U.S. Wheat and Barley Scab Initiative
FY02 Final Performance Report (approx. May 02 – April 03)
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Cover Page

PI:	**Nilgun Tumer**
Institution:	**Rutgers, The State University of New Jersey**
Address:	**Biotech Center** **59 Dudley Rd.** **New Brunswick, NJ 08901-8520**
E-mail:	**tumer@aesop.rutgers.edu**
Phone:	**732-932-8165 x215**
Fax:	**732-932-6535**
Year:	**FY2002 (approx. May 02 – April 03)**
Grant Number:	**58-5325-2-758**
Grant Title:	**Fusarium Head Blight Research**
FY02 ARS Award Amount:	**$ 52,683**

Project

Program Area	Project Title	USWBSI Recommended Amount
BIO	Modification of the Ribosomal Target to Enhance Resistance to Trichothecene Mycotoxins.	$54,000
	Total Amount Recommended	**$54,000**

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Principal Investigator Date

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?

Our primary goal in this project was to determine if overexpression of wild type or trichothecene resistant alleles of the yeast ribosomal protein L3 gene in transgenic plants will confer resistance to trichothecene mycotoxins. To determine if overexpression of the yeast L3 gene in transgenic plants will confer resistance to trichothecenes, we made expression vectors containing the wild type yeast L3 gene and a N-terminal truncated form of this gene and transformed them into tobacco plants either by themselves or together with pokeweed antiviral protein (PAP), a ribosome inactivating protein that confers resistance to viral and fungal infection. We confirmed the presence of the yeast L3 and PAP genes in transgenic plants by Southern blot analysis. Expression of yeast L3 and PAP mRNA was determined by real time PCR analysis and protein expression was analyzed by immunoblot analysis. Transgenic tobacco plants were evaluated for resistance to the trichothecene mycotoxin deoxynivalenol (DON) and 4,15-diacetoxyscirpenol (DAS) using a seed germination assay.

In addition to expressing the yeast L3 genes in transgenic plants, we designed constructs to inhibit expression of the endogenous tobacco L3 genes to determine if down-regulation of L3 expression will confer resistance to the trichothecene mycotoxins. RNAi vectors were constructed by cloning each tobacco L3 gene in sense and antisense orientation, separated by an intron in the middle. Transgenic tobacco plants were generated with these constructs, expression of endogenous L3 genes were analyzed by Northern blot analysis and by real time PCR analysis and trichothecene resistance was evaluated by a seed germination assay.

2. What were the most significant accomplishments?

We have expressed the full-length and N-terminal truncated forms of yeast L3 in transgenic tobacco plants and demonstrated that overexpression of wild type or mutant forms of L3 will not have any detrimental effects on the growth and development of transgenic plants. Analysis of wild type plants indicated that 1 μM DAS and 10 μM of DON were the concentrations that would give the best inhibition of germination of wild type tobacco seeds.

All transgenic lines exhibited resistance to 1 μM DAS as indicated by higher germination rates on media containing the toxin and higher root length. Resistance to 10 μM DON was also observed in majority of the transgenic lines. The highest level of DON resistance was obtained in transgenic plants containing both the N-terminal truncated form of L3 and the PAP gene. We are in the process of analyzing the expression levels of the endogenous L3 genes in these lines to determine how DON resistance correlates with L3 expression. The yeast L3 constructs that showed the highest level of DON resistance were provided to Dr. Ann Blechl for transformation into wheat.

Analysis of transgenic tobacco plants containing the RNAi vectors indicated that transgenic plants containing NT482, which consisted of the L3B gene in sense and antisense orientation separated by an intron, showed higher tolerance to 8μM DAS compared with the wild type plants.

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.

Manuscripts:

Popescu, S. and Tumer, N. E. 2003. Silencing of ribosomal protein L3 genes interferes with ribosome biogenesis, cell proliferation and plant development in *N. tabacum*. Submitted.

Di, R. and Tumer, N. E. 2003. Transgenic tobacco plants expressing the yeast L3 gene and pokeweed antiviral protein confer resistance to trichothecene mycotoxins. In preparation.