

**U.S. Wheat and Barley Scab Initiative  
 FY01 Final Performance Report (approx. May 01 – April 02)  
 July 15, 2002**

**Cover Page**

<b>PI:</b>	<b>Subbaratnam Muthukrishnan</b>
<b>Institution:</b>	<b>Kansas State University</b>
<b>Address:</b>	<b>Dept. of Biochemistry 103 Willard Hall Manhattan, KS 66506-3702</b>
<b>Email:</b>	<b>smk@ksu.edu</b>
<b>Phone:</b>	<b>785-532-6939</b>
<b>Fax:</b>	<b>785-532-7278</b>
<b>Year:</b>	<b>FY2001 (approx. May 01 – April 02)</b>
<b>Grant Number:</b>	<b>59-0790-0-066</b>
<b>Grant Title:</b>	<b>Fusarium Head Blight Research</b>
<b>FY01 ARS Award Amount:</b>	<b>\$ 58,408</b>

**Project**

<b>Program Area</b>	<b>Project Title</b>	<b>Requested Amount</b>
Biotech	Enhanced resistance to scab by genetic engineering with genes for PR proteins	\$ 60,296
	<b>Total Amount Requested</b>	<b>\$ 60,296</b>

\_\_\_\_\_  
Principal Investigator

\_\_\_\_\_  
Date

### **Project 1: Enhanced resistance to scab by genetic engineering with genes for PR 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 and evaluating transgenic plants generated by this approach in greenhouse and in scab nurseries.

2. What were the most significant accomplishments?

We have generated several lines of transgenic wheat containing combinations of genes encoding pathogenesis-related proteins (PR-proteins). Twenty-six transgenic wheat lines containing different combinations of PR-protein genes have been generated so far. Of these, six are found to be stably expressing the bar gene and chitinase and/or glucanase as revealed by PCR, RNA analysis and western blot analyses. Four of these lines have been carried through the T4 generation and identified to be homozygous for the transgene locus. These four homozygous lines (T3 and T4) were tested for scab resistance by the single floret inoculation assay. A line expressing a combination of a wheat chitinase (wheat *chi383*) and a wheat glucanase (wheat *glu638*) was found to be more resistant than the line expressing a chitinase or glucanase alone under greenhouse trials. The transgenic lines and a control susceptible line and two resistant check lines were tested in the scab nursery at Rocky Ford (in collaboration with Dr. William Bockus) for resistance to scab. It was found that the transgenic lines were not statistically more resistant to scab compared to controls while the resistant check varieties showed good resistance. It is concluded that this combination of chitinase and glucanase does not confer resistance to scab under field conditions.

Additional crosses involving an advanced line expressing a thaumatin-like protein (TLP) and the chitinase/glucanase transformed line described above has been made. These plants have been selfed to obtain F1 progeny and characterized for the presence of the transgenes and expression of the three different transgene-encoded proteins. Currently the F2 plants are being propagated for identifying the homozygous line with all three transgene loci. These plants will be evaluated next spring.

We have recently regenerated seven transgenic plants containing a gene for la wheat lipid transfer protein (TLP). These lines are being propagated to identify stably expressing lines for testing against scab.

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.

Muthukrishnan, S., Liang, G. H., Trick, H. N., and Gill, B. S. (2001). Pathogenesis-related proteins and their genes in cereals. *Plant Cell, Tissue and Organ Culture*. 64:93-114.

Jackson, S. A., Zhang, P., Chen, W., Phillips, R. L., Friebe, B., S. Muthukrishnan, S., and Gill, B. S. (2001) High resolution structural analysis of biolistic transgene integration into nuclear genome of wheat. *Theor. Appl. Genet.* 103:56-62.

Anand, A., Janakiraman, V., Zhou, T., Trick, H.N., Gill, B.S. and Muthukrishnan, S. (2001) Transgenic wheat overexpressing PR-proteins shows a delay in Fusarium Head Blight Infection. 2001 National Fusarium Head Blight Forum, Cincinnati. pp 2-6.

Anand, A., Jayaraj, J., Yi, H., Krishnaveni, S., Jeoung, JM., Essig, JS., Trick, H.N., Liang, GH and Muthukrishnan, S. (2002) Genetic Engineering of Wheat and Sorghum with Genes Encoding Pathogenesis-related Proteins (PR) to Disease Resistance. pp 42a, Society for In vitro Biology, Orlando, FL