U.S. Wheat and Barley Scab Initiative
FY02 Final Performance Report (approx. May 02 – April 03)
July 15, 2003

Cover Page

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Year:	**FY2002 (approx. May 02 – April 03)**
Grant Number:	**58-5430-2-324**
Grant Title:	**Fusarium Head Blight Research**
FY02 ARS Award Amount:	**$ 48,780**

Project

Program Area	Project Title	USWBSI Recommended Amount
BIO	Enhanced resistance to scab by genetic engineering with PR-protein genes.	$50,000
	Total Amount Recommended	**$50,000**

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Principal Investigator Date

Project 1: Enhanced resistance to scab by genetic engineering with PR-protein genes.

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

Scab is a major disease of wheat that results in substantial yield loss each year. We have adopted a gene pyramiding approach to generate transgenic wheat plants expressing different combinations of genes encoding antifungal proteins. Transgenic wheat expressing the desired combinations of genes are evaluated in greenhouse and field trials and compared with resistant and susceptible checks.

A second approach involves identification of specific combinations of antifungal proteins that are effective in inhibiting the growth of *Fusarium graminearum*, the scab pathogen using in vitro antifungal assays.

2. What were the most significant accomplishments?

Greenhouse testing was carried out in spring 2001 and fall 2002 with 4 independent homozygous transgenic lines stably expressing either the 638 glucanase alone (1 line); 289 glucansae / 383 chitinase in combination (2 independent lines) and one transgenic line over-expressing the 383 chitinase / 638 glucanase (*chi/glu*) combination. The line over-expressing the *chi/glu* transgenes (#32A) showed a delay in the development of disease symptoms, and fewer infected spikes compared to the control and silenced transgenic plants (Table 1A). Three other transgenic lines with moderately elevated levels of glucanase alone or a combination of chitinase and glucanase were also tested in the greenhouse.

Field trials were carried out in spring 2002 in a scab nursery in Manhattan, Kansas with transgenic wheat lines that had moderate resistance to scab in previous greenhouse trials. The plants included in this study were homozygous progeny from the rice *tlpD34* transgenic line and line 32A with a high level expression of wheat 383 chitinase/638 glucanase combination. Non-transgenic Bobwhite and Wheaton, a susceptible control, along with the two resistant checks, MN99112 and Scab-7 were included in the trial. The transgenic lines did not have any enhanced resistance against scab, suggesting that these lines could not withstand continuous pathogen pressure encountered in the field where both the type I and type II resistance is required for survival

From the apoplastic fluid of transgenic line 32A2-3 several PR-proteins were purified to near homogeneity. These proteins were tested in different combinations for their ability to inhibit *Fusarium graminearum*. One combination of a wheat chitinase and glucanase and another combination of a chitinase, glucanase and thaumatin-like protein were found to be inhibitory to the scab pathogen at concentrations that are likely to be present in transgenic wheat plants. These experiments have pointed to specific combinations of genes that are most likely to be effective against the scab pathogen.

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.

Anand, A., Trick, H. N., Gill, B. S., and Muthukrishnan, S . (2002). A systematic approach for identifying antifungal proteins with enhanced resistance to scab. 2002 National Fusarium Head Blight Forum Proceedings, 4-8.

Anand, A., Zhou, T., Trick, H. N., Gill, B. S., and Muthukrishnan, S . (2003). Greenhouse and field testing of transgenic wheat against *Fusarium graminearum*. J. of Exp. Bot. 54:1101-1111.

Anand, A., Trick, H. N., Gill, B. S., and Muthukrishnan, S . (2003) Stable and random gene silencing in transgenic wheat. Plant Biotechnol. J. (In Press)

Ajith Anand[1], Eric A. Schmelz[2] and Subbaratnam Muthukrishnan[1*] Development of lesion-mimic phenotype in a transgenic wheat line over-expressing genes for pathogenesis-related (PR) proteins is dependent on salicylic acid concentration. Mol Plant Microbe Interactions (accepted).

Ajith Anand, Yasuyuki Arakane, William Bockus, Subbaratnam Muthukrishnan. Pathogenesis-Related Proteins from Apoplastic Fluid of a Transgenic Wheat Line shows combinatorial antifungal activity. Plant Physiol. (submitted)