USDA-ARS / USWBSI
FY03 Final Performance Report (approx. May 03 – April 04)
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Cover Page

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Year:	**FY2003 (approx. May 03 – April 04)**
FY03 ARS SCA ID:	**58-5325-2-758**
FY03 ARS Agreement Title:	**Modification of the Ribosomal Target to Enhance Resistance to Trichothecene Mycotoxins.**
FY03 ARS Award Amount:	**$ 52,848**

USWBSI Individual Project(s)

USWBSI Research Area*	Project Title	ARS Adjusted Award Amount
BIO	Modification of the Ribosomal Target to Enhance Resistance to Trichothecene Mycotoxins.	$ 52,848
	Total Amount Recommended	**$ 52,848**

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Principal Investigator Date

* BIO – Biotechnology
CBC – Chemical & Biological Control
EDM – Epidemiology & Disease Management
FSTU – Food Safety, Toxicology, & Utilization
GIE – Germplasm Introduction & Enhancement
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?

To develop resistance to DON, we generated transgenic tobacco plants expressing full length and truncated forms of yeast L3 genes either alone or together with pokeweed antiviral protein (PAP), a ribosome inactivating protein that confers broad-spectrum viral and fungal resistance. We examined expression of the yeast L3 and PAP genes and evaluated the resistance of these plants to trichothecene mycotoxins, DON and 4,15-diacetoxyscirpenol (DAS).

To determine if alteration of tobacco L3 gene would confer resistance to trichothecene mycotoxins, we altered expression of the large subunit ribosomal protein L3 genes in *N. tabacum* using post-transcriptional gene silencing. L3 is encoded by two genes, *RPL3A* and *RPL3B*, with 80.2% amino acid sequence identity in tobacco. Two types of "hairpin" RNA vectors carrying the *RPL3A* or *RPL3B* sequences in both sense and antisense orientation were generated in order to alter the expression level of both *RPL3* genes and transformed into tobacco plants. Transgenic plants were analyzed for the expression of the endogenous L3 genes, for effects on growth and development, ribosome biogenesis and resistance to trichothecene mycotoxins.

2. What were the most significant accomplishments?

Our results indicate that transgenic tobacco plants expressing a truncated from of ribosomal protein L3 (L3Δ) together with wild type pokeweed antiviral protein (PAP) are resistant to DON and DAS. Co-expression of L3Δ and PAP led to a dramatic increase in PAP mRNA and protein expression. Transgenic plants expressing high levels of PAP were phenotypically normal, indicating that L3Δ eliminated the cytotoxicity of PAP. Ribosomes from plants expressing PAP and L3Δ were not depurinated, even though PAP was associated with ribosomes. Since PAP accesses ribosomes by binding to ribosomal protein L3, binding of PAP to L3 may have protected ribosomes from the translation inhibitory effects of DON. Expression of the endogenous tobacco ribosomal protein genes, *RPL3A* and *RPL3B* was also upregulated in the transgenic lines containing L3Δ. These results demonstrate that expression of yeast L3Δ leads to upregulation of both PAP and endogenous L3 expression and confers resistance to DON, providing evidence that L3 is the ribosomal target of DON.

Tobacco plants transformed with a vector containing a 5'- terminal fragment of *RPL3A* gene displayed decreased *RPL3A* mRNA levels and a marked increase in the abundance of *RPL3B* mRNA. These results indicated that expression of the *RPL3* genes is coordinately regulated in tobacco. The transgenic plants that contained higher levels of *RPL3B* mRNA exhibited leaf overgrowth and mottling. Epidermal cells of these plants were increased in number and decreased in size. The rRNA precursor and the mature rRNAs accumulated in these plants, suggesting that ribosome biogenesis is upregulated. Tobacco plants transformed with a "hairpin" RNA vector harboring the full-length *RPL3B* cDNA exhibited efficient silencing of both *RPL3A* and *RPL3B* genes, reduced L3 levels and an abnormal phenotype characterized by a delay in development, stunting and inhibition of lateral root growth. L3 deficiency led to a reduction in cell number and an increase in cell size, suggesting that L3 positively regulates cell division. Decreasing *RPL3* gene expression resulted in resistance to high levels of DAS.

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 you grant. Please reference each item using an accepted journal format. If you need more space, continue the list on the next page.

Popescu, S. and Tumer, N. E. 2004. Silencing of ribosomal protein L3 genes in *N. tabacum* reveals coordinate expression and significant alterations in plant growth, development and ribosome biogenesis. *The Plant Journal*, 39:29-44.

Di, R. and Tumer, N. E. 2004. A truncated form of yeast L3 eliminates ribosome depurination and cytotoxicity of pokeweed antiviral protein and confers resistance to the trichothecene mycotoxins. In preparation for Nature/Biotechnology

Di, R. and Tumer, N. E. 2003. Expression of the yeast L3 and the pokeweed antiviral protein genes confers resistance to trichothecene mycotoxins. National Fusarium Head Blight Forum Proceedings, Bloomington, MN p. 13.