsLTP gene and demonstrated that expression of this gene in yeast confers resistance to trichothecenes. We are in the process of constructing wheat transformation vectors with this gene to determine if overexpression of the wheat ns LTP in transgenic wheat will improve resistance to DON and FHB.

We showed that treatment of Arabidopsis with DON and Tcin induces reactive oxygen species (ROS), indicating that trichothecenes cause oxidative stress. ROS induction by DON

and trichothecin in wild type Arabidopsis plants was associated with chloroplasts and mitochondria by confocal microscopy. Similarly, treatment of yeast with Tcin, DON and DAS increased ROS levels by DCFH-DA staining using flow cytometry. Both AtLTP4.4 and AtLTP4.5 exhibited antioxidant properties by reducing basal ROS levels and inhibiting ROS induction by trichothecene treatment in yeast. Expression of AtLTP4.4 and AtLTP4.5 also protected yeast against the oxidative stressors, paraquat and hydrogen peroxide, indicating that they have antioxidant properties. Our results suggest that AtLTP4.4 and AtLTP4.5 associate with yeast mitochondria and selectively protect mitochondria against translation inhibition and oxidative stress caused by trichothecenes. We are using fluorescent dyes to quantitatively measure ROS levels in transgenic Arabidopsis plants to determine if overexpression of AtLTP4.4 and AtLTP4.5 reduces ROS levels due to trichothecenes in plants.

Using yeast viability assays developed in our lab, we screened the entire yeast overexpression library against trichothecenes and identified several novel genes that enhance resistance to trichothecenes, including an unknown peptide that provides high level of resistance to Tcin.

Impact: Our results demonstrate that two nsLTP genes identified from an activation tagging screen in Arabidopsis confer resistance to trichothecenes in Arabidopsis and in yeast. We show for the first time that trichothecenes cause oxidative stress by increasing ROS levels and that the two AtLTP genes, which improve resistance to trichothecenes have antioxidant properties. The two AtLTP genes also protect yeast against mitochondrial translation inhibition and disruption of mitochondrial membrane integrity by trichothecenes. These results demonstrate that Arabidopsis and yeast are powerful models for understanding the basis for trichothecene sensitivity. They allowed us to rapidly identify plant genes that can confer trichothecene resistance and to understand the molecular basis of resistance at the single cell level. Characterizing the mechanism of action of nsLTPs in transgenic wheat and barley plants will help plant breeders increase wheat and barley resistance to FHB.

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

Manuscripts in preparation:

Bin-Umer, A. McLaughlin, J.E., Basu, D. McCormick, S. and N.E. Tumer. Novel insights into the mechanism of nsLTP-mediated resistance to trichothecene toxicity suggest a critical role for mitochondria. Manuscript in preparation.

McLaughlin, J.E., Bin-Umer, A., Widiez, T., Salmon-Denikos, E., Basu, D., McCormick, S., Gregory, B., and N.E. Tumer. Activation tagging in Arabidopsis identifies two novel non-specific lipid transfer proteins which provide enhanced resistance to a trichothecene mycotoxin. Manuscript in preparation.

Gnecco, J., McLaughlin, J.E., Bin-Umer, A. McCormick, S. and N.E. Tumer. The identification of trichothecene resistance genes by screening an overexpression ORF Collection in *Saccharomyces cerevisiae*. Manuscript in preparation.

Presentations:

Bin Umer A., McLaughlin J.E., and N.E. Tumer. Lipid Transfer Proteins Confer Resistance to Trichothecenes. Annual meeting of the National Fusarium Head Blight Forum, Orlando, FL. 4-6 December, 2012. Invited talk.

Bin-Umer, A. McLaughlin, J.E., Basu, D. McCormick, S. and N.E. Tumer. Lipid transfer protein-mediated resistance to a trichothecene mycotoxin- Novel players in FHB resistance. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri. Orlando, FL. 4-6 December, 2012. Poster 62.

McLaughlin, J.E., Bin-Umer, A., Widiez, T., Salmon-Denikos, E., Basu, D., McCormick, S., Gregory, B., and N.E. Tumer. Activation tagging in Arabidopsis identifies two novel non-specific lipid transfer proteins which provide enhanced resistance to a trichothecene mycotoxin. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri. Orlando, FL. 4-6 December, 2012. Poster 72.