

**U.S. Wheat and Barley Scab Initiative
 FY00 Final Performance Report (approx. May 00 – April 01)
 July 30, 2001**

Cover Page

PI:	Subbaratnan Muthukrishnan
Institution:	Kansas State University
Address:	Dept. Of Biochemistry Willard Hall Manhattan, KS 66506
Email:	smk@ksu.edu
Phone:	785-532-6939
Fax:	785-532-7400
Year:	FY2000 (approx. May 00 – April 01)
Grant Number:	59-0790-0-066
Grant Title:	Fusarium Head Blight Research
2000 ARS Award Amount:	\$39,024

Project

Program Area	Project Title	Requested Amount
Biotechnology	Enhanced resistance to scab by genetic engineering with genes for defense proteins.	\$64,812.00
	Requested Total	\$64,812.00¹

Principal Investigator

Date

¹ Note: The Requested Total and the Award Amount are not equal.

Project 1: Enhanced resistance to scab by genetic engineering with genes for defense proteins.

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

Scab disease of wheat leads to substantial yield loss every year. We are attempting to reduce the yield loss by genetically increasing the resistance to scab. The specific approach involves the introduction of genes for pathogenesis-related (PR-) proteins in different combinations into wheat by biolistic transformation. These genes will be under the control of promoters that will result in constitutive high level expression of the protein products of these genes in all parts of the plant. We will identify the specific combinations of genes for PR-proteins that are most effective against scab using bioassays of transgenic plants in laboratory and field conditions.

2. What were the most significant accomplishments?

By biolistic transformation, we have regenerated 24 wheat plants after selection in presence of glufosinate. These primary transgenics have been propagated to obtain progeny lines. From these, we have identified 6 transgenic lines expressing either single PR-protein or combinations of different PR-proteins (class IV or class VII wheat chitinases or a wheat glucanase) and combinations of them. One line has two different glucanase genes (289:383) and two have a glucanase and a chitinase genes (638:383).. We have followed their inheritance and expression in the T₂ progenies. The genes were detected using PCR and Southern techniques and their expression was followed using reverse transcriptase/PCR and western blotting techniques using appropriate antibodies. These experiments have confirmed that the genes were inherited by the progeny and expressed in a stable manner. *In situ* hybridization was performed with a few lines to localize the transgenes on the wheat chromosomes. Homozygous lines were identified for the lines with 383 chitinase and 638 glucanase / 383 chitinase gene combination and are being grown for seed increase and possible field-testing. The TLP transgenic line was crossed with the commercial line Heyne and the resulting F₁plants were selected based on resistance to the herbicide, Liberty.

In a preliminary bioassay against scab in the greenhouse, we compared a line harboring the chitinase/glucanase gene combination (383:638) with the line containing the 383 chitinase gene alone. The former line performed better and showed a delay in the spread of infection for over two weeks after inoculation.

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.

Engineering disease resistance in wheat by cloning defense genes. Anand, A., Zhou, T., Walmsley, R. D., Janakirman, V., Prakash, P., Li, W., Chen, W.P., Sakthivel, N., Gill, B., Shah, J., Trick, H. N., and Muthukrishnan, S. (2000). In Vitro 36: P-1022 Abstract

Isolation and characterization of novel cDNA clones of acidic chitinases and β -1,3-glucanases from wheat spikes infected by *Fusarium graminearum* Li, W.L., Faris, J.D., Muthukrishnan, S., Liu, D.J., Chen, P.D., Gill, B.S. Theor. Appl. Genet.102:353-362