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

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Year:	**FY2000**
Grant Number:	**59-0790-9-076**
Grant Title:	**Fusarium Head Blight Research**
Amount Granted:	**$35,000.00**

Project

Program Area	Objective	Requested Amount
Biotechnology	Develop rapid testing of anti-fungal proteins against Fusarium graminearum.	$56,355.00
	Requested Total	$56,355.00[1]

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Principal 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.

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

We are speeding genetic engineering of wheat and barley for scab (FHB) resistance by pretesting antifungal protein plasmid constructs in a plant cell culture system.

Objectives and procedures were:

Objective I. To devise a rapid, plant tissue cell-based (Black Mexican Sweet Corn - BMS) assay system using two common and one new promoter [1].

- Cauliflower mosaic virus 35S promoter (CaMV 35S)
- Maize ubiquitin protein promoter (Ubi)
- Sugarcane bacilliform badnavirus promoter (ScBV) [5]

Objective II. To make and proof expression plasmids containing antfungal protein AFP gene sequences driven by the ScBV promoter and test their effect on growth of the FHB fungus.

Objective III. To make and proof a variety of AFP constructs for whole plant transformation

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

Accomplishments

The three promoters are equally efficacious in BMS. Stable BMS suspension cell culture lines were subcultured for eight months without loss of transgene (GUS) activity. Thus BMS liquid suspension cultured cells are stable enough for testing against the growth of *Fusarium graminearum*.

The ScBV promoter - AFP constructs for rice and barley chitinase, beta 1,3 glucanase from barley and oat TLP1were tested for transcription in each BMS cell line using RT-PCR. In preliminary studies BMS lines with these 4 AFPs were not effective in preventing or slowing growth of *Fusarium graminearum*.

We made or modified ScBV and other promoter driven constructs of GUS and the AFPs wheat a-thionin, barley ribosome inactivating protein (RIP), barley PR-5 (thaumatin-like protein-1) and barley class-II beta 1,3-glucanase. These were used in wheat plant transformation by Gary Muehlbauer's group at Minnesota. For each construct 80 to 300 plants were regenerated. Our RT-PCR primers were used on T0 and T1 leaf RNA to confirm tanscription of AFPs.

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

Most objectives were met or exceeded. However, to date none of the four antifungal genes tested in the BMS transformed cell lawns showed significant ability to inhibit the growth of the headblight fungus *Fusarium graminearum*. More AFPs in biolistic plasmid constructs need to be tested in 2000-2001.

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

- Establishing basic protocols necessary for handling the BMS cells and the FHB fungus to begin the rapid testing of AFPs [see reference 1 – Hilburn *et al*.].

- Creating plasmid constructs containing a new and potentially more potent promoter ScBV that apparently is expressed very well in the floral parts of barley.

- Creating potent new plasmid constructs with the ScBV promoter that were used with several AFPs in whole plant transformations [see reference 2 – Smith].

- Gaining valuable experience with handling and manipulating BMS cultues and the FHB fungus.

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.

1. Hilburn, K.L.B., W.R. Bushnell, G.D. Baldridge, R.J. Zeyen. 2000. Toward a plant suspension cell assay for eukaryotic antifungal protein constructs used in cereal transformation. American Phytopathological Society, New Orleans - Phytopathology Supplement (Abstract).

2. Smith, L, M. Wyckoff, G. Baldridge, R. Zeyen and G.J. Muehlbauer. 2000 Antifungal protein gene expression in transgenic wheat (Triticum aestivum). Agronomy Abstracts.