USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY10 Final Performance Report
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Cover Page

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Fiscal Year:	FY10
USDA-ARS Agreement ID:	59-0790-6-069
USDA-ARS Agreement Title:	Modification of the Ribosomal Target to Enhance Resistance to Trichothecene Mycotoxins.
FY10 USDA-ARS Award Amount:	$ 47,527

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Award Amount
GDER	A Genome Wide Screen to Identify Novel Genes for FHB Resistance.	$ 47,527
	Total ARS Award Amount	**$ 47,527**

Nilgun Tumer 7-12-11

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?**

   A primary goal in our laboratory is to identify novel genes for resistance to trichothecene mycotoxins and to engineer resistance to FHB. Our work has shown that trichothecene mycotoxins affect not only cytosolic translation, but also mitochondrial translation and membrane morphology, and has identified mitochondria as a primary target. To identify novel genes for trichothecene resistance, we used activation tagging approach to identify plant genes which confer resistance to trichothecenes. Activation tagging permits screening for both loss-of-function and gain-of-function (overexpression) mutants. It allows the identification of essential genes that may impact the phenotype of interest were traditional T-DNA knockouts would lead to lethal phenotypes. A further advantage of the activation-tagging based mutagenesis approach is that recovery of the insertion site is relatively straightforward either by plasmid rescue or TAIL-PCR. We have applied the activation tagging approach in *Arabidopsis* to identify genes that confer resistance to trichothecene mycotoxins.

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:

   Ongoing work with yeast has focused on the effects of trichothecenes on mitochondria. We have used both *in organello* and flow cytometric approaches to measure the impact of these toxins on mitochondrial function. Trichothecene-treated yeast cells were stained for mitochondrial membrane potential, ROS generation, and cell death. Treated cells were analyzed using flow cytrometry. Median fluorescence intensities for all three markers increased when yeast cells were treated with trichothecenes. These results further demonstrated a direct role for mitochondria in trichothecene toxicity. Furthermore, flow cytometry results indicated that trichothecenes trigger ROS generation resulting in hyperpolarization of the mitochondrial membrane, which eventually leads to cell death. This work was presented as a poster at the 2010 Annual meeting of the National Fusarium Head Blight Forum in Milwaukee, WI. The results of this work have been prepared as a manuscript to be submitted.

   In collaboration with Brian Gregory at The University of Pennsylvania, we have screened a large activation tagged population of Arabidopsis (~250,000 plants) for resistance to trichothecene mycotoxins. We have developed germination screen, which permits the identification of activation tagged mutants that are able to form roots on media containing the mycotoxin. To date, we have identified 15 mutants from the screen that exhibit resistance (root growth on media containing the toxin) relative to Col-0, the wild type plant. The resistant plants were able to form roots on 4 µM Tcin and were indistinguishable from the untreated wild type plants, except for one dwarf mutant identified. Both mutations

mapped to chromosome 5. Sequence analysis of two the resistant lines by TAIL-PCR demonstrated T-DNA insertions in two novel genes. *AtTRRF1* is an unknown gene and *AtTRRF5* is a member of the TBL (Trichome birefringence-like) gene family. qRT-PCR analysis revealed that the activation tag caused a knockout in *AtTRRF5*. Up-regulation of neighboring genes was not detected in this mutant line. qRT-PCR analysis revealed the upregulation of two upstream lipid transfer genes in *AtTRRF1*. Verification of both mutants using independently generated knockouts and overexpression lines is currently in progress. This work was presented at the 2010 Annual meeting of the National Fusarium Head Blight Forum in Milwaukee, WI.

We developed a collaboration with Michael Ayliffe (CSIRO Plant Industry, Australia) to screen activation tagged populations of both barley and wheat. We obtained the seeds and plan to screen this material for resistance to trichothecenes as we did with *Arabidopsis*.

Impact:

Our studies identified novel plant genes that confer resistance to trichothecene mycotoxins. Our activation tagging approach identified a valuable collection of previously unknown and uncharacterized plant genes that can be manipulated in wheat for improvement of the existing germplasm for resistance to FHB and DON. The novel plant genes identified through the activation tagging approach will reveal potentially new resistance mechanisms against FHB. These genes may be used by plant breeders to help increase plant resistance to *F. graminearum*.

Our results demonstrated that trichothecenes have a direct affect on mitochondria. They inhibit mitochondrial translation independent of cytosolic translation and mitochondrial membrane potential.

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.

McLaughlin JE., Salmon-Denikos E., Bin Umer A., Basu D., McCormick S., Gregory B., and Tumer NE. Activation tag screening to identify novel genes for trichothecene resistance. Annual meeting of the National Fusarium Head Blight Forum, Milwaukee, WI. 7-9 December, 2010. Invited talk.

Bin Umer A., McLaughlin JE. Basu D., McCormick S., Tumer NE. Identification of a direct role for mitochondria in trichothecene resistance. Annual meeting of the National Fusarium Head Blight Forum, Milwaukee, WI. 7-9 December, 2010. Poster 1.

Bin Umer A., McLaughlin JE. Basu D., McCormick S., Tumer NE. Identification of a direct role for mitochondria in trichothecene resistance. In preparation.

Di, R., Blechl, A., Dill-Macky, R., Tortora, A. and Tumer N. E. 2010. Expression of a truncated form of yeast ribosomal protein L3 in transgenic wheat improves resistance to Fusarium Head Blight. Plant Science 178:374-380.

McLaughlin, JE, Bin Umer, A, Tortora, A., Mendez, N., McCormick, S. and Tumer, NE. 2009. A genome-wide screen in *S. cerevisiae* reveals a critical role for the mitochondria in the toxicity of a trichothecene mycotoxin. PNAS. 106:21883-21888.