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Research Category: **GDER** **Duration of Award: 1 Year**

Project Title: A Genome Wide Screen to Identify Novel Genes for FHB Resistance.

PROJECT 1 ABSTRACT

(1 Page Limit)

With funding from USWBSI, we have screened the entire collection of yeast deletion mutants for resistance to a trichothecene mycotoxin and identified a valuable collection of novel targets. The largest group of resistant strains affected mitochondrial function, suggesting a role for fully active mitochondria in trichothecene toxicity. Tcin inhibited mitochondrial translation in the wild type strain to a greater extent than in the most resistant strains, implicating mitochondrial translation as a previously unrecognized site of action. The Tcin-resistant strains were cross-resistant to anisomycin and chloramphenicol, suggesting that Tcin targets the peptidyltransferase center of mitochondrial ribosomes. Tcin induced cell death was partially rescued by mutants that regulate mitochondrial fusion and maintenance of the tubular morphology of mitochondria. Treatment of yeast cells with Tcin led to the fragmentation of the tubular mitochondrial network, supporting a role for Tcin in disruption of mitochondrial membrane morphology. These results provide genome-wide insight into the mode of action of trichothecene mycotoxins and uncover a critical role for mitochondrial translation and membrane maintenance in their toxicity. These results were published in PNAS (McLaughlin, J. E., Bin-Umer, M. A., Tortora, A., Mendez, N., McCormick, S. and N. E. Tumer (2009). A genome-wide screen in *S. cerevisiae* reveals a critical role for the mitochondria in the toxicity of a trichothecene mycotoxin. Proc. Natl. Acad. Sci. USA, 106:21883-8).

To identify the genes involved in trichothecene metabolism, we screened the yeast deletion strains for enhanced sensitivity to a trichothecene mycotoxin and identified the components of pathways that play a role in trichothecene metabolism. The new genes identified from the genome-wide screens will provide novel targets for engineering FHB resistance in cereals and for reducing mycotoxin contamination. Furthermore, this approach will provide important new insights into the mode of action of trichothecene mycotoxins and trichothecene metabolism. The primary goal of this project is to determine if manipulating expression of the orthologs of the genes identified in yeast will confer resistance to trichothecenes in plants.

The specific objectives of this proposal are:

1. Characterize the trichothecene resistance in yeast and determine if trichothecene resistance can be engineered by manipulating expression of the orthologs of the yeast genes in plants. We will use *Arabidopsis* and *Physcomitrella* knockout lines to determine if the genes identified in yeast will confer trichothecene resistance in plants.
2. Determine if genes that confer trichothecene sensitivity when deleted in yeast will confer trichothecene resistance when overexpressed. We will determine if overexpression of the genes identified in the genome-wide screen for trichothecene sensitivity in yeast will confer trichothecene resistance. To obtain a comprehensive picture of the pathways involved in trichothecene metabolism and resistance, the overexpression library, which represents 93% of all coding sequences in yeast, will be screened for trichothecene resistance.

This project fulfills the following research priorities of the GDER: "Increased efficiency of identification of candidate genes for resistance against FHB and reduced DON accumulation" and to "develop effective FHB resistance through transgenic strategies."