USDA-ARS/
U.S. Wheat and Barley Scab Initiative
FY08 Final Performance Report (approx. May 08 – April 09)
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Cover Page

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Fiscal Year:	2008
USDA-ARS Agreement ID:	59-0790-6-069
USDA-ARS Agreement Title:	Modification of the Ribosomal Target to Enhance Resistance to Trichothecene Mycotoxins.
FY08 USDA-ARS Award Amount:	$ 60,057

USWBSI Individual Project(s)

USWBSI Research Category*	Project Title	ARS Adjusted Award Amount
GDER	A Genome Wide Screen to identify Novel Genes for FHB Resistance.	$60,057
	Total Award Amount	**$ 60,057**

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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
HWW-CP – Hard Winter Wheat Coordinated Project
VDHR – Variety Development & Uniform Nurseries – Sub categories are below:
SPR – Spring Wheat Region
NWW – Northern Winter Wheat Region
SWW – Southern Sinter 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?

The overall goal of this project was to understand the genetic basis of susceptibility and resistance to trichothecene mycotoxins and to identify novel genes for trichothecene resistance.

The objectives of this project were:

1. To carry out a genome-wide screen of the yeast deletion library to isolate mutants that are resistant to trichothecenes.

2. To carry out a genome-wide screen to isolate mutants, which are hypersensitive to trichothecenes to identify the genes that will confer trichothecene resistance when over-expressed.

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:

To define the set of genes involved in trichothecene mechanism of action, we carried out a systematic screen of a yeast deletion library, which contains 4720 unique strains representing 76% of the total known open reading frames for resistance to trichothecin (T-cin), which has the same mechanism of action as DON. We identified 138 deletion strains as resistant to 4 µM Tcin from three independent experiments. The largest group of gene deletions (89/138 or 64%) that showed resistance to 4 µM Tcin encoded proteins associated with the mitochondria. 43% of the mitochondrial genes identified were associated with ribosomes, 17% with genome maintenance, 16% with respiration, 12% with translation, 6% with membrane and 4% with gene regulation. These results revealed a specific requirement for mitochondria in the sensitivity to a trichothecene mycotoxin. In addition, a number of cellular pathways which have not previously been connected to trichothecene sensitivity were identified. Resistant strains were placed in six functional categories, which include the following: cell cycle, cytosolic ribosome, mitochondria, membrane/phospholipids, ubiquitination/proteasome, and an unclassified group. *In vivo* assays demonstrated that T-cin inhibits mitochondrial translation and affects mitochondrial morphology.

The T-cin sensitivity screen took place using 2 µM T-cin, which did not inhibit the growth of BY4743. The screen of the entire library was repeated twice. A total of 84 deletion strains were identified that showed either sensitivity or hypersensitivity to T-cin. The identified genes encoded components of signal transduction, translation/transcription, vacuolar protein sorting and degradation. Preliminary results demonstrated that the genes that showed increased sensitivity to T-cin, were also more sensitive to DON. The sensitivity screen

identified a valuable collection of genes that may play a role in trichothecene metabolism and represent novel candidates for engineering FHB resistance in plants.

Impact:

Trichothecene mycotoxins are thought to inhibit protein synthesis by targeting the peptidyltransferase center of eukaryotic ribosomes. Trichothecenes have been reported to have diverse roles in the cell that are not limited to the inhibition of protein synthesis. However, it is not known if these effects are cellular responses to protein synthesis inhibition or if trichothecenes have multiple modes of action. To develop a better understanding of the cellular components required for sensitivity to trichothecenes, we have screened the deletion collection of *Saccharomyces cerevisiae* to systematically identify genes whose deletion confers resistance to trichothecin. The largest group of resistant strains identified affected mitochondrial function, suggesting a specific requirement for mitochondria in trichothecene sensitivity. *In vivo* assays demonstrated that T-cin inhibits mitochondrial translation in the wild type strain, but not in the most highly resistant deletion strains, providing the first evidence that mitochondrial translation is the site of action of trichothecene mycotoxins. These results provided insight into mechanisms that control susceptibility of eukaryotic cells to trichothecene mycotoxins. The identification of genes whose loss of function leads to enhanced trichothecene resistance in yeast may provide novel approaches to investigate the orthologs of these genes in plants leading to new opportunities for resistance to FHB in cereals.

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.

J. E. McLaughlin, Bin Umer, A., Mendez, N., McCormick, S. and Tumer, N. E. (2009) A chemical genomic screen in *Saccharomyces cerevisiae* reveals mitochondrial translation as the site of action of a trichothecene mycotoxin. In preparation.

R. Di, N. E. Tumer, A. Blechl, R. Dill-Macky and A. Tortora (2009). Expression of a truncated from of yeast ribosomal protein L3 in transgenic wheat confers resistance to Fusarium head blight. Submitted.

A.Bin Umer, J. McLaughlin, D. Pu, N. Mendez, S. McCormick and N. Tumer (2008). A genomics approach to characterize trichothecene mode of action reveals a cellular wide response in yeast. 2008 National Fusarium Head Blight Forum, 2-4 Dec. 2008, Indinanapolis, IN. Poster # 105.

J. McLaughlin, A. Bin Umer, J. Schifano, A. Tortora, S. McCormick and N. Tumer (2008). A genome-wide screen in yeast to identify potential targets of trichothecene mycotoxins. 2008 National Fusarium Head Blight Forum, 2-4 Dec. 2008, Indinanapolis, IN. Poster #54.

J. McLaughlin, A. Bin Umer, J. Schifano, A.Tortora and N. E. Tumer (2008) A genome wide screen to identify the potential targets of trichothecene mycotoxins. XII. International Congress of Mycology, 5-9 August 2008, Istanbul, Turkey, BP-183.

If your FY08 USDA-ARS Grant contained a VDHR-related project, include below a list all germplasm or cultivars released with full or partial support of the USWBSI. List the release notice or publication. Briefly describe the level of FHB resistance. If this is not applicable (i.e. no VDHR-related project) to your FY08 grant, please insert 'Not Applicable' below.

Not applicable.