

**USDA-ARS/  
U.S. Wheat and Barley Scab Initiative  
FY06 Final Performance Report (approx. May 06 – April 07)  
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**Cover Page**

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<b>Fiscal Year:</b>	2006
<b>USDA-ARS Agreement ID:</b>	59-0790-6-069
<b>USDA-ARS Agreement Title:</b>	Modification of the Ribosomal Target to Enhance Resistance to Trichothecene Mycotoxins.
<b>FY06 ARS Award Amount:</b>	\$ 48,404

**USWBSI Individual Project(s)**

USWBSI Research Area*	Project Title	ARS Award Amount
GET	Modification of the Ribosomal Target to Enhance Resistance to Trichothecene Mycotoxins.	\$ 48,404
	<b>Total Award Amount</b>	<b>\$ 48,404</b>

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Principal Investigator

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Date

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\* CBCC – Chemical, Biological & Cultural Control  
EEDF – Etiology, Epidemiology & Disease Forecasting  
FSTU – Food Safety, Toxicology, & Utilization of Mycotoxin-contaminated Grain  
GET – Genetic Engineering & Transformation  
HGR – Host Genetics Resources  
HGG – Host Genetics & Genomics  
PGG – Pathogen Genetics & Genomics  
VDUN – Variety Development & Uniform Nurseries

**Project 1:** *Modification of the Ribosomal Target to Enhance Resistance to Trichothecene Mycotoxins.*

**1. What major problem or issue is being resolved and how are you resolving it?**

The major problem addressed in this project is to determine if resistance to Fusarium Head Blight (FHB) can be achieved in transgenic wheat plants by expressing DON resistant forms of the yeast ribosomal protein L3 genes.

**2. List the most important accomplishment and its impact (how is it being used?). Complete all three sections (repeat sections for each major accomplishment):**

**Accomplishment:**

The most important accomplishment is that we have produced transgenic wheat plants expressing a truncated form of the yeast L3 gene (L3 $\Delta$ ) that are resistant to FHB and accumulate significantly lower levels of DON. FHB is a devastating disease worldwide, caused mainly by *Fusarium graminearum*. The *Fusarium*-infected grain is contaminated with potent mycotoxins, especially deoxynivalenol (DON), which poses a great threat to human and animal health. There are very limited sources of genetic resistance available against this devastating disease. Our group has demonstrated that ribosomal protein L3 is the target of DON and identified a truncated form of yeast L3 (L3 $\Delta$ ) that confers a high level of resistance to DON. During this grant period, we have demonstrated that expression of the yeast L3 $\Delta$  in transgenic wheat plants confers resistance to FHB and leads to a significant reduction in DON levels. The susceptible spring wheat cultivar, Bobwhite was transformed with the yeast L3 $\Delta$  under the control of the barley floret-specific *Lem1* or the maize constitutive *Ubi1* promoter. Seeds from the homozygous lines containing each construct were able to germinate on media containing DON, unlike the seeds of the wild type Bobwhite plants. To determine if these lines were resistant to FHB, five different homozygous lines were evaluated for resistance to FHB in greenhouse tests by inoculating a single spikelet at the central node of the main spike of each plant with a macroconidial spore suspension of *F. graminearum*. All spikelets of inoculated wild type plants turned brown at 21 days after the inoculation. In contrast, only the inoculated spikelets of the transgenic lines turned brown; the uninoculated spikelets remained green in most of the transgenic plants. The disease severity was reduced by 48-56% in four different transgenic wheat lines compared to the untransformed Bobwhite plants. The reduction in disease severity correlated well with the level of expression of L3 $\Delta$  mRNA. These results demonstrated that transgenic wheat plants expressing the yeast L3 $\Delta$  showed improved resistance to FHB over the untransformed Bobwhite plants. To determine if resistance to FHB would result in a reduction in DON levels, the mature kernels above and below the inoculated spikelets were analyzed for DON levels. There was a 63-76% reduction in DON levels in the four different FHB resistant transgenic lines. The DON levels in one transgenic line were lower than the DON levels in the resistant line, Alsen.

**Impact:**

This is the first time that FHB resistance has been shown in transgenic wheat plants by expressing a modified form of the ribosomal protein L3 gene. Our results also provide the

first evidence that resistance to DON correlates with resistance to FHB and results in reduced accumulation of DON in transgenic wheat plants.

**As a result of that accomplishment, what does your particular clientele, the scientific community, and agriculture as a whole have now that they didn't have before?**

The transgenic wheat lines could be used as breeding material for introduction of the FHB resistance into elite wheat cultivars or breeding lines.

**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.**

### **Publications**

Di, R., A. Blechl, R. Dill-Macky, A. Tortora, and N. E. Tumer. 2007. Expression of the N-terminal 99 amino acids of yeast ribosomal protein L3 in transgenic wheat confers resistance to *Fusarium* head blight. *Mol. Plant Breeding* 5:283.

Di, R., and N. E. Tumer. 2005. Expression of a truncated form of ribosomal protein L3 confers resistance to pokeweed antiviral protein and the *Fusarium* mycotoxin deoxynivalenol. *Mol Plant Microbe Interact* 18:762-70.

### **Oral presentations in conferences**

Di, R., A. Blechl, R. Dill-Macky, A. Tortora, and N. E. Tumer. 2007. Expression of the N-terminal 99 amino acids of yeast ribosomal protein L3 in transgenic wheat confers resistance to *Fusarium* head blight. The 2<sup>nd</sup> International Conference of Plant Molecular Breeding, Hainan, China.

Di, R., Blechl, A., Dill-Macky, R., Tortora, A. and Tumer, N. E. 2006. Expression of a truncated form of ribosomal protein L3 in transgenic wheat confers resistance to Deoxynivalenol and *Fusarium* Head Blight. Proceedings of the 2006 National *Fusarium* Head Blight Forum, Research Triangle Park, North Carolina Dec. 10-12, 2006, Abstract 5.

R. Di and N. E. Tumer 2005. Expression of a truncated form of ribosomal protein L3 confers resistance to pokeweed antiviral protein. American Society for Virology 24<sup>th</sup> Annual Meeting, The Pennsylvania State University, University Park, Pennsylvania. June 18-22. Abstract No. 29-1.

### **Manuscripts in preparation**

Di, R., and N. E. Tumer. The N-terminal 99 amino acids of yeast ribosomal protein L3 reduce the toxicity of pokeweed antiviral protein in *Saccharomyces cerevisiae*.

### **Patent Applications**

Tumer, N. E. and R. Di. Sept. 8, 2005. Transgenic tobacco plants expressing truncated proteins L3 and pokeweed antiviral protein are resistant to trichothecene fungal toxins. U.S. Application #11/010,795.