USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY11 Final Performance Report
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Cover Page

PI:	Nilgun Tumer
Institution:	Rutgers University
Address:	Biotech Center - Cook College 59 Dudley Rd. New Brunswick, NJ 08901-8520
E-mail:	tumer@aesop.rutgers.edu
Phone:	732-932-8165 x215
Fax:	732-932-6535
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USDA-ARS Agreement Title:	A Genome-Wide Screen to Identify Novel Genes for FHB Resistance.
FY11 USDA-ARS Award Amount:	$ 61,209

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	A Genome-Wide Screen to Identify Novel Genes for FHB Resistance.	$ 61,209
	Total ARS Award Amount	**$ 61,209**

Nilgun Tumer July 19, 2012
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 primary goal is to identify plant genes that provide resistance to trichothecene mycotoxins and Fusarium Head Blight (FHB). We have used *Saccharomyces cerevisiae* as a model to identify the specific cellular targets of trichothecene mycotoxins and identified mitochondria as a primary target (McLaughlin et al. 2009. PNAS 106: 21883-21888). Using isolated mitochondria we demonstrated that mitochondria are a direct target of trichothecene mycotoxins (Bin-Umer et al. 2011. Toxins 3: 1484-1501). Our work has shown that trichothecene mycotoxins affect mitochondrial translation and mitochondrial membrane morphology. Importantly, we showed that inhibition of mitochondrial translation is a primary target of trichothecenes and is not secondary to the disruption of mitochondrial membranes or inhibition of cytosolic translation (Bin-Umer et al. 2011. Toxins 3: 1484-1501).

We have applied an activation tagging approach to identify plant genes that confer resistance to trichothecene mycotoxins. Activation tagging is a mutation generation technique whereby plants are transformed with T-DNAs engineered to contain enhancer sequences to identify both loss-of-function and gain-of-function (overexpression) mutants. Using this approach, we have screened ~250,000 activation tagged *Arabidopsis* seeds for resistance to trichothecin and identified 30 lines that showed resistance. These plants were able to form roots on 4 μM Tcin, a concentration which severely inhibits germination and prevents root formation of the wild type Col-0. Characterization of one of these lines using quantitative RT-PCR identified an activation tagged line, termed *Arabidopsis thaliana resistant root formation1* or *AtRRF1*. In AtTRRF1, two novel non-specific lipid transfer protein (nsLTP) genes, designated as LTP4.4 and LTP4.5, were overexpressed compared to the wild-type control. Both LTPs belong to type IV nsLTPs (Boutrot et al., 2005 BBA 1730:114-125).

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):

<u>Accomplishment:</u>

Two novel nsLTPs, LTP4.4 and LTP4.5 were overexpressed in the activation tagged Arabidopsis line, *AtRRF1,* which showed resistance to trichothecene mycotoxins. To verify resistance, both genes were cloned into the Gateway overexpression vectors as C-terminal fusions with GFP downstream of the CaMV35S promoter. Expression of LTP4.4:GFP and 4.5:GFP was examined by transient expression in tobacco leaves by confocal microscopy. The LTP4.4 and 4.5 proteins were found to localize to the extracellular space near the cell wall. In addition, coexpression analysis using an ER-mcherry marker indicated that LTP4.4 may also localize to the ER. The wildtype Col-0 Arabidopsis was transformed with both expression vectors independently to test for resistance to trichothecin. Overexpression of

