U.S. Wheat and Barley Scab Initiative
Annual Progress Report
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Cover Page

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Year:	**FY1999**
Grant Number:	**59-0790-9-076**
Grant Title:	**Fusarium Head Blight Research**
Amount Granted:	**$34,146.00**

Project

Program Area	Objective	Requested Amount
Biotechnology	Develop rapid testing of anti-fungal proteins against Fusarium graminearum.	$35,000
	Requested Total	$35,000[1]

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Principle Investigator Date

[1] Note: The Requested Total and the Amount Granted are not equal.

Project 1: Develop rapid testing of anti-fungal proteins against Fusarium graminearum.

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

Speeding progress of genetic engineering of wheat and barley for scab resistance.

Given the inefficiency of whole plant transformation of wheat and barley, it is too expensive and time-consuming to test all available and new antifungal proteins against FHB. A more rapid testing of AFP's against the FHB fungus was needed. At its fastest, whole plant transformation takes 9-12 months, with and additional 2-3 months needed for disease screening adult plants. Our experience with genetically engineering AFP's into oat, a more efficient system, showed that it cost more than $75,000 per AFP to test each individual AFP for efficacy in stable, whole plant oat transformants.

Therefore we are transforming maize callus cells (Black Mexican Sweet Corn - BMS), and testing various constitutive promoters linked to a GUS reporter gene to gain understanding of promoter efficacy.

Once we have established the types of promoters that work best we will create AFP-expressing suspension cell cultures. Liquid suspensions of the transformed plant cells will be poured on nylon membranes and the liquid drained off. Thus we create a "lawn" of transformed plant cells that are excreting the AFP to be tested against *Fusarium graminearum.* Such "lawns" will survive for several days if given correct temperature and light conditions.

Simultaneously with creating AFP expressing suspension plant cell cultures, we will grow hyphal colonies of the head blight fungus *Fusarium graminearum on* defined solid nutrient media. Discrete plugs of hyphae will be removed from the solid media and inserted into pure, solid agar sheets lacking fungal nutrients. The fungal plugs embedded in the agar sheets will be placed under a microscope fitted with a television camera and an image of the fungus will be recorded. Then the solid agar sheets with their fungal plugs will be placed face down on a "lawn" of transformed plant cells. This "sandwich" approach ensures that the fungus is in contact with the plant cell surfaces that are excreting the AFP of interest.

Finally, after several days the solid agar sheets will be removed from the "sandwich" and the fungal plugs will be placed under the microscope and another TV image will be recorded. The "before" and "after" images will be compared to see if the fungus continued to grow or was stopped or killed by the excreted AFP.

The results from this rapid testing procedure will determine which AFP-expression systems should be most actively pursued in the more costly and time-consuming whole plant transformation systems.

2. Please provide a comparison of the actual accomplishments with the objectives established.

Objective I. Devise a rapid, plant cell based assay system for antifungal proteins (AFP's) against *Fusarium graminearum*.

Established a black Mexican sweet corn (BMS) cell suspension culture.

a. Made plasmid constructs for testing efficacy of constitutive promoters in BMS cells.
b. Used the GUS reporter gene in conjunction with different promoters to test promoter efficacy.

Established that three promoters are equally effective. Maize UBI, CaMV 35 S, and the Badnavirus promoter from the sugar cane badnavirus.

Established physical and cultural conditions for introduction of F. graminearum to transformed BMS cells. *F. graminearum* can grow on MS media used to culture the BMS cells and can withstand the temperatures used for BMS culturing.

Objective II. Make and proof AFP-containing constructs for genetic engineering against FHB.

1. Made Badnavirus promoter driven constructs for the hydrolytic, antifungal cell wall enzymes, chitinase (from rice) and beta 1,3 glucanase (from alfalfa).
2. Made a Badnavirus promoter driven construct for the antifungal oat thionin protein.

Objective III. Pass proofed and FHB tested AFP constructs onto whole plant transformation groups.

3. What were the reasons established objectives were not met? If applicable.

Because funding did not arrive until May of 1999 there was a delay in the project start and in getting personnel to begin work. Therefore work on Objective II, which is necessary before we can test the first AFP in our cell suspension system is just now beginning.

4. What were the most significant accomplishments this past year?

a. Finding that several promoters can be used with the BMS cell line. Thus, we can test constructs that are built in other laboratories and not have to cut out the AFP and place it in a particular construct with a particular promoter.
b. Finding that F. graminearum can grow on MS media used to culture the BMS cells and can withstand the temperatures used for BMS culturing.

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.

None to date.