either LTP4.4 or LTP4.5 gene in transgenic *Arabidopsis* provided resistance to trichothecin when seedlings were plated on media containing 4 µM Tcin. The mechanism by which nsLTPs enhance tolerance to fungal and bacterial pathogens remains to be determined. To address the mechanism of trichothecene resistance, we have transformed each LTP gene into the yeast, *S. cerevisiae*. Both LTPs conferred resistance to lethal doses of trichothecin upon overexpression in yeast. Overexpression of these LTPs did not confer resistance against other known inhibitors of yeast growth (cyloheximide, anisomycin and chloramphenicol). Furthermore, overexpression of a different LTP (LTP1.1) also failed to provide resistance to trichothecin, suggesting that trichothecene resistance is a unique feature of LTP4.4 and LTP4.5 and not a general response of all LTPs. In vivo translation assays showed a significant inhibition (>75%) of total translation by trichothecin in cells overexpressing the LTPs. Mitochondrial translation, however, was only minimally inhibited (<23%). LTP-overexpressing yeast cells also had an active mitochondrial membrane potential when grown against trichothecin. These results provide new insights on the defensive role of LTPs against trichothecenes and their potential role in developing transgenic crop plants with enhanced tolerance to FHB.

For functional genomics of cereals such as wheat and barley, maize transposable elements have been used for generating large collection of gene knockouts because high-throughput T-DNA transformation is not a viable approach. Ayliffe et al. (Plant Mol Biol. 2007. 64: 329-347), reported a novel Ac-Ds system in which a modified Ds element (UbiDs) carrying two maize ubiquitin 1 promoters are used for high throughput transcription of adjacent flanking sequences in barley. In the presence of Ac transposase, this modified Ds element undergoes transposition to new genomic locations with a majority of (75%) of new UbiDs insertions initiating high levels of adjacent sequence transcription. To determine if this activation tagging system has the potential to generate overexpression and gene silencing mutants in wheat, we have developed a high-throughput seed germination assay using hydroponics, which should allow us to screen 60,000 activation tagged F2 seedlings from F1 wheat (Bobwhite background) provided by Mick Ayliffe.

Impact:

Our activation tagging screen has identified two novel plant nsLTP genes that confer resistance to trichothecene mycotoxins. We have confirmed that overexpression of each gene provides resistance to trichothecin in *Arabidopsis* and in yeast. The finding that these two specific nsLTPs provide resistance in two independent systems indicates that these genes work by a conserved mechanism, which is likely to function in higher plants. Our yeast studies showed that both genes protect mitochondrial translation and membrane potential against trichothecenes, demonstrating that the novel plant genes identified through the activation tagging approach provide potentially new resistance mechanisms against FHB. We will determine if expression of LTP4.4 and/or LTP4.5 will provide resistance to *F. graminearum* in wheat and barley. These genes may be used by plant breeders to help increase wheat and barley resistance to *F. graminearum*.

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.

Bin-Umer, M.A., McLaughlin, J.E., McCormick, S., Tumer, N.E. 2012 Overexpression of Arabidopsis Lipid Transfer Proteins confers resistance to trichothecenes in *Saccharomyces cerevisiae*. In preparation.

McLaughlin, J. E., Bin-Umer, M. A., Widiez, T., Basu, D., Salmon-Denikos, E., McCormick, S., Gregory, B. and Tumer, N. E. 2012 Activation tagging in Arabidopsis identifies two novel non-specific lipid transfer proteins which provide resistance to a trichothecene mycotoxin. In preparation.

Bin-Umer, M.A., McLaughlin, J.E., Basu, D., McCormick, S., Tumer, N.E. Trichothecene Mycotoxins Inhibit Mitochondrial Translation—Implication for the Mechanism of Toxicity. 2011 Toxins 3, 1484-1501.

McLaughlin, J.E., Bin-Umer, M.A., Basu, D., McCormick, S., Tumer, N.E. An activation tagging screen to identify novel genes for Fusarium Head Blight (FHB) resistance. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri. 4-6 December, 2011. Poster 56.

Bin Umer A., McLaughlin JE. Basu D., McCormick S., Tumer NE. Trichothecene mycotoxins inhibit mitochondrial translation- Implications for FHB resistance. Annual meeting of the National Fusarium Head Blight Forum, St. Louis, Missouri. 4-6 December, 2011. Poster 49